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Journal of Dairy Science Volume 100 Special Issue: Introduction

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Humans have used cattle for food, power, fertilizer, clothing, and medicines for at least 10,000 years. The domestication of cattle allowed early humans to settle in one location or to move while maintaining an adequate food supply. As humans migrated around the planet, they took cattle with them. It is not by chance that cattle became and continue to be symbols and items of wealth and power in many cultures and regions. The ability to find and manage pasture, to store dried forages and grains, to implement systems for breeding, and to harvest both milk and meat sustainably were key in the advancement of civilizations that depended on cattle for food.

In the last 3 centuries, humankind developed sufficient technology for scientific investigation and adequate "leisure time" for detailed studies of cattle as a food source. By the middle of the 19th century, the urbanization of civilization proceeded to the point that greater efficiency of food production was needed. The cow stood ready to produce. Early empirically based genetic selection, improved nutrition, "rapid transit" along rail lines, and finally the development of refrigeration and sanitation procedures formed the basis for the nascent dairy industry. The dairy farmer took a leading role supplying food for hundreds of millions and then billions of people worldwide. Scientists initiated a widespread and systematic study of the basic science behind the production and processing of milk. The practical application of scientific knowledge and new technology led to an abundant supply of nutritious food derived from milk that was biologically efficient and environmentally sustainable.

In 1862, President Lincoln and the United States Congress recognized the importance of agricultural research, practice, and education and passed the *Morrill Act*, "An Act donating Public Lands to the several States and Territories which may provide Colleges for the Benefit of Agriculture and the Mechanic Arts." Soon, many states' colleges had departments of animal husbandry and dairy husbandry, studying the production and processing of food. The *Hatch Act* of 1887 added "... agricultural experiment stations in connection with the colleges established in the several States..." Several cheese schools were established for the safe and effective production of cheese for transport to urban populations and storage when effective refrigeration was not in place.

During this time, colleges and research stations were joined by industry people who championed modern dairy production standards. W. D. Hoard was clearly a prominent leader in these endeavors. Hoard noted, "The cow is *the foster mother of the human race*. From the day of the ancient Hindoo to this time have the thoughts of men turned to this kindly and beneficent creature as one of the chief and sustaining forces of human life." Acting as a private citizen and governor of Wisconsin, W. D. Hoard encouraged, planned, and funded what became the basis of modern dairy production practices. Included in his efforts were cow feeding and housing standards and the beginning of genetic selection standards, including the forerunner of the modern Dairy Herd Improvement Association (DHIA) program. Standards for production and processing of alfalfa for feed were developed, as were standards for milk to detect and prevent adulteration. He fought to protect dairy products through his support of the "Oleomargarine Act." Disease was a priority, as Hoard led a 50-year effort to eradicate tuberculosis from the national dairy herd. Education of the farmer was central to Hoard's philosophy for a successful dairy industry. In his speech in the early 1900s, he wrote, "[Wisconsin State Dairy Association] has held steadily to the one purpose of promoting dairy knowledge and improved dairy cattle... Make the farmer an intelligent, reading, thinking dairyman and all other material results will follow as supply follows demand" (Hoard Library; https://www.wisconsinhistory.org/turningpoints/search.asp?id=973).

By 1907, the need for organized support and communication of the dairy sciences across the United States resulted in the founding of the Official Dairy Instructors' Association. In his 1913 presidential ad-

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dress to the association, Julius H. Frandsen, professor of animal husbandry and dairy husbandry and head of the department, University of Massachusetts, wrote

> "In order that the association may accomplish the great tasks that lie before it, it should have the hearty support of all who are engaged in the various instructional and investigational lines of dairy work. In my estimation this can best be accomplished by some method which will give the association more prominence and publicity than it has enjoyed in the past. . . In my estimation, a journal published by our Association, either quarterly or monthly, would be of almost as great service to its members. It would give the Association an opportunity to publish committee reports, outlines for courses of study, and new official methods without needless delay. It would also provide for the publication of such original data as the members of the Society may care to contribute immediately upon its completion. It would also afford an excellent opportunity for the publication of such experimental work and observations as may be of interest to all engaged in this line of work, but of such nature that it may not be desirable to publish in bulletin form."

Within 4 years, the first issue of the *Journal of Dairy Science* was published, with Professor Frandsen as the editor-in-chief. He wrote in the opening announcement (Frandsen, 1917), "The American Dairy Science Association, formerly the Official Dairy Instructors' Association, has established THE JOURNAL OF DAIRY SCIENCE as the official organ of the Association and as a medium for the discussion of general and technical problems relating to the science of dairying, which confront the worker in every field of this important industry."

There were about 6.4 million farms and just under 100 million people in the United States when Frandsen wrote these words. Most farms had at least one cow, and about 3 million farms reported being primarily a dairy farm, with almost 20 million cows in the United States. The oldest land-grant college was barely 50 years old and many were only 20 to 30 years old with small enrollments. Nevertheless, a massive increase in scientific research and practical application to improve the human condition was beginning. As the population and economy expanded, departments of dairy husbandry were expanded from the old cheese schools and new ones were created, along with departments of animal and poultry husbandry in many states.

The number of dairy farms is in the United States is now approximately 40,000, with approximately 9 million dairy cows. Total production per cow in the United States has increased 4- to 5-fold since the first issue of the *Journal of Dairy Science* was published. The US trends in cow numbers, farms, and production are mirrored in other countries with modern production practices. Throughout the last 100 years, the *Journal of Dairy Science* has been, and remains, a running record of scientific and technological advancement in dairy production and science. Each issue, volume, and decade built on the last to answer relevant scientific questions and to report developments and technological advancements. In 2016, the journal published more than 700 articles and 10,000 pages from more than 1,000 authors and co-authors from all over the world. Topics included cheese quality, gene transcription in the mammary gland, the effect of milking parlor activity on milk production and quality, and the improvement of animal comfort and welfare. Without doubt, the original vision of J. H. Frandsen has been fulfilled.

This special issue represents our attempt to summarize and highlight 100 years of scientific and technical progress in dairy science. It was made possible through the financial support of the ADSA Foundation. Thirty separate but related papers, with 1,000 unique references, provide the dedicated reader an opportunity to learn where we are now and how we got here. Included in each article is a timeline of the major findings in each area. This series of papers can be used by anyone interested in the role that scientific research and technological advancement has played in efforts to make a safe and plentiful food supply for a growing population.

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Journal of Dairy Science Volume 100 Special Issue: Summary

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The *Journal of Dairy Science* has been a driving force for dairy research and application over the last century. All aspects of dairy science are represented in the journal, from the production and harvesting of milk to its processing into a safe and affordable human food. In discussing the purpose and composition of this 100-year issue, attention was paid to the breadth of disciplines and fields encompassed by "dairy science." It was a challenging task to determine an appropriate level of detail for the anniversary issue. "Dairy nutrition," for example, was too broad, but a single chapter on "Vitamins" and another on "Minerals" was too narrow. An initial list of 40 subject areas was refined to the 30 subject areas that are included here. There is overlap between articles but that is true in science as well. Nutrition affects reproduction and milk composition; genetics affects milk production and nutrient requirements, and so on. Authors were selected based on their in-depth knowledge of current as well as historical aspects of a field. The entire field of dairy science owes a great debt to the authors who selflessly donated their time in the preparation of papers that are published herein. Individuals whose careers were defined by detail in their research efforts were forced to be summative and discriminatory in their presentation of 100 years of scientific literature.

Identifying 10 to 20 major findings or advances to be included in the timelines was not always easy or, in some cases, possible for the individual topic areas. Despite the challenges, an outstanding series of articles that identified and summarized key findings in dairy science was prepared. Students and researchers of the present need a solid foundation of what we know now so that we do not repeat the past instead of creating the future. We hope that the articles themselves and the associated timelines become valuable resources for this purpose. We also hope that funding bodies and elected decision-makers take note of the papers because they demonstrate a strong example of what 100 years of direct public–private partnerships within the agricultural research sector can accomplish.

The *Journal of Dairy Science* has seen steady growth over its first 100 years (Figure 1). In total, almost 30,000 articles and 200,000 pages have been published. Major findings published in the journal in its first century of publication, as summarized in the 30 timelines in this issue, clearly demonstrate the wide and deep influence that dairy science research and application have had on the production of a safe and affordable food supply for humanity. Any attempt to estimate the economic impact of 100 years of dairy science would be futile. It is a simple fact that dairy science research, teaching, and application has made the world a healthier place by supplying milk for human consumption.

Many of the topic areas in dairy science today were well known to dairy scientists in 1917. For example, age at first calving was a topic addressed in the second issue of the journal and continues to be researched today. In 1917, the conclusion was that heifers should be fully grown to maximize production. Advances in nutrition and genetics over the past 100 years have made the conclusions from 1917 a reality in 2017. Topics such as genetic subtypes of casein, flavored cultured yogurt, artisan cheeses, antibiotic resistance, genetic selection for reproduction, balancing rations for amino acids, feeding for reduced environmental impact, animal welfare, and cow comfort were identified as relatively new fields by the authors. High-throughput measurement techniques, big data, modeling, genomics, systems biology, advanced statistics, rumen monitoring, and activity monitoring were not known at the 50-year mark and are now standard in our field. As in all scientific fields, dairy science demonstrates the continuous loop between the initial question, the testing of the hypothesis (research), the defense and publication of the results, and the creation of new questions based on the original research. This constant cycle of scientific discovery fueled by the inborn curiosity of the human condition has led to a century of dairy science research and vast improvements in the production and processing of milk.

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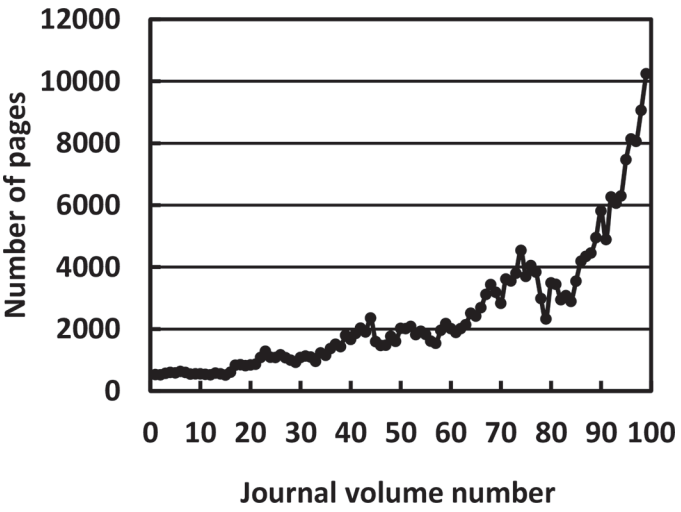

Figure 1. Number of pages per volume published in the *Journal of Dairy Science* in its first 100 years.

We will not attempt to predict the next 100 years in detail. It is completely reasonable to expect that we will see more and better integration of what we now still consider "separate" disciplines of dairy science. Research quality will continue to improve as demanded by our rising expectations of scientific endeavors and by our clients—farmers, consumers, and funding agencies. Fewer "university" and more "on-farm" studies will be conducted for practical reasons of cost and animal availability. At the same time, more and better laboratory (in vitro) studies will be conducted as we continue to probe in more detail at the cellular and molecular levels. Complex data sets will be the norm. Studies will be published that expand our understanding of the complex system that includes the cow, the farm, the industry, and society. Research will regularly be published that relates the specific genetics, nutrition, and housing of the cow to meet a specific consumer demand for the product, the production system, and the environment.

Will the *Journal of Dairy Science* publish an additional 100 volumes? It is hard to say. What we can say is that 100 volumes of the *Journal of Dairy Science* demonstrate something unique and special about dairy scientists and their desire to create and share new knowledge. The *Journal of Dairy Science* was conceived and launched during the First World War. Indeed, dairy science has been done and manuscripts published through the most significant world-changing events of the 20th and now 21st centuries. It is not the requirement of "publish or perish" that explains the legacy of 100 years of dairy science and the peer-reviewed scientific literature that tells its story. Rather, the human spirit is defined in part by our desire to pursue new knowledge through scientific investigation and to use this information to help others. New discoveries motivate scientists to share their results with their generation and with many future generations.

Science faces many threats today, as do the journals that have served our scientific enterprise so well. Science and the scientific literature will change and the *Journal of Dairy Science* will change as well. Nonetheless, there will be dairy scientists actively working in the future to ensure the safe, efficient, and affordable production and processing of milk for human consumption. What we know from the first 100 years is that dairy scientists will tell others what they have learned through the pages of our journal and they will continue the significant scientific legacy of the *Journal of Dairy Science*.

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A 100-Year Review: Historical development of female reproductive physiology in dairy cattle[1]

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ABSTRACT

The objective of this historical review in female reproductive physiology is to encapsulate major advancements since the founding of the American Dairy Science Association in 1906. The emphasis is on landmark publications in the *Journal of Dairy Science* since its first volume in 1917. A historical perspective and inferences are made in forecasting evolution of female reproduction and links between physiology of reproduction and the mammary gland. Subsequent sections are focused into main physiological categories and the temporal advancements within these physiological windows. Time points of understanding vary considerably within categories due to various advancements in technology, biological techniques, experimental design, data collection, statistical analyses, and computational forecasting. The physiological windows examined are hypothalamic and pituitary control of the ovary related to estrous behavior and cycle; differential control of the corpus luteum and uterus as influenced by cycling and pregnancy statuses; peripartum and postpartum programing of reproduction; and scientific foundation for the next century. The importance of interdisciplinary programs and integration of reproduction, nutrition/metabolism, genetics, health, and management are emphasized. The modern dairy cow of 2017 exemplifies excellence in both reproductive and lactational performance and is the foundation biological model for the next century.

Key words: reproductive physiology, pregnancy, estrous behavior, 100-year review

INTRODUCTION

It is quite appropriate to reflect on the landmark contributions over the last 100 years of the *Journal of Dairy Science* related to female reproductive physiology in dairy cattle. The modern dairy cow has been selected and managed for high production and efficiency. Indeed, it is a particular challenge to optimize the fertility of the lactating dairy cow, but major advancements have been made that are operational and based on our understanding of the underlying processes of reproductive physiology. These advancements provide the basic technology that has been transferred to the modern day dairy producer (Appendix Table A1). This historical review of female reproduction sets the stage for current and future advancements in female reproduction.

Of course, not all original advancements were published in the *Journal of Dairy Science*, and the dairy cow animal model is not necessarily the all-encompassing original animal model for initial advancements in female reproductive physiology. However, there is indeed a legacy of advancements, based on the scientific method, that have been published in the *Journal of Dairy Science*. The foundation of knowledge in the bovine provides unique insights that benefit not only the health and well-being of cattle species but other species as well, including humans. It is indeed insightful as to how accurate early sequential descriptions of female reproduction (Willett, 1956) predicted our current in-depth understanding of reproductive function utilizing the temporal advancements in the array of scientific tools and methodology developed to date. The classical areas of reproductive physiology focused on disciplines of anatomy and physiology and expanded with advancements in development of knowledge and technology such as branches of microscopy (i.e., optical, electron, and scanning probe microscopy), chemistry, biochemistry, endocrinology, hormonal measurements, tissue/cell culture, quantitation of DNA/RNA, in vitro maturation, fertilization, and development of embryos, experimental models, ultrasonography, statistics combined with computer technology, sequencing of the bovine genome, functional quantitation of the transcriptome (i.e., RNA microarrays and deep RNA sequencing), genomic selection, and gene editing. These historical and temporal advancements are instrumental to understanding the fundamental makeup and complexity of reproductive processes subsequently described.

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HISTORICAL INSIGHTS FORECASTING STRATEGIES AND WINDOWS OF INVESTIGATION IN FEMALE REPRODUCTION

Gaines (1927) hypothesized that the service period (between date of calving and date of successful conception; comparable to days open) would be related to high milk production during the first calendar month of lactation. He pointed out that lactation is known to affect the reproductive cycle in humans and some of the lower animals such as the rabbit, whereby lactation proceeding at a high level inhibited pregnancy. Nevertheless, the coefficient of variation for service period was high and not associated with yield during the first calendar month of lactation. He noted that these 2 responses appeared to be independent of each other. The mean estimate for service period was 170 d in 1927 (Gaines, 1927). The mean estimate published in 2010 (2.1 million observations between 2001 and 2007) was 146.5 d and the 50th percentile was 119 d (Pinedo and De Vries, 2010). The processes of lactation, homeorhetic metabolic changes, postpartum diseases, and so on may contribute to timing of first estrus, ovulation, and pregnancy at first service. Indeed, the specific level of production early in lactation may not be the sole source associated with fertility.

At the time of these early scientific investigations, it is useful to reference the earlier publications of Halban (1905), an obstetrician/gynecologist in Vienna who linked and described in women the dynamics of mammary gland and uterine development throughout their lifespan (i.e., embryonic to postmenopausal senescence) and conducted early experimental investigations in animal models. This is a vivid example of the past that retrospectively provided the framework for reproduction and lactational physiology for dairy cattle during the subsequent 112 years captured in the publications of the *Journal of Dairy Science*. "Embryonal impulse" represented rapid growth of the fetal mammary gland and uterus in the 8th and 9th months of pregnancy. Neonatal swelling and regression of the mammary gland and uterus were due to active substances from the placenta and their withdrawal. There was a "puberty impulse" on the mammary gland and uterus due to activity of the ovaries, and this was further characterized by ovariectomy and re-transplantation experiments. After puberty, periodic swelling of the mammary gland and uterus occurs with re-occurring estrous cycles (i.e., in humans, the "menstrual impulse"). There is the "pregnancy impulse" of rapid proliferation, with hyperplasia of the glandular tissue but at a much greater rate than postpubertal changes, leading to the inference that the placenta produces more regulatory substances than does the ovary. Pregnancy changes were not due to the fetus but to the placenta because mammary growth proceeded after death of the fetus and subsided with loss of placenta. Secretions produced by epithelium of the placenta (i.e., trophoblast and chorionic epithelium) and not stromal tissue were inferred. The corpus luteum (**CL**) persisted during pregnancy under influence of the placenta. Changes in the maternal and fetal uterus were correlated because both regress in the postpartum or puerperium period. Puerperal involution of the maternal uterus occurred only after delivery of the placenta and was considered a true atrophy. Emptying of the uterus was considered critical to onset of milk secretion (i.e., 3 to 4 d after birth). Secretion from the mammary gland before placental delivery was characterized as colostrum, not milk. Suckling did not induce milk secretion and only maintained secretion after the uterus was emptied, and suckling was associated with quiescence of the ovary. Distinct differences or uncoupling were observed between uterus and mammary gland following pregnancies in the nonsuckled and suckled states. Nonsuckled or nonlactating state resulted in a coupled decrease in both the uterus and mammary gland and an earlier recrudescence of ovarian cycles. In contrast, the suckled or lactating state resulted in a greater rate of uterine regression and a marked delay in recrudescence of ovarian cycles.

Willett (1956) noted, "A pygmy sitting on the shoulders of a giant can see farther than the giant." Due to the background of fundamental research during the half-century preceding 1906, physiologists thereafter have been pygmies or giants able to see and understand phenomena that were beyond the view of the giants of yesteryear and were able to broaden ever further the horizons of our knowledge. When Asdell (1949a,b) reviewed the studies dealing with hormonal and nutritional treatments of sterility in dairy cattle, he emphasized the need to develop more sensitive tools of investigation and specified the need for adequate controls to account for spontaneous recovery of experimental animals and the need for the biochemist or nutritionist to work closely with the physiologist. These basic principles have been emphasized extensively within the *Journal of Dairy Science* with publication of strategies for design and statistical analyses of sensitive experiments across a wide array of experimental variables (discrete and continuous) with chosen confidence (α) and power (β) to detect treatment differences (Tempelman, 2009; Lean et al., 2016).

A classical experiment begun in 1947 and completed over a 7-yr period (Reid et al., 1957, 1964; Sorenson et al., 1959) examined the influence of 3 planes of nutrition fed to heifers from birth until first calving. The dietary treatments provided approximately 65% (low), 100% (medium), and 140% (high) of Morrison's TDN

standards. After first calving, all cows were fed adequately. Age of puberty was greater in the low group (20.2 mo) compared with the medium (11.2 mo) and high (9.2 mo) groups. However, both wither height and BW at the time of puberty were comparable between dietary treatments. Consequently, cow BW at first calving was lower (440, 539, and 614 kg) and occurrence of calving difficulty was greater (48, 27, and 25%) for the low, medium, and high diet groups, respectively. Reproductive responses such as services per conception and percent conception on first service did not differ. However, postpartum intervals to first estrus or ovulation were not reported, which could influence service period. These findings, and others reviewed by Schultz (1969), set the stage for subsequent investigations of basic physiology and nutritional regulation of gestation and postpartum periods on reproductive performance. With the greater milk production of recent generations of dairy cows, Butler and Smith (1989) documented that both milk production per day and days to ovulation during the first 20 d postpartum were associated negatively with average energy balance (Mcal/d) and predicted potential physiological and endocrine control mechanisms.

HYPOTHALAMIC AND PITUITARY CONTROL OF THE OVARY RELATED TO ESTROUS BEHAVIOR AND CYCLE

Hypothalamic and pituitary control of reproductive and metabolic hormones are pivotal to the integration of the reproductive system and its function during key physiological periods such as the estrous cycle/pregnancy and the transition/postpartum phases leading to restoration of reproductive competence. The ability to quantify hormones using specific, sensitive, and accurate techniques coalesced many advancements in our understanding of the endocrine system. Trenkle (1972) described the overall basic principles allowing for the use of valid radioimmunoassays (RIA) to measure hormones in plasma precisely and accurately. Indeed, such principles are applicable and essential to current systems of biological measurements based on RIA, ELISA, mRNA expression via PCR, and protein abundance. It is well established that GnRH secreted from hypothalamic neurosecretory neurons terminating in the median eminence passes into the blood vessels of the hypophyseal portal system and is transported to the anterior pituitary. The ultimate action of GnRH is to induce secretion of FSH and LH from gonadotrophs that are important for follicular growth, maturation, and ultimately ovulation with subsequent formation of the CL. Several previous temporal reviews have characterized pituitary ovarian relationships (Hansel and Snook, 1970) and further temporal advances in reproductive physiology including physiological responses to a synthetic GnRH (Britt et al., 1981).

Failure to express estrus, detect estrus, or both are major impediments to herd fertility. With use of either collar-mounted activity meters in a pasture-based production system (Kamphuis et al., 2012) or devices composed of a pressure-sensitive radiotelemetric transmitter on cows in a freestall barn (Lopez et al., 2005), approximately 28.6 and 28.5% of the cows failed to be detected in estrus, respectively. In all likelihood, anovulatory cows or cows with insufficient plasma estradiol fail to express sufficient estrus responses for detection. High-producing dairy cows have shorter durations of estrus, fewer standing events and lesser standing times, lower plasma estradiol concentrations, and greater occurrence of multiple ovulations (Lopez et al., 2005). The importance of estradiol to influence the acute release of LH in response to an injection of GnRH is demonstrated vividly in an experimental model with lactating dairy cows during a timed AI (**TAI**) program (Stevenson and Pulley, 2016). Cows with a low progesterone (**P4**) concentration at the time of a GnRH injection released more LH then cows classified as having medium to high P4 concentrations in plasma; within each progesterone classification (i.e., medium or high versus low), cows with high estradiol concentrations released more LH in response to GnRH compared with those with low estradiol concentrations in plasma. Thus, the ratio of estradiol to progesterone influences responsiveness of the pituitary to GnRH. This same phenomenon was evident in characterizing the responsiveness of the pituitary to GnRH given at different times after a luteolytic injection of $PGF_{2\alpha}$ to cows with a CL. The LH response to GnRH increased as the estradiol (pg/mL):progesterone (ng/mL) ratios increased in plasma at 0 (0.47), 12 (2.03), 24 (5.99), 48 (12.99), and 60 (14.04) h after injection of $PGF_{2\alpha}$ (Thatcher and Chenault, 1976). The preovulatory follicle increase of estradiol secretion increases sensitivity of the anterior pituitary to GnRH.

The actions of estradiol to enhance responsiveness of the pituitary to exogenous GnRH does not preclude effects at a higher level to enhance endogenous hypothalamic GnRH secretion or higher loci in the brain to regulate estrous behavior (**EB**). Kommadath et al. (2013) utilized a systems biology approach relating quantitative EB scores in cows to identified genes and biological processes shared among the anterior pituitary (**AP**) and 4 brain areas: dorsal hypothalamus (**DH**), ventral hypothalamus (**VH**), amygdala (**AM**), and hippocampus (**HC**) of lactating dairy cows. Quantitative values of EB expression in 14 cows were recorded during multiple consecutive cycles per cow beginning

at 30 DIM, and cows were euthanized on the day of estrus between 77 and 139 DIM. Tissues from the AP and the higher brain areas (i.e., DH, VH, AM and HC) were collected and underwent transcriptome analyses with a bovine oligonucleotide microarray system. For the respective tissue pairs, numbers of consensus modules of co-expressed genes and numbers of genes within the consensus modules were identified between the networks: AP–DH (23; 1,904), HC–DH (10; 846), AM–HC (8; 843), AM–DH (3; 225) and VH–DH (2 197), respectively.

The correlation between the module's eigengene (weighted average gene expression profile representative of the gene expression profiles in a module) and levels of EB exhibited by the experimental cows were tested (eigengene significance). One of the strengths of this method is that the problem of multiple testing corrections is eliminated because the correlation is between the EB trait of the cow and the module eigengene (i.e., not to individual gene expression values). Estrous behavior–correlated modules were found to be enriched for gene ontology terms such as glial cell development and regulation of neural projection development, as well as pathway terms related to brain degenerative diseases. Indicative of increased transcription and protein synthesis, cellular biosynthetic processes such as oxidative phosphorylation and ribosomal RNA synthesis were enriched in several correlated modules.

In rodent studies, stimulation of ribosomal RNA synthesis is a primary event in the activation of neuronal cells and pathways associated with female reproductive behavior. This activation precedes the estrogen-driven expansion of dendrites and synapses. Similar processes also appear to operate in cows to affect EB. Hub genes (i.e., genes in the network highly connected with other genes) within EB-correlated modules are strong candidate genes for regulating EB expression. The following hub candidate genes in the AP–DH tissue pair were notable: *NEFL* [neurofilament protein, intracellular transport to axons and dendrites, targeting and regulation of phosphatase(s) within neurons], *NDRG2*, *THY1*, and *GAP43* (involved in neural growth and plasticity), and *TCF7L2* (effector in the Wnt signaling pathway, which is active in development of blood brain barrier), *OXY* (oxytocin prepropeptide), and *AVP* (interacting to influence sexual behavior).

These findings enhance our understanding of the genomic regulation of EB in dairy cows, and provide insights into genes and biological processes shared among the bovine AP and brain areas functioning together to regulate EB. Such analytical approaches may lead to development of new biomarkers for strategic utilization to improve reproductive performance. Identification of genes associated with EB may be incorporated into development of DNA and phenotypic databases utilizing genome-wide analyses to improve fertility.

In the early 1970s, only limited reports were available regarding blood concentrations of reproductive hormones (e.g., estradiol, progesterone, LH, and FSH) due to a lack of sensitive and accurate assays. Technical tools involved gas chromatography, gonadotrophin assays with high cross-reactivities with other hormones, and bioassays for hypothalamic peptides and pituitary hormones (Gomes and Erb, 1965; Hansel and Snook, 1970). However, very insightful relationships were documented or inferred such as (Hansel and Snook, 1970): increases in estimates of pituitary FSH and LH secretion and hypothalamic content of LH releasing factor during proestrus and estrus, respectively; preovulatory surge of plasma LH; identification of LH as the luteotrophin for CL growth and progesterone secretion; coordination of plasma LH and progesterone peaks during the luteal phase of the estrous cycle; precipitous decline in plasma progesterone associated with CL regression in late diestrus followed by a sharp increase in plasma LH; no detection of a preovulatory increase of progesterone to induce a LH surge; and inference for a role of estradiol for induction of EB and ovulation. Subsequent studies characterized extensively the temporal changes in progesterone, estradiol, and LH in plasma for the late diestrus through the periovulatory periods (Chenault et al., 1975, 1976).

Ireland et al. (2000) encapsulated historically the evolved experimental approaches to the turnover of dominant follicles during the bovine estrous cycle. Two salient experimental approaches emerged in the 1980s, which permitted investigators to access health status of individual follicles and dynamics of antral follicle development on a within-animal basis. Measurements of intrafollicular ratios of estradiol:progesterone within antral follicles allowed for the classification of follicles as estrogen-active (i.e., healthy; estradiol > progesterone or high estradiol:progesterone ratio) or estrogen-inactive (i.e., atretic; progesterone > estradiol or low estradiol:progesterone ratio (Badinga et al., 1992; Ireland et al., 2000). The 2-cell system (i.e., theca interna and granulosa cells) for estrogen biosynthesis of the follicle was modeled by J. E. Fortune (Hansel and Convey, 1983). Indeed, the follicular wall of dominant follicles at d 5 and 8 had high aromatase activities, but follicular fluid concentrations of estradiol were high at d 5 and markedly reduced by d 8 (Badinga et al., 1992). This was indicative that thecal cell androgen availability was likely limited at d 8, accounting for the decrease in the estradiol:progesterone ratio as an atresia marker of the dominant follicle.

Such markers of follicle quality, coupled with the use of ultrasonography to measure follicle diameter (Pier-

son and Ginther, 1984), facilitated the investigation of temporal follicle dynamics on a within-cow basis. Waves of follicular development comprise a dominant follicle (**DF**) and several subordinate or secondary follicles (Ireland et al., 2000). Follicular waves comprise periods of emergence, deviation (DF selected from the cohort), dominance (health DF sustains preferential bilateral growth over all follicles on the ovaries), and atresia of DF coupled with emergence of a new follicular wave. As cited by Ireland et al. (2000), transient peaks of FSH occur with each wave of follicular development at the time of follicular emergence. As FSH concentrations descend from peak (i.e., likely due to inhibin produced by follicles in the emerging wave), deviation occurs with continued sustained growth and dominance of the DF due to acquisition of LH responsiveness. Luteinizing hormone receptors develop on granulosa cells of the healthy DF on d 4, 6, and 8 (Xu et al., 1995). However, expression of LH receptor mRNA in granulosa cells was not detected in regressing dominant follicles collected on d 10. Pulsatile perfusion of LH in the first wave of postpartum lactating cows (7 to 12 d postpartum) caused ovulation to occur 14 d earlier than in control cows (Hampton et al., 2003). The ultimate fate of the DF (i.e., turnover during diestrus or continued development as a preovulatory follicle) depends on availability of LH receptors and basal LH (enhanced either following regression of CL or following infusion in anovulatory cows). Diestrus progesterone concentrations are associated with a lower LH pulse frequency.

Ultrasonographic analyses indicate that cattle usually have 2 and 3 waves of follicular development during the estrous cycle (Ireland et al., 2000). An understanding of ovarian follicular and CL functional dynamics is essential for the use of physiological pharmaceuticals such as GnRH, P4, and $PGF_{2\alpha}$ in development of reproductive management programs that achieve high fertility (see Stevenson and Britt, 2017).

Within the basic life cycle of female reproductive competence, the corpora lutea "cometh and goeth." Secretions from the hypothalamus, pituitary gland, ovary, uterus, embryo, and fetal-placental unit control presence of the CL and its capability to secrete progesterone.

Corpus Luteum Development

The CL is a continuation of follicular development in which the preovulatory surge of LH induces luteinization of granulosa and theca cells. Smith (1986) reviewed a cross-section of physiological and molecular determinants that influence CL function, drawing on an extensive review of the literature among ruminant and nonruminant species. Following ovulation, subsequent growth and differentiation of small (theca interna) and large (granulosa) cells become the principal luteal cell components for synthesis of P4. Luteinizing hormone is the principal luteotrophin in the bovine CL (Donaldson and Hansel, 1965). Small luteal cells are responsive to LH, whereas large luteal cells have a high basal P4secretion rate. Indeed, the majority of recurring pulses of P4 in the luteal phase are coupled with stimulatory peaks of LH, and additional pulses may be associated with peak occurrences of FSH and oxytocin. The potential functional capacity of the CL is programmed to some degree by prior preovulatory programming associated with a sequential exposure to diestrus P4 and proestrus secretions of gonadotrophins (i.e., FSH and LH) and estradiol. This programming influences size and estrogenic health status of the preovulatory follicle, subsequent size of the CL, and whether subsequent CL lifespan is shortened or normal due to potential programming of the uterus (Bisinotto et al., 2013). The secretory granules in large luteal cells contain P4 and oxytocin (Fields et al., 1992). The greatest percentage of large luteal cells with a cluster of large granules, containing oxytocin and neurophysin, occurs mid-cycle on d 7 (84%) and 11 (64%) and decreases progressively on d 14 (26%), 17 (16%), and 19 (8%). From d 7 to 14, the 69% decline in large luteal cells containing oxytocin-laden secretory granules occurs before the reported increases in uterine oxytocin receptors and luteolytic pulses of $PGF_{2\alpha}$. The early concurrent granule release of oxytocin and P4 into the inferior vena cava as pulses may account for spikes of P4 that are not necessarily associated with LH pulses (Walters et al., 1984).

With LH being the luteotrophic hormone in the dairy cow, cellular mechanisms associated with regulating progesterone secretion (+/−) are critical to understanding the transition of the mature steroidogenic CL to a regressing CL. The concentrations of receptors vary in the 2 cell types; LH receptors are primarily on small luteal cells, whereas prostaglandin and estradiol receptors are located primarily with large luteal cells. Basal progesterone production is greater in large luteal cells. Protein kinase A (**PKA**) and protein kinase C (**PKC**) are 2 intracellular effector systems that regulate luteal P4 production. Each of the enzymes are present in both luteal cell types. Binding of LH to the membrane receptor of small luteal cells activates adenylate cyclase to increase intracellular cAMP and activation of PKA. The 2 intracellular effector systems, PKA and PKC, have opposing effects on P4 production. Protein kinase A is responsive to LH in small luteal cells and is a potent stimulator of P4 production, whereas PKC in large luteal cells is responsive to $PGF_{2\alpha}$ as a potent inhibitor of P4 production.

In addition to large and small luteal cells, the CL also contains endothelial, immune, and fibroblast cells. Luteal steroidogenesis depends on steroidogenic acute regulatory protein (STAR) protein to transport cholesterol from the outer to the inner mitochondrial membrane. The cholesterol side-chain cleavage enzyme (P-450scc), located on the inner membrane of mitochondria, converts cholesterol to pregnenolone. The 3β- hydroxysteroid dehydrogenase (3β-HSD) enzyme converts pregnenolone to P4 (Niswender, 2002).

Garverick et al. (1985) characterized changes and interrelationships in CL tissues of cows at 6 stages (d 4, 7, 10, 13, 16, and 19) for LH receptors, adenylate cyclase activity, and phosphodiesterase activity. Mean CL weight, luteal and plasma concentrations of P4, and unoccupied LH receptors increased from d 4 to d 10, plateaued (d 10 to 16), and then declined following luteal regression (d 19). Total number of occupied receptors/CL increased 4-fold from d 4 (47 fmol/CL) to d 10 (221 fmol/CL) and remained similar thereafter. Activities of basal adenylate cyclase, LH-activated adenylate cyclase, and guanylyl imidodiphosphate [Gpp(NH)p]-activated adenylate cyclase were greatest on d 7 to 16, compared with d 4 and 19. Luteinizing hormone stimulated ($P < 0.05$) adenylate cyclase activity relative to basal activity on d 7, 10, 13, and 16, whereas Gpp(NH)p stimulated ($P < 0.05$) adenylate cyclase activity at each period. Thus, adenylate cyclase activity is capable of being regulated in regressing CL at d 19. Phosphodiesterase was 46% greater on d 19 than on d 4. Furthermore, plasma P4 concentrations were correlated positively ($P < 0.01$) with unoccupied LH receptor concentrations (r = 0.64), and activities of basal adenylate cyclase (r = 0.46), LH-activated adenylate cyclase (r = 0.52), and Gpp(NH)p-activated adenylate cyclase (r = 0.47). A prior in vivo experiment monitoring plasma P4 concentrations following GnRH stimulation of plasma LH injected at 0, 12, 24, 48, and 60 h after injection of $PGF_{2\alpha}$ indicated that functional CL regression was fully complete between 48 and 60 h (Thatcher and Chenault, 1976).

Uterine-Corpus Luteum Regression

Luteolysis is a uterine and neuroendocrine-mediated event. Functional luteolysis denotes the decline in P4 production by the CL. Structural luteolysis denotes the physical involution and regression of luteal tissues. The interrelationships between uterus and ovary have been well documented with experimentation and development of collateral models in sheep and cattle. The crossover of investigations in reproduction with these 2 species as models is reflected in *Journal of Dairy Science* and *Journal of Animal Science* publications, symposia, and joint meetings for the last 100 years. This is further reflected through recent reviews of 2 interdisciplinary efforts in ruminant female reproduction related to control of the CL during the cycle and early pregnancy that entails multiple failsafe mechanisms to ensure success (Arosh et al., 2016; Spencer et al., 2016).

The phenomena in cattle controlling regression of the CL involve the effect of estradiol produced by a dominant follicle acting on a P4-primed uterus to induce endometrial secretion of $PGF_{2\alpha}$, which is the luteolytic hormone causing functional and structural luteolysis. Injection of estradiol in hysterectomized heifers failed to completely regress the CL compared with cows with a uterus, indicating that estradiol exerts its luteolytic effect by acting through the uterus (Brunner et al., 1969). Estradiol stimulates $PGF_{2\alpha}$ at d 18 of the estrous cycle with maximal uterine production and metabolism at 6 h after injection (Knickerbocker et al., 1986a). The $PGF_{2\alpha}$ travels to the ovary via a uterine venous transfer to the ovarian artery ipsilateral to the CL (Wolfenson et al., 1985). The estradiol acting in conjunction with oxytocin secreted by the posterior pituitary, by the ovary, or both (Fields et al., 1992) induces $PGF_{2\alpha}$ availability for regression of the CL.

In intensive studies with sheep (see review by Arosh et al., 2016), at the time of luteolysis, $PGF_{2\alpha}$ is released from the endometrium in a pulsatile pattern. Continuous exposure of endometrium to P4 for 8 to 10 d downregulates expression of nuclear progesterone receptor (PGR) in luminal epithelial (LE) cells between d 11 and 13, thereby allowing a rapid increase in expression of estrogen receptorα (**ESR1**) after d 13, followed by an increase in expression of oxytocin receptor (**OXTR**) after d 14 of the estrous cycle. Pulsatile release of oxytocin after d 13 to 14 of the estrous cycle acts on endometrial OXTR to induce the luminal endometrial epithelium to secrete 5 luteolytic pulses of $PGF_{2\alpha}$ between d 14 and 16 of the estrous cycle. Luteal oxytocin acts as a supplemental source of oxytocin to boost the oxytocin pulse from the posterior pituitary during luteolysis. Binding of oxytocin to its receptor on LE cells activates phospholipase C (**PLC**), leading to increases in Ca^{2+} and PKC, which activates the $PGF_{2\alpha}$ biosynthetic pathway (Figure 1A). Five $PGF_{2\alpha}$ pulses of 1 h duration over a period of 48 h at 8-h intervals are required to consistently cause complete CL regression. Endometrial $PGF_{2\alpha}$ pulses are transported from the uterine luminal cells through a prostaglandin transporter (**PGT**)-dependent mechanism, which increases local $PGF_{2\alpha}$ availability in the utero-ovarian vein for countercurrent exchange in the uterine-ovarian plexus (**UOP**) via a simple diffusion to the ovarian artery. Local transport of $PGF_{2\alpha}$ through the UOP is essential for regression of the CL, because 99% of $PGF_{2\alpha}$ secreted by

the endometrium is catabolized into its inactive stable metabolite (PGFM) after a single systemic passage through the lungs. Endometrial luteolytic $PGF_{2\alpha}$ pulses act through the $PGF_{2\alpha}$ receptor (**FP**) of large luteal cells to activate Ca^{2+} and PKC pathways to stimulate $PGF_{2\alpha}$ biosynthetic machinery, as well as suppressing survival and activating apoptotic pathways to decrease P4 biosynthesis (see Figure 1B). Collectively, these coupled systems lead to regression of the CL.

Understanding the mechanisms of controlling follicle development, ovulation, CL development, and regression of the CL has resulted in development of the physiological pharmaceutical agents (i.e., GnRH, P4 intravaginal devices, and $PGF_{2\alpha}$) to control induction of estrus and ovulation for optimal fertility in dairy heifers and lactating dairy cows with the use of AI or embryo transfer (see Stevenson and Britt, 2017).

Uterine–Conceptus–Corpus Luteum Interactions to Sustain Pregnancy

Long-term cooperation between the laboratories of F. W. Bazer, R. M. Roberts, and W.W. Thatcher, as well as several distinct generations of academic descendants and cooperators, led to intensive mechanistic/molecular models and approaches to comprehend establishment of pregnancy in sheep and cows (reviews: Bazer et al., 2009; Arosh et al., 2016; Spencer et al., 2016).

Interferon-τ (**IFNT**), a type 1 interferon, is secreted by conceptus mononuclear cells of the trophectoderm and is the antiluteolytic factor to prevent regression of the CL in ruminants. Interferon-τ interacts locally within the conceptus–uterine–ovarian (**CUO**) complex such that the CL is maintained to sustain pregnancy due to differentials in uterine secretion and transport of $PGF_{2\alpha}$ and prostaglandin (PG)E_2 within the CUO complex. Differences in prostaglandin production are coupled with localized transport systems to the ovary bearing the CL that sustains secretion of P4.

Interferon-τ acts on uterine luminal epithelia (**LE**) and superficial glandular epithelia (**sGE**) to inhibit transcription of *ESR1* and *OXTR* genes and abrogate development of the endometrial luteolytic mechanism (Spencer and Bazer, 1996). The increases in expression of *ESR1* and *OXTR* mRNA in uterine luminal and superficial glandular epithelia between d 11 and 17 after estrus in cyclic sheep do not occur in pregnant ewes (Spencer and Bazer, 1995) or in cyclic ewes in which recombinant ovine IFNT is injected into the uterine lumen (Spencer et al., 1995). Thus, oxytocin is unable to induce secretion of luteolytic pulses of $PGF_{2\alpha}$. However, basal production of $PGF_{2\alpha}$ is greater in pregnant than in cyclic ewes, as IFNT does not inhibit expression of prostaglandin-endoperoxide synthase 2 (**PTGS2**) in uterine LE or sGE (Charpigny et al., 1997). The molecular mechanisms involved in IFNT silencing expression of ESR1 are likely due to IFNT inducing expression of IFN regulatory factor 2 (**IRF2**), a potent repressor of transcription, in uterine LE and sGE. In the absence of ESR1, ovine uterine epithelia do not express OXTR (Fleming et al., 2006).

In contrast to the ovine *OXTR* gene, the bovine *OXTR* gene lacks a classical palindromic estrogen response element (ERE). However, there are 3 ERE halfsites (Telgmann et al., 2003) such that estradiol activation of the OXTR may require either steroid receptor co-factors or the transcription factor SP-1. Nevertheless, IRF2 can regulate expression of the bovine *OXTR* gene (Telgmann et al., 2003). The IFNT induction of IRF-2 serves a common role in inhibiting presence of both ESR1 and OXTR; IRF2 is the primary effector of IFNT antiluteolytic actions in bovine endometria.

A novel alternative and complementary noncanonical mechanism leading to suppression of $PGF_{2\alpha}$ in pregnancy is described by Arosh et al. (2016; Figure 1C). Interferon-τ activates the JAK-SRC-EGFR-RASRAF-ERK1/2-EGR-1 module signaling pathway in ovine endometrial LE cells in vitro. The IFNT treatment acts through extracellular signal-regulated protein kinases 1 and 2 (**ERK1/2**) and early growth response protein 1 (**EGR-1**) to phosphorylate PGT protein at tyrosine and threonine residues and concurrently dephosphorylate PGT protein at serine residues. Alterations in phosphorylation of PGT inhibits PGT function to suppress PGT-mediated transport of pulsatile release of $PGF_{2\alpha}$ from the endometrium. This proposed pathway (Figure 1C) to inhibit $PGF_{2\alpha}$ release occurs without necessarily being totally dependent on suppressing endometrial expression of either *ESR1* or *OXTR*. Concurrently, IFNT acts on both endometrial and stromal cells to increase net production of PGE_2, and PGE_2 from the stroma cells acts in a paracrine manner through PGE_2 receptors (**EP**), EP2/EP4, to further enhance PGE_2 production by endometrial cells. As a consequence of these coordinated PG responses to IFNT, a greater amount of PGE_2 is transported through the UOP to the CL. Greater concentrations of PGE_2, acting through the EP2/EP4 membrane receptors on large luteal cells (Figure 1D), activate the cAMP/PKA pathway: increasing PGE_2 secretion and autocrine uptake in large luteal cells, activation of antiapoptotic and survival pathways, and suppression of proapoptotic pathways. Collectively, these effects maintain survival of luteal cells and maintain P4 secretion.

A series of experiments published over the last 3 decades in cows paralleled investigations in sheep in examining the functional interrelationships of IFNT and prostaglandins. A variety of experimental models

documented in vivo and in vitro alterations in transport and attenuation of $PGF_{2\alpha}$ responses between cyclic and pregnant cows, at the time the CL regresses or is sustained, respectively (see reviews by Thatcher et al., 1984a,b). In pursuit of the antiluteolytic agent, temporal characterization (Bartol et al., 1985), and intrauterine administration (Knickerbocker et al., 1986b) of bovine conceptus proteins, bovine trophoblast protein-complex (Helmer et al., 1989) and recombinant bovine (b)IFNT (Meyer et al., 1995) clearly documented the antiluteoytic effects of bIFNT to maintain the CL. Both in vivo and in vitro experimental models documented that bIFNT attenuates uterine secretion of $PGF_{2\alpha}$. Mechanistic experimental approaches compared expression and abundance of candidate molecules in the endometrium, such as ESR1 and OXT receptors, at d 17 post-insemination between pregnant and cyclic cows that were treated with or without bST and were nonlactating (Guzeloglu et al., 2004) or lactating (Bilby et al., 2006). Gene expression and protein abundance or quantities in the uterine lumen of luteolytic and antiluteolytic agents were evaluated. Quantities of *ESR1* mRNA, ESR1 protein, ESR1 immunostaining of LE, and *OXTR* mRNA were decreased in endometrium of pregnant cows, whereas quantities of PGR immunostaining in endometrial glands, PGHS-2 protein, PGHS-2 immunostaining of LE, and total quantities of both $PGF_{2\alpha}$ and PGE_2 ($PGF_{2\alpha} > PGE_2$) in the uterine flush were greater in pregnant cows. These findings further document suppression of the endometrial luteolytic mechanism to maintain ovarian P4 production in cattle.

Uterine–Conceptus–Corpus Luteum Interactions to Enhance Growth and Development of the Conceptus

Beyond the mechanisms dealing with maintenance of the CL, an understanding of the factors controlling the coordinated growth and development of the conceptus is essential to improving fertility. An optimal level of luteal P4 during the cycle before ovulation, the rise in P4 following ovulation, and sustained luteal-phase concentrations of P4 are essential to program the endometrium to produce an enriched histotrophic environment within the uterine lumen to support the developing blastocyst and differentiation of the conceptus. The enriched pool of histotroph at the critical period of pregnancy recognition (i.e., maintenance of the CL) contains embryotrophic factors (e.g., amino acids, sugars, proteins, prostaglandins, lipids). Certain components alter gene expression of the endometrium and conceptus to support development of a conceptus–maternal unit approaching the transitional processes of implantation and placentation. Rapid elongation of the conceptus and extraembryonic membranes is a dynamic and extensive process with major alterations of endometrial biosynthetic and metabolic pathways to support conceptus growth and development. Alterations in immune function within the endometrium appear to be driven in a manner to prevent rejection of the conceptus. These vital interrelationships have been reviewed extensively in sheep and cattle (Dorniak et al., 2011; Spencer et al., 2016).

Application of transcriptomics allows for the complete identification of RNA transcripts that are differentially expressed from the genome of a population of cells from different states that control conceptus–endometrial interactions. The major changes required to drive conceptus elongation and uterine receptivity for the gradual process of implantation occur between d 7 and 13 in response to P4 from the CL. This occurs regardless of whether an appropriate embryo or developing conceptus is present. Comparisons of the endometrial transcriptome between cyclic and pregnant heifers showed no difference before pregnancy recognition, indicating that P4 programming sets the stage for subsequent differential responses associated with or without presence of a conceptus (Forde et al., 2011).

An experiment was designed to determine effects of pregnancy and lactation on endometrial gene expression on d 17 of the estrous cycle and pregnancy in primiparous nonlactating and lactating cows (Cerri et al., 2012). Regulation of different immune functions is an extremely important event within the d 17 endometrium in the presence of a conceptus (pregnancy). Results from Gene Ontology (**GO**; http://www.geneontology.org/) analyses show an upregulation of many genes related to the immune system such as defense response (GO:0006952), interferon regulatory factor (IPR001346), and immune effector process (GO:0002252).

A great portion of these genes (the vast majority upregulated by pregnancy) were in fact IFN-stimulated genes (ISG) such as myxovirus resistance 1, interferon-inducible protein p78 (*MX1*), myxovirus resistance 2 (*MX2*), similar to putative ISG12(a) protein (*ISG12*), ISG15 ubiquitin-like modifier (*ISG15*), IFN-stimulated exonuclease gene (*ISG20*); IFN regulatory factors (*IRF1*, *IRF3*, *IRF5*, *IRF6*, *IRF7*, *IRF8*, *IRF9*), IFN induced with helicase C domain 1 (*IFIH1*); chemokines chemokine (C-C motif) ligand (*CCL2*, *CCL8*, *CCL11*), chemokine (C-X-C motif) ligand (*CXCL2*, *CXCL10*), chemokine (C-C motif) receptor 7 (*CCR7*); signal transducer and activator of transcription (*STAT1*, *STAT2*), with many of them previously described in sheep (Spencer et al., 2008).

The genes that encode for the Fc fragment of IgG (*FCGRT*), T-cell receptor delta chain (*TRD*), immu-

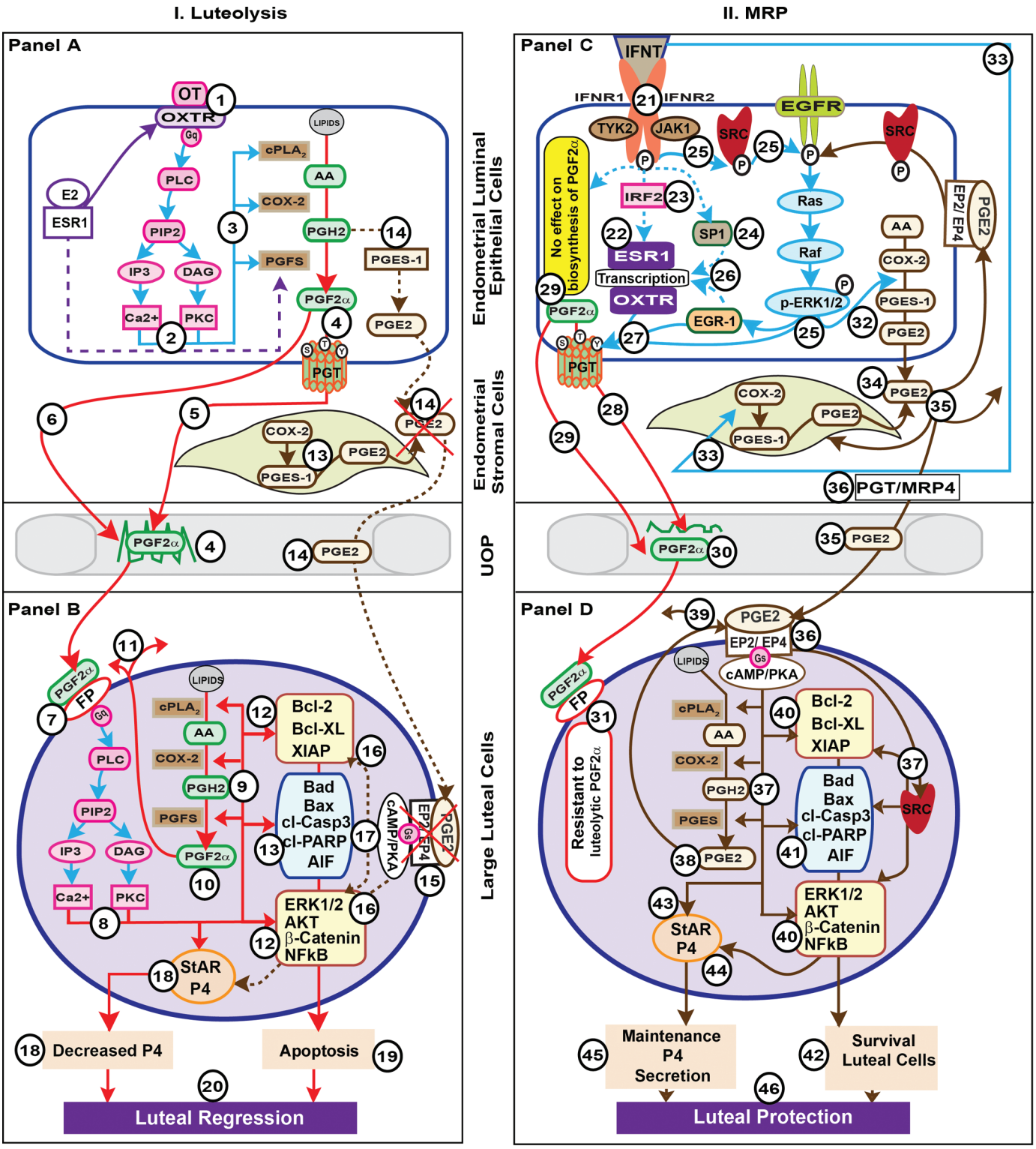

Figure 1. Caption on facing page.

noglobulin heavy constant gamma 1 (*IGHG1*), and immunoglobulin light chain, lambda gene cluster (*IGLL1*) were all upregulated (approximately 2- to 8-fold increases) by lactation but were not altered by pregnancy (Cerri et al., 2012). These differences in immune responses to the physiological states of lactation and

Figure 1. Working model on novel concepts on action of prostaglandins (PG) on luteal maintenance and maternal recognition of pregnancy (MRP) and establishment of pregnancy (ESP) in ruminants: (I) Luteolysis (panel A): (1) Oxytocin (OT) acts on its receptor OXTR, (2) activates Ca^{2+} and protein kinase C (PKC) pathways, (3) which in turn activates the endometrial $PGF_{2\alpha}$ biosynthetic machinery, and (4) induces endometrial $PGF_{2\alpha}$ production. Luteolytic $PGF_{2\alpha}$ pulses are transported through (5) prostaglandin transport (PGT)-dependent mechanisms and basal release of $PGF_{2\alpha}$ is mediated through (6) PGT-independent mechanism or simple diffusion from the endometrial epithelial cells to the corpus luteum (CL) through the utero-ovarian plexus (UOP). (Panel B) Endometrial luteolytic $PGF_{2\alpha}$ pulses (7) act on the $PGF_{2\alpha}$ receptor (FP) in large luteal cells, (8) activate Ca^{2+} and PKC pathways, (9) which in turn activates the luteal $PGF_{2\alpha}$ biosynthetic machinery, and (10) induces intraluteal $PGF_{2\alpha}$ production, and (11) further auto-amplifies luteal $PGF_{2\alpha}$ production by autocrine and paracrine mechanisms. In addition, $PGF_{2\alpha}$–FP signaling (12) suppresses survival pathways and (13) activates apoptotic pathways. (14) Importantly, endometrial PGE_2 production is suppressed at the time of luteolysis, which leads to (15) repressed PGE_2–EP2/EP4 (PGE_2 receptor) signaling in large luteal cells, thus expediting (16) suppression of survival and (17) activation of apoptotic pathways in large luteal cells. Together, activation of FP and suppression of EP2/EP4 signaling (18) decrease progesterone (P4) biosynthesis and secretion, (19) induce apoptosis of large luteal cells, which eventually culminates in (20) functional and structural luteolysis. II. MRP/ESP: (panel C) (21) Interferon-tau (IFNT) acts on its receptors IFNR1 and IFNR2 and (22) suppresses estrogen receptor α (ESR-1) and oxytocin receptor (OXTR) through (23) IFN regulatory factor-2 (IRF-2) or (24) specificity protein 1 (SP1) (sequential signaling cascades from IFNR to IRF2 or SP1 is yet to be confirmed). In parallel, IFNT activates (25) the IFNR-JAK-SRCEGFR-RAS-RAF-ERK1/2-EGR1 module. (26) Early growth response protein 1 (EGR1) and SP1 are competing for the same GC-rich elements, and EGR1 may compete or replace binding with SP1 and suppresses OXTR (this mechanism yet to be confirmed). (27) ERK1/2 and EGR1inhibits PGT function and (28) suppresses PGT-mediated transport of pulsatile release of $PGF_{2\alpha}$ from the endometrium to the CL through the UOP. Interestingly, (29) IFNT does not inhibit basal endometrial production of $PGF_{2\alpha}$, which is transported by PGT-independent mechanism or simple diffusion from the endometrium to the UOP. It supports the increased basal concentration of $PGF_{2\alpha}$ (30) found in the UOP at the time of ESP. (31) $PGF_{2\alpha}$ acts on the FP in the large luteal cells but the CL of early pregnancy is resistant to both basal and luteolytic $PGF_{2\alpha}$ action. Concurrently, IFNT acts on (32) the endometrial epithelial cells and (33) stromal cells through multiple mechanisms, (34) increases net endometrial PGE_2 production, (35) which further auto-amplifies endometrial PGE_2 production through autocrine/paracrine EP2/EP4 signaling. (36) PGE_2 is transported from the endometrium to the CL through the UOP via PGT- or MRP4-mediated mechanisms. (Panel D) (37) Endometrial PGE_2 acts on the EP2/EP4 in the large luteal cells, activates cAMP/PKA and SRC pathways, and in turn auto-amplifies (38) intraluteal PGE_2 production via (39) autocrine and paracrine EP2/EP4 signaling. In parallel, EP2/EP4 signaling activates (40) antiapoptotic or survival pathways, (41) suppresses proapoptotic pathways, thus protecting the CL from structural luteolysis. PGE_2 through (42) cAMP/protein kinase A (PKA) and (43) SRC-ERK1/2 or SRC-AKT pathways may drive the (44) constitutive production of progesterone by the large luteal cells and thus protects the CL from functional luteolysis. Collectively, (45) IFNT or pregnancy-induced endometrial PGE_2 and luteal PGE_2 promote resistance of the CL against $PGF_{2\alpha}$ through multiple intracellular mechanisms and protect the CL from regression during MRP or ESP. Note: Considering the focus of this review, we are not able to include other important signaling pathways such as nitric oxide, endothelin, cytokines, and antioxidants in the large luteal cells. Given the complexity of the signaling network, we limited our model to large luteal cells for clarity and readability. Color version available online. Reproduced from Arosh et al. (2016) with permission.

pregnancy provide some new insight into how lactation could affect the normal mechanism of early embryonic development. Except for *TRD*, these genes are related to immunoglobulins and indicate a possible increase in B-lymphocyte and γδT-cell activity or an increase in the endometrial B-lymphocyte population in lactating dairy cows. None of these immunoglobulin genes were affected by pregnancy and no interaction with lactation was observed. Lactation could promote an imbalance in the immune system in the peri-implantation period, with potential negative effects on conceptus survivability. Several chemokines and cytokines are responsible for the stimulation of B-lymphocyte plasma cells to produce antibodies; however, conclusions on how lactation affects immunoglobulin production require further investigation. The gene *TRD* encodes for a cell receptor delta locus associated specifically with the γδT-cell, which is an important cell regulating the embryo–maternal relationship. The immune system is perhaps the most affected system in the endometrium when the conceptus is present. A vast number of genes with distinct immune functions (i.e., pro- and anti-inflammatory) may serve to create a uterine environment that is sufficiently protected against viral and bacterial infections but still tolerant enough to accept the implantation of the embryo. Lactation is likely to affect this balanced immune environment in the endometrium with potential negative effects on embryo survivability.

Pregnancy increased expression of Dickkopf homolog 1 (*DKK1*) and decreased expression of the Wnt signaling pathway, indicative of an important feature of uterine regulation to accommodate the conceptus (Cerri et al., 2012). Furthermore, DKK1 of endometrial origin may affect embryo development through its antagonism of the Wnt signaling pathway, which ultimately serves a key role in heart, head, and forelimb development during morphogenesis of the embryo. A downregulation of *DKK1* expression by lactation counters the positive effect of pregnancy on *DKK1* expression indicative of a powerful candidate gene mediating the effects of lactation on endometrium transcriptome. An important pregnancy-lactation process, involving differential effects on WNt signaling pathway and *DKK1* expression in the endometrium, may regulate embryonic loss in lactating dairy cows. Such insight provides a platform of candidate genes for subsequent manipulation to improve fertility. In addition, the negative effect of lactation on *RELN* (i.e., involved in embryonic brain development) expression in pregnant lactating cows but not nonlactating cows reinforces the potential effect of lactational status on embryonic differentiation and development.

Cell adhesion (represented by cadherins, claudins, collagens, and L-galectins) is a function mostly downregulated by pregnancy at d 17. This may reflect strong tissue remodeling, as well as a window of time that facilitates the flow of substrates to the uterine lumen to support the elongating embryo. Because of these activities within endometrial tissue on d 17 of gestation, genes related to carbohydrate, lipid, and AA metabolism and transport are upregulated by pregnancy. However, upregulation of pyruvate dehydrogenase kinase, isozyme 4 (*PDK4*) by lactation indicates that lactation exerts a negative effect on glucose homeostasis (see Cerri et al., 2012 for descriptive metabolism gene responses). Carbolic acid, cellular lipid, cellular amino acid and derivative, fatty acid, and glucose metabolic processes were upregulated by pregnancy (Cerri et al., 2012). These coordinated gene expression responses indicate that the uterus is in a high metabolic state to support anabolic and catabolic activities during the period of conceptus implantation. Several transcripts are responsive to pregnancy at d 17 in relation to lipid metabolism and transport: fatty acid binding protein 3 (*FABP3*), acetyl-CoA acyltransferase 1 (*ACAA1*), acetyl-CoA acetyltransferase 2 (*ACAT2*), carnitine/choline acetyltransferase family (*CPT1B*), carnitine palmitoyltransferase 1C (*CPT1C*), carnitine acetyltransferase (*CRAT*), and solute carrier family 27 (fatty acid transporter), member 2 (*SLC27A2*), which may reflect endometrium support in development of extraembryonic membranes during elongation of the embryo.

The extensive and novel investigation of Ribeiro et al. (2016b) examined changes in the bovine transcriptome of preimplantation conceptuses at the onset of elongation on d 15 and associated changes in uterine histotroph composition and endometrial physiology. Transcriptome analyses revealed drastic changes in the transition from ovoid to tubular and from tubular to filamentous conceptuses. Differentially expressed genes were associated with cellular movement, cell-to-cell signaling, cellular assembly and organization, lipid metabolism, small molecule biochemistry, and molecular transport. Specific changes included reorganization of cytoskeleton and cell migration, arginine metabolism, growth factor signaling, and lipid metabolism. Functional analysis revealed fatty acids and peroxisome proliferator activated receptor gamma (**PPARG**), as upstream regulators of transcriptome changes. Expression of *PPARG* increased 17-fold during the onset of elongation and was highly correlated with expression of genes involved in lipid metabolism. Figure 2 depicts potential upstream regulators (i.e., fatty acid, PPARG, and PTGS2) and their target genes that were differentially expressed between ovoid and filamentous conceptuses. Expression of *PPARG* was positively and significantly correlated with *PTSG2* (r = 0.70), *IFNT* (r = 0.73), and pregnancy-associated glycoprotein 2 (*PAG2*; r = 0.88). The histotroph is rich in amino acids, lipids, saccharides, and other intermediate metabolites, and important changes in composition occur in the presence of a conceptus. Pregnancy has a major impact on the concentrations of important lipids in the uterine fluid and expression of genes in the endometrium. Collectively, conceptus elongation involves remarkable changes in transcriptome, composition of the histotroph, and endometrial physiology, which help elucidate important events in uterine and conceptus biology at the onset of elongation.

PERIPARTUM AND POSTPARTUM PROGRAMMING OF REPRODUCTION

The phenotypic decline in daughter pregnancy rate (**DPR**) from the mid-1970s to a nadir in the late 1990s has been followed by an increase of DPR to a level in 2017 comparable to that of the late 1970s (https://www.uscdcb.com/eval/summary/trend.cfm?R_Menu=HO.d#StartBody). This dynamic trend of reproductive performance occurred concomitantly with a steady increase in milk production per cow. The restoration of improved reproductive performance reflects advances made in reproductive management (i.e., application of timed AI protocols, assisted technology for detection of estrus), effective use of computer technology to monitor reproductive performance on a cow and herd basis, and genetic selection (e.g., DPR). In a national survey to demonstrate excellence in reproductive performance, Ferguson and Skidmore (2013) indicated that excellent herd reproductive performance was associated with reproductive management that resulted in high insemination rates combined with average conception rate. The featured column of the Dairy Cattle Reproduction Council's 2016 award winners for reproduction excellence had herd 21-d pregnancy rates between 26 and 39%, with high levels of milk production (http://www.dcrcouncil.org/newsletters/newsletter-2016-december/). It has become clear that the next leaps in reproductive performance will come with improving the reproductive competence of the lactating dairy cow presented for insemination at the designated voluntary waiting period.

Postpartum Diseases Associated with Reproduction

Morrow (1968) described a coordinated herd health program to maximize profit, which included a program for reproductive health. A major issue facing dairy cows under intensive management systems is the high incidence of health problems, particularly those that

affect the reproductive tract and those of metabolic origin that affect subsequent reproductive performance. With the current use of sound reproductive programs for insemination, estimates of peripartum or postpartum incidence of diseases on subsequent fertility are documented clearly. Under large herd intensive management environments, data were compiled from 5,719 postpartum dairy cows evaluated daily for health disorders from 7 dairy farms (Santos et al., 2011). Cows were subjected to presynchronized timed AI programs. Only 55.8% were considered healthy and did not develop any disease in the first 60 d postpartum. The incidence of clinical diseases (calving-related problems, 14.6%; metritis, 16.1%; clinical endometritis, 20.8%;

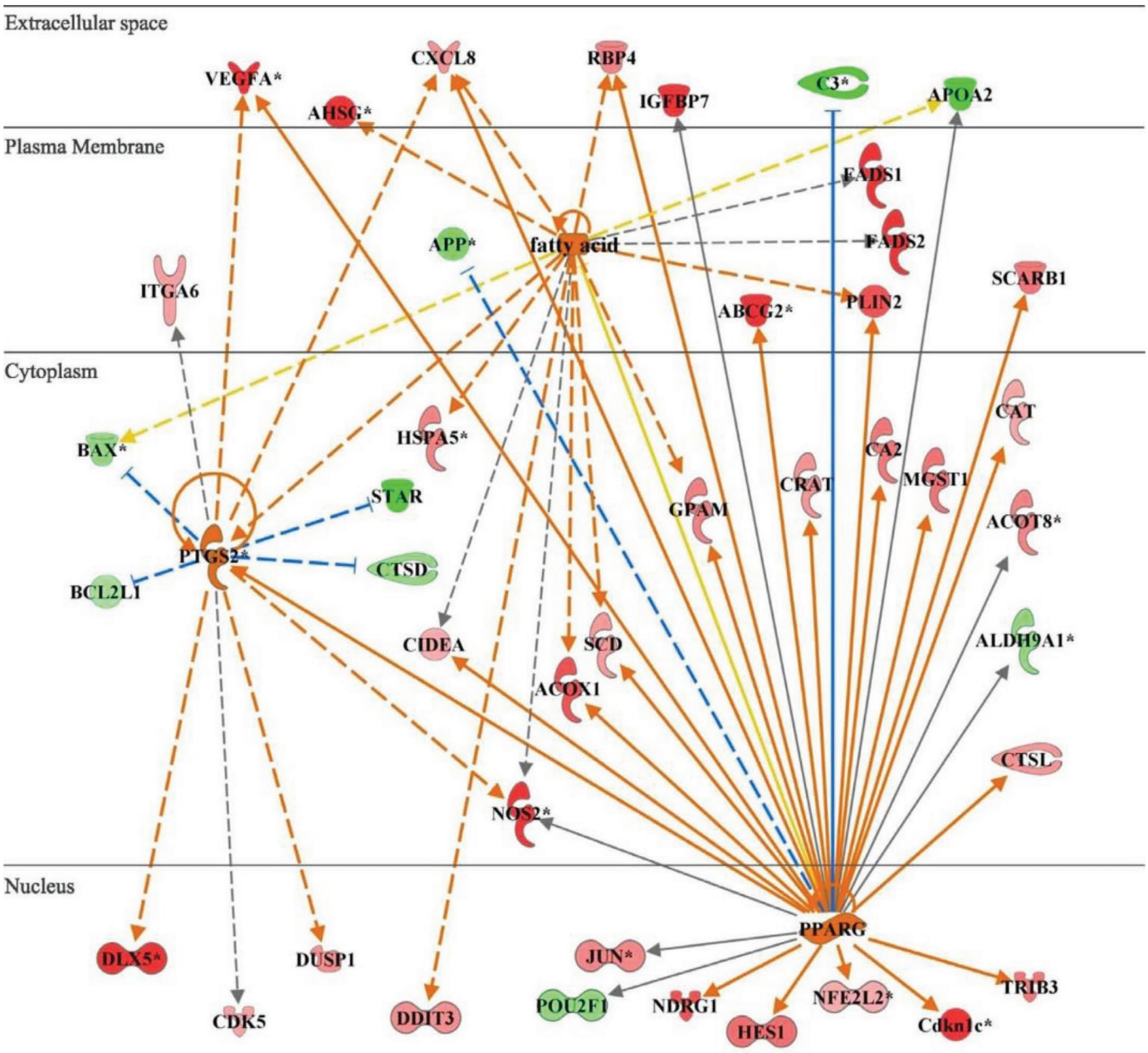

Figure 2. Potential upstream regulators fatty acid, PPARG (peroxisome proliferator-activated receptor gamma), and PTGS2 (prostaglandin-endoperoxide synthase 2), and their target genes that were differentially expressed between OV (ovoid) and FIL (filamentous) conceptuses. Red denotes genes upregulated and green denotes genes downregulated in FIL. Orange and blue represent predicted increased and predicted decreased activation, respectively. Solid and dashed lines imply direct and indirect relationships, respectively. The pointed and blunted arrowheads represent activating and inhibitory relationships, respectively. Color version available online. Reproduced from Ribeiro et al. (2016b) with permission.

fever, 21.0%; mastitis, 12.2%; ketosis, 10.4%; lameness, 6.8%; digestive problems, 2.8%; pneumonia, 2.0%) was high. Moreover, 27.0% of the cows were diagnosed with a single disease event, whereas 17.2% had at least 2 disease events in the first 2 mo of lactation. In spite of similar milk yield, cows diagnosed with health problems were less likely to be cyclic (i.e., anovulatory) at 65 d postpartum. Calving-related disorders and those that affect the reproductive tract were the major contributors for the depression in cyclicity (cyclic: healthy 84.1% vs. 70.7% $>$ 1 disease). Diagnosis of health disorders markedly depressed the risk of cows to become pregnant at the first postpartum AI (pregnancy per TAI: healthy 51.4% vs. 34.7% $>$ 1 disease), and increased the risk of pregnancy loss in the first 60 d of gestation (pregnancy loss %: healthy 8.9% vs. 15.8% $>$ 1 disease). Comparable associations between clinical and subclinical postpartum diseases with reductions in reproductive performance were detected in large herd systems of extensive seasonal grazing that underwent an intensive seasonal insemination program (Ribeiro et al., 2013). Cows managed extensively are not immune from the subsequent infertility syndrome associated with peripartum or postpartum clinical and subclinical diseases. These responses indicate that reduction in morbidity by prevention of periparturient or postpartum diseases has the potential to enhance fertility of dairy cows by improving resumption of postpartum ovulation, increasing pregnancy per AI, and minimizing the risk of pregnancy loss. Epidemiological data analyses are a powerful tool to identify reproductive inefficiencies and potential causative associations but do not prove cause and effect. Conceptus development and transcriptome arrays at preimplantation stages differed between distinct genetic groups of lactating dairy cows (Ribeiro et al., 2016c). Furthermore, postpartum inflammatory diseases altered both subsequent developmental biology of the conceptus and fertility (Ribeiro et al., 2016a). Restoration of uterine or ovarian function and optimization of immune function are considered important researchable areas to sustain reproduction in lactating dairy cows.

Maternal–Conceptus Unit Programs Potential Calf Welfare and Cow Performance

The initial vulnerability of the cow immediately after calving to postpartum clinical and subclinical diseases is likely influenced by the functional dialog of the maternal–conceptus unit. The optimal unit leads to successful birth of the calf and programming of the maternal unit to support the needs of the calf and the production goals of the producer (i.e., milk production and re-establishment of a pregnancy).

Genetic and environmental differences among cows in the peripartum period are associated with various endocrine and biochemical systems regulating parturition, postpartum milk secretion, ovarian recrudescence, uterine regression, and health of the maternal unit as well as the newborn. The conceptus (i.e., fetus and placenta) and maternal dialog is central to regulation of these various processes (Thatcher et al., 1980). A series of experiments demonstrated quantitative hormonal and physiological differences (1) among cows in the peripartum/postpartum period attributable to Jersey cows selected for milk yield versus a nonselected control line (Eley et al., 1981a,b); (2) in first-calf Holstein cows bearing Holstein, Holstein × Angus, or Holstein × Brahman conceptuses (Guilbault et al., 1985a,b); and (3) in cows under a shade heat-abatement system versus a control no-shade environment (Collier et al., 1982; Lewis et al., 1984) or current systems of forced ventilation and intermittent sprinkling (Tao and Dahl, 2013). Distinct differences in free and sulfated estrogens, P4, prolactin, 13,14 dihydro-15 keto $PGF_{2\alpha}$, thyroxine, and triiodothyronine, as well as physiological differences in conceptus birth weights, postpartum milk yield, and uterine regression were detected in response to these various conceptus–maternal experimental models in the peripartum and postpartum period.

Whether the mean transfer of 248 g of IgG or 7 kg of colostrum to the newborn (Morin et al., 2010) contributes to the period of postpartum immunosuppression of the mother and is related to peripartum reproductive disorders (e.g., puberal metritis, endometritis, subclinical endometritis) warrants investigation (Herr et al., 2011). Colostrum contains a plethora of growth factors defined as lactocrines (e.g., epidermal growth factor, IGF-1, IGF-2, and other unidentified factors) that target the neonate to affect differentiation of anterior pituitary mammotropes, gastrointestinal tract development, and maturation of the immune system (Chen et al., 2011). Lactocrine-acting factors, whether natural or synthetic, may affect developmental events associated with programming of female reproductive tract tissues and subsequent reproductive competence, and warrant extensive investigation in dairy cattle.

Postpartum Coordination of Reproductive Processes and Lactation

Bauman and Currie (1980) established the concept of homeorhesis (i.e., coordinated control of metabolism in body tissues necessary to support a physiological state) in which metabolism is altered drastically to support lactation. Furthermore, this metabolic change for lactation followed the homeorhetic processes of supporting the needs for growth and development of the calf in

utero. The control systems in lactation are appreciably different from the processes during pregnancy with the presence of a placenta programming both maternal and fetal function. The processes are linked with development of the mammary gland in unison with development of the calf to ensure postnatal nourishment of the calf. Transition from a nonlactating to a lactating state and sustaining a high level of milk production is a drastic challenge and occurs at the expense of reducing immune function and increasing incidence of clinical and subclinical production disorders, metabolic diseases, and temporal infertility. The coordinated biochemical and metabolic responses reflect changes in energy balance of the lactating dairy cow. Butler and Smith (1989) described the biological responses controlling energy balance during lactation and their impact on the neuroendocrine and endocrine systems that partially account for variability in recrudescence of reproductive cyclicity and fertility.

After the gradual withdrawal of progesterone due to metabolism and then its acute decline with regression of the CL, coupled with high placental secretion of estrogens and their abrupt decline with parturition, the dairy cow escapes from negative steroid feedback. Consequently, an early increase in plasma FSH concentrations occurs during d 1 to 5 postpartum (Beam and Butler, 1997). Most cows have a first follicle wave occurring within the first 14 d postpartum but only about 40% ovulate. Whether the first follicular wave is estrogenic and ovulates (ovulatory vs. nonovulatory) depends on greater LH pulse frequency, greater follicular fluid concentrations of estradiol and androstenedione, greater plasma estradiol and lower glucose concentrations, higher ME balance (Mcal/d; –12.0 vs −18.0), and decreased insulin resistance postpartum compared with nonovulatory cows (Cheong et al., 2016). Postpartum plasma concentrations of LH are low early postpartum with minimal release of LH in response to GnRH on d 3 and an attenuated response on d 10, and are not fully restored until d 20 postpartum (Fernandes et al.,1978). Collectively, these findings indicate that cows ovulating the first postpartum dominant follicle have a greater glucose utilization, supporting higher LH pulsatility and enhanced sensitivity to gonadotrophins accounting for greater steroidogenic activity. Cows that fail to ovulate may have recurring nonovulatory follicles or develop large persistent follicles that are not ovulatory. Hampton et al. (2003) demonstrated that pulsatile infusion of LH stimulated follicular growth and steroidogenesis and decreased time to first ovulation in anestrous postpartum cows.

Lucy et al. (2014) describes the vital mechanisms in place that center on the control of glucose availability and its role in increasing insulin and re-coupling the somatotropic axis through effects on GH receptor 1A (GHR1A) to restore availability of IGF-1. The central role of glucose metabolism and the possible importance of a glucose set point at 3 d postpartum for subsequent recrudescence of both reproductive function and fertility is intriguing (Garverick et al., 2013).

A novel approach to examine ovum quality at different days postpartum within cows programmed to have distinct differences in energy balance was conducted with the use of transvaginal follicular aspiration twice weekly from 30 to 100 d postpartum (Kendrick et al., 1999). Oocytes were classified into 4 categories depending on ooplasm homogeneity and cumulus investment: 1 (good +), 2 (good), 3 (good −), and 4 (poor), and number of oocytes in each class for each aspiration was determined. Cows fed a high-energy (HE) diet had a slight increase in good quality (+) embryos per aspiration session compared with cows in the low-energy (LE) diet (1.53 > 1.37), but this difference was of questionable biological advantage. Cows on the HE diet had an increase of 1 good quality oocyte from 30 to 100 d postpartum (1.1 to 2.1), whereas those on the LE diet had an increase of 0.2 good quality (−) oocyte from 30 to 100 d postpartum (1.3 to 1.5). In contrast, cows on the HE diet had a much greater occurrence of poor quality embryos during the first 2 wk of aspiration and between 10 to 13 wk of lactation. The higher incidence of poor quality oocytes could be attributable to the HE diet increasing insulin secretion (Thatcher et al., 2011).

Beyond the issue of oocyte quality in lactating cows, the ability of the reproductive tract to support normal embryo development may be compromised. One of the earliest techniques of assisted reproductive technology was the successful surgical transplantation of single embryos (i.e., containing 8, 10, and 12 cells) to 3 un-bred recipients that resulted in 3 calves, normal at birth and in later development (Willett et al., 1953). Rizos et al. (2010) used an endoscopic embryo transfer technique to compare the ability of the reproductive tract of postpartum dairy cows to support development of early embryos to the blastocyst stage, compared with nulliparous heifers. Bovine embryos of 2 to 4 cells were produced by in vitro maturation and fertilization of oocytes derived from the ovaries of slaughtered cattle. On d 2 of a synchronized estrous cycle, 100 embryos were transferred endoscopically to the oviduct ipsilateral to the CL of Holstein heifers (n = 10) and postpartum Holstein cows (n = 8, approximately 60 d postpartum). On d 7, the oviduct and uterus were flushed nonsurgically to recover the embryos. The percent of recovered embryos was greater for heifers than cows (79.0 ± 7.0 vs. 57.2 ± 11.4%). Of the embryos recovered, 33.9 ± 3.6% had developed to the blastocyst stage in the heifer compared with 18.3 ± 7.9% in the postpartum cow.

There was no difference in total cell number of blastocysts (71.2 ± 5.7 vs. 67.0 ± 5.3, respectively). Rizos et al. (2010) concluded that the reproductive tract of the postpartum lactating dairy cow might be less capable of supporting early embryo development than that of the nonlactating heifer.

SCIENTIFIC FOUNDATION FOR THE NEXT CENTURY

Our deep and broad knowledge in many aspects of animal biology requires increasingly complex scientific hypotheses and experiments. With the evolution of extensive and intensive scientific tools that are now available for use across tissues, cells, and intracellular organelles, as well as the integration of groups and their management, scientific investigation has become much more interdisciplinary. This complexity requires computer programs for storage and analysis of data, and the use of biomathematical models to describe the processes. A full history of systems research and modeling in ruminant animals is available (Baldwin, 1995), and specific examples in nutrition and reproductive processes can be found here (McNamara and Shields, 2013; McNamara, 2015). The process of investigation comprises the traditional scientific method, whereby the experimenter gathers data to reveal whether a hypothesis is verified or not (i.e., hypothesis is rejected or not); with that data, a mathematical description is then built to describe the processes involved.

Using a systematic research approach, systems biology, and modeling constructs models based on verified experimental results, and in the case where the model adequately explains what we know, we can have increased confidence in our knowledge. When the model "fails" and cannot explain what we know, we have a clear focus for further experimentation. Advancements in computer technology allow for the integration of large databases within and across the areas described above. The systems biologist and biomathematician use computational and computer development to integrate large biological data sets from responses associated with genomics, transcriptomics, proteomics, metabolomics, and lipidomics to develop predictive biological response models. The multidisciplinary field of systems biology requires an understanding of both biological and mathematical concepts to predict outcomes. Results from such analyses and system simulation models are used to test and extend biological understandings and to suggest new hypotheses or experiments. Modeling with large libraries of data permits a scientist to form a hypothesis, utilize the available data and associations to test the hypothesis, and verify the new concept. Bioinformatics research involves pathway analyses that usually develop from high throughput biology, and entail use of pathway collections and interaction networks related to structure and functionality of cells and tissues. Such analytics are powerful tools to determine the significance of "-omics" data and identify new targets or potential biomarkers within biological systems of interest. These scientific approaches further our understanding of the physiology of dairy cattle and can predict performance or, conversely, how the evolving dairy cow acquires new horizons for performance. It is exciting to see the marriage of scientific foundations and disciplines being applied in the various areas showcased in this special issue of the *Journal of Dairy Science* to celebrate its 100th year of publishing.

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APPENDIX

Table A1. Major advancements in female reproductive physiology in the past 100 years

Year	Milestone	Reference
1905	Integration of uterine and mammary gland development from embryo to senescence.	Halban, 1905
1927	Days open is not associated with rate of milk yield during first month.	Gaines, 1927
1949	Nutrition/reproduction needs include sensitive tools, adequate controls, and integration of physiologist, biochemist, and nutritionist.	Asdell, 1949a,b
1951	Plane of nutrition of heifers (birth to calving) influenced age of puberty, and calving difficulty. High level of feeding is uneconomical.	Reid et al., 1957 (1951), 1964
1953	Calves born after surgical transplantations of fertilized bovine eggs.	Willett et al., 1953
1963	Gas chromatographic estimates of cyclic progestins in blood, CL and ovaries.	Gomes et al., 1963
1965	LH is luteotrophin for the bovine CL.	Donaldson and Hansel, 1965
1968	Integration of Postpartum Herd Reproductive Management.	Morrow, 1968
1969	Luteolytic action of estradiol acts through uterus.	Brunner et al., 1969
1970	Integration of hypothalamic, pituitary control of FSH and LH with dynamics of progesterone secretion during estrous cycle.	Hansel and Snook, 1970
1972	Valid use of radioimmunoassay.	Trenkle, 1972
1976	Physiological effects and transitional progesterone, estradiol and LH responses to $PGF_{2\alpha}$.	Thatcher and Chenault, 1976
1978	Restoration of LH responsiveness to GnRH during the postpartum period.	Fernandes et al., 1978
1981	Genetic (1981, 1985) and Environmental (1982, 1984, 2013) effects peripartum on endocrine and biochemical systems, milk secretion, ovarian-uterine restoration, and health of cow and calf.	Eley et al., 1981a,b; Guilbault et al., 1985a,b; Collier et al., 1982; Lewis et al., 1984; Tao and Dahl, 2013
1982	Assessment of antral follicle health and turnover based on intra-follicular concentrations of estradiol and progesterone.	Ireland and Roche, 1982

Continued

Table A1 (Continued). Major advancements in female reproductive physiology in the past 100 years

Year	Milestone	Reference
1984	Identification of bovine Interferon Tau (IFNT) in early pregnancy (1984) and anti-luteolytic effect of recombinant IFNT (1995).	Thatcher et al., 1984a,b; Meyer et al., 1995
1984	Ultrasonography of ovary to monitor follicle and CL development.	Pierson and Ginther, 1984
1985	LH receptor and signal transduction of CL.	Garverick et al., 1985
1989	Interrelationships of energy balance (EB) on reproductive function.	Butler and Smith, 1989
1992	Evolution of first wave follicle (1992); follicular cell dynamics of LH and FSH receptor gene expression (1995).	Badinga et al., 1992; Xu et al., 1995
1992	Oxytocin localized in secretory granules of large steroidogenic luteal cells of cycle but not pregnancy.	Fields et al., 1992
1997	A first wave follicle developed following the early postpartum rise in FSH. Follicular competence was associated with higher plasma IGF-1 and shorter intervals to EB nadir.	Beam and Butler, 1997
1999	Oocyte quality following transvaginal follicular aspiration was related to EB.	Kendrick et al., 1999
2005	Multiple ovulations are associated with high milk production.	Lopez et al., 2005
2005	With activity monitors, approximately 28.5 % of cows failed to be detected in estrus likely due to anovulatory cows and insufficient plasma estradiol.	Lopez et al., 2005; Kamphuis et al., 2012
2010	Postpartum reproductive tract reduced embryo development compared to dairy heifers following transfer of 2-4 cell embryos (n=100).	Rizos et al., 2010
2012	Endometrial transcriptone analyses detected effects of pregnancy on immune, glucose and fatty acid regulatory pathways, which were antagonized by lactation.	Cerri et al., 2012
2013	Gene co-expression network analysis identified genes and biological processes shared among anterior pituitary and brain areas that affect estrous behavior.	Kommadath et al., 2013
2013	Prevalence of peri-parturient diseases alters fertility in grazing and intensely managed cows.	Ribeiro et al., 2013
2013	Targeted progesterone supplementation to anovulatory cows during follicular recruitment improves fertility.	Bisinotto et al., 2013
2013	Periparturient blood concentrations of NEFA and glucose are indicative of pregnancy at first service (2013), and glucose availability increases insulin and recoupling of GH axis (2014).	Garverick et al., 2013; Lucy et al., 2014
2013	Integrating nutritional aspects of reproductive control during lactation utilizing a systems research approach.	McNamara and Shields, 2013

Continued

Table A1 (Continued). Major advancements in female reproductive physiology in the past 100 years

Year	Milestone	Reference
2016	Distinct metabolic and endocrine differences between cows that ovulate or do not ovulate first postpartum dominant follicles.	Cheong et al., 2016
2016	Integration of molecular pathways associated with regression and maintenance of the CL (2016), and roles of progesterone and conceptus-derived factors in early pregnancy (2016).	Arosh et al., 2016; Spencer et al., 2016
2016	Recommendations for improving design, analysis, and interpretation of research on reproductive performance.	Lean et al., 2016

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A 100-Year Review: A century of change in temperate grazing dairy systems[1]

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ABSTRACT

From 1917 to 2017, dairy grazing systems have evolved from uncontrolled grazing of unimproved pastures by dual-purpose dairy-beef breeds to an intensive system with a high output per unit of land from a fit-for-purpose cow. The end of World War I signaled significant government investments in agricultural research institutes around the world, which coincided with technological breakthroughs in milk harvesting and a recognition that important traits in both plants and animals could be improved upon relatively rapidly through genetic selection. Uptake of milk recording and herd testing increased rapidly through the 1920s, as did the recognition that pastures that were rested in between grazing events yielded more in a year than those continuously grazed. This, and the invention and refinement of the electric fence, led to the development of "controlled" rotational grazing. This, in itself, facilitated greater stocking rates and a 5 to 10% increase in milk output per hectare but, perhaps more importantly, it allowed a more efficient use of nitrogen fertilizer, further increasing milk output/land area by 20%. Farmer inventions led to the development of the herringbone and rotary milking parlors, which, along with the "unshortable" electric fence and technological breakthroughs in sperm dilution rates, allowed further dairy farm expansion. Simple but effective technological breakthroughs in reproduction ensured that cows were identified in estrus early (a key factor in maintaining the seasonality of milk production) and enabled researchers to quantify the anestrus problem in grazing herds. Genetic improvement of pasture species has lagged its bovine counterpart, but recent developments in multi-trait indices as well as investment in genetic technologies should significantly increase potential milk production per hectare. Decades of research on the use of feeds other than pasture (i.e., supplementary feeds) have provided consistent milk production responses when the reduction in pasture intake associated with the provision of supplementary feed (i.e., substitution rate) is accounted for. A unique feature of grazing systems research over the last 70 yr has been the use of multi-year farm systems experimentation. These studies have allowed the evaluation of strategic changes to a component of the system on all the interacting features of the system. This technique has allowed excellent component research to be "systemized" and is an essential part of the development of the intensive grazing production system that exists today. Future challenges include the provision of skilled labor or specifically designed automation to optimize farm management and both environmental sustainability and animal welfare concerns, particularly relating to the concentration of nitrogen in each urine patch and the associated risk of nitrate leaching, as well as concerns regarding exposure of animals to harsh climatic conditions. These combined challenges could affect farmers' "social license" to farm in the future.

Key words: rotational grazing, set-stocking, supplementary feeds, future issues

PILLARS OF THE MODERN GRAZING SYSTEM

Increased interest in grazing because of ease of establishment and a lower requirement for capital infrastructure, low operating expenses per kilogram of milk, and potential access to high-value markets because of perceived animal welfare benefits has led to a range of grazing systems being developed globally. However, the archetypal modern grazing system is that synonymous with New Zealand, Australia, and western Europe (e.g., France, Ireland, and the UK), all of which have a rich

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history of agricultural science that has contributed to the development and refinement of a very efficient system over the last century.

Dairy grazing systems are designed to grow large yields of digestible forage, generally grass-legume mixtures, and harvest a high proportion of the "pasture" grown directly by the cow. The requirement for machinery and housing and associated expenses are minimized (Roche et al., 2017b). This is primarily achieved by (1) ensuring optimal soil fertility and appropriate use of nitrogenous fertilizers; (2) matching the feed demand of the herd with the annual pasture supply profile (i.e., seasonal milk production) through strategic decisions around calving date and stocking rate; and (3) designing the farm infrastructure of paddocks and cow tracks or lanes to facilitate easy access to pasture (Roche et al., 2017b). Even with all these, success is still dependent on adequately drained soil types resistant to treading damage. In many countries, significant government investment in large-scale drainage schemes and irrigation channels has increased the possibilities for grazing systems in otherwise unsuitable areas.

Ideally, more than 90% of the herd (including replacement heifers) calve within a 50- to 60-d period, with the end of calving coinciding with the time that pasture growth equals herd demand (Dillon et al., 1995; Roche et al., 2017b). Virtually all modern intensive grazing systems use rotational grazing practices, wherein the cows "rotate" around a sequence of paddocks (i.e., an enclosed grazing area) linked by farm tracks, allowing each paddock to recover from the stress of grazing and take advantage of the exponential phase of growth, at least for temperate grass species (Figure 1). This system also has the labor advantages of collecting cows for milking from a small confined area (e.g., 1 ha) compared with a system where cows have free access to the entire farm or large areas of it (the alternative grazing method of "set-stocking"). Feeds imported from off-farm (i.e., supplementary feeds) are incorporated into grazing systems to provide nutrients during periods when pasture growth rates are less than feed demand. Pasture surplus to requirements in spring and summer can be conserved as silage or hay and offered to cows during periods of low pasture growth, particularly to nonlactating cows during winter. For this system to function optimally, the cow must be highly fertile and have good grazing behavior characteristics.

This system evolved over the last 100 yr through independent and collaborative research undertaken by many researchers in many countries (Appendix Table A1). Key principles contribute to the biological and economic success of this grazing systems and these were described by C. P. McMeekan in his landmark treatise, *Grass to Milk* (McMeekan, 1960):

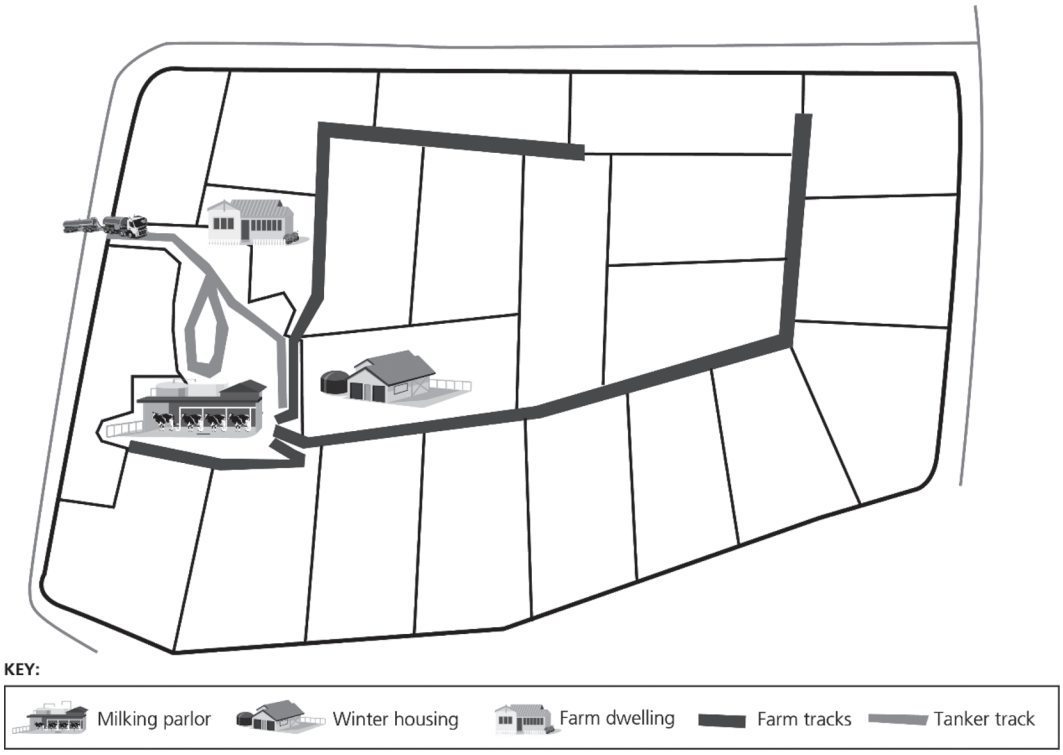

Figure 1. A typical layout of a modern grazing dairy farm. Reproduced with permission from Roche et al. (2017b).

- grow as much pasture as economically practicable;
- carry enough stock to use all the grass grown;
- adjust the variable supply of fodder to the needs of the herd;
- use animals that will process the grass efficiently; and
- minimize herd wastage by control of disease.

The research that led to the development and the continual refinement of these principles over the last 100 years is the subject of this review.

WHERE IT ALL BEGAN: GRAZING DAIRY SYSTEMS IN 1917

The majority of dairy systems in 1917 involved some level of grazing, but there had been little gain in the efficiency of this component of the system in the previous 200 yr (Blaxter and Robertson, 1995). Most farms tended to have a few fields, but, unlike the modern rotational grazing system, these were typically "night and day" fields, to reduce the amount of walking done by the cows and by the farmer in the morning rather than for any biological advantage. In fact, such a practice often led to the transfer of nutrients from the "day" field to the "night" field. In many countries, the majority of cows were still milked by hand, limiting the number of cows on any farm to the number of people available for milking. Calving was year-round to ensure a continuous milk supply to the creamery or milk factory. Grazing practices were rudimentary and a key aim was to conserve hay during late spring and summer to use as feed in winter when pasture growth was slow or zero.

Over the last 100 yr, grazing research programs have addressed key aspects of pasture-based systems of milk production, including grazing management, stocking rate, dairy cow genetics, dairy cow reproduction, milking and milk quality, dairy cow health and welfare, as well as bio-economic modeling. A unique feature of the research undertaken in grazing systems has been the use of multi-lactation "farm systems" experiments, wherein small farms (i.e., farmlets of 20–50 cows) are established for 3 or more years and changes to a particular component of the system evaluated (e.g., stocking rate; Macdonald et al., 2008a; McCarthy et al., 2016). This research methodology has provided valuable insight into the interactions between components of a farm and enabled an understanding of the farm system implications from changing a single component of a grazing system.

A further principle, particularly evident in grazing systems today, which was eloquently relayed by C. P. McMeekan, was that "research was useless unless it was applied" (Scott, 1997). This tradition of connectedness to the farmer has continued in much of the grazing research undertaken, with a strong focus on applied research questions and trial design, the testing of component-level changes in multi-year farm systems experiments, demonstration and farmer-partner farms, and the dissemination of experimental results directly by scientists.

INFRASTRUCTURE DEVELOPMENTS

Although grazing is a relatively simple and technology-free system of farming, key developments in the evolution of the grazing system were borne out over the past 100 yr and were instrumental in its success. These developments facilitated intensive management strategies (Macdonald and Penno, 1998) and, importantly, the expansion of grazing dairy farms to maintain viability at scale. The 3 most important technological breakthroughs did not occur in the laboratory or in some engineering factory, but were the product of necessity and farmer ingenuity.

The most obvious example is in the development of the electric fence, which in effect allowed the corralling of dairy cows on small areas and facilitated the resting of grazed pastures. This technological development paved the way for rotational grazing. The manufacture and sale of electric fence "chargers" began in the United States in the early 1930s and an estimated 70,000 were in operation by 1938 (Jones, 1988). In 1936, Bill Gallagher, a New Zealand farmer, read about the development of these energizers and made one to control the dairy cows on his farm. Two years later, he sold the farm and started manufacturing the energizers commercially; in 2017, Gallagher is, arguably, the most globally recognized name in electric fence manufacture and sales.

However, the fences were prone to "shorting" (losing power) and could not be used over long distances, therein limiting their usefulness to small farms. If farms were to increase in size, a new type of fence had to be developed. In the 1950s, Doug Phillips at Ruakura (Waikato, New Zealand) developed the "unshortable" fence, so named because it could be used without any insulated supports (Jones, 1988). The Waikato energizer, as Phillips' invention came to be known, allowed large areas of pasture to be fenced for rotational grazing but at approximately 10% of the cost of conventional fencing. Large areas could be fenced, allowing large numbers of cows to be managed on one farm; in Phillips' own words, "it was a revolution in grassland farming."

Two other farmer inventions also pioneered the way for increasing scale in grazing systems. At the beginning of the 20th century, most cows in most countries

were still hand milked, and the number of cows in the herd was dependent on the number of people available for milking. Feeding, although laborious in winter when cows were stanchioned, was "self-service" during the grazing season. Therefore, most grazing farms needed less labor per cow than an equivalent housed system for most of the year. Nevertheless, the milking routine was time consuming. Although prototype milking machines had been developed, they were, for the most part, unaffordable and relatively inefficient.

Many improvements to the reliability of the vacuum pump and pulsators during the early part of the 20th century and an increased requirement for milk cleanliness and concerns around the spread of zoonoses improved the efficiency and uptake of the technology. For example, it is estimated that more than half of New Zealand's cows were milked by machine by 1920 (McCloy, 2014). Even with perfect technology, however, the design of the milking sheds or parlors slowed the milking routine. The "walk-in" shed, wherein cows were led into the bail by the milker, tethered, milked, released, and reversed out of the bail, and kept separate from herd mates waiting to be milked, led to a slow and labor-intensive procedure. The walk-in shed was replaced by the "walk-through" milking parlor, where the process was the same except that cows did not have to reverse out of the stall after milking and could exit through a gate at the front of the stall and return to the pasture. This simple change in parlor design reportedly allowed a single milker to machine milk up to 42 cows in an hour.

Despite the large improvement, the walk-through milking parlor did not allow for a significant increase in farm size. The economic turmoil associated with the Great Depression in the 1930s and World War II in the 1940s limited investment and innovation. A serendipitous discovery in the early 1950s, however, was to revolutionize milk harvesting. Ron Sharp, a dairy farmer from near Hamilton (Waikato, New Zealand) noticed the new 60°-angle car parking system that was introduced to the city after the war. He went home and investigated the application of a similar design in his milking parlor. Groups of cows would file through the alley and "park" themselves at approximately 60° angles to each other. The milker would stand in a sunken pit behind the cows and attach the milking cluster. Once milking was finished, the cows would exit from the other end of the shed and another batch would come in to take their place (McCloy, 2014). The herringbone milking parlor was born. Sharp's design was so successful that, within 15 yr, 70% of milking parlors in New Zealand used a similar design, and milking efficiency increased from 42 to 70 cows/milker per hour. This was the first parlor design to take the "spancel" off the farmer and allow them to manage a much larger herd. (A rope "spancel" is used to restrain a cow with a propensity to kick during milking, by tying a back leg to the railing.)

In a similar vein of ingenuity, another New Zealand farmer, Merv Hicks, decided that cows were not comfortable parked up against each other in the herringbone design; he believed that they needed to be separated during milking. He designed a milking parlor in which each cow had its own bail, like the slow and laborious walk-in milking parlor design, but that rotated the cows in a circle during milking, like a carousel in a children's playground. At the completion of one full rotation, cows would reverse off the turning platform. In such a simple but ingenious way, the rotary or carousel dairy was invented.

All of these inventions have been upgraded with improvements in engineering and design and the ability to add complementary technologies, such as automatic cow identification and in-parlor individual cow or flat-rate feeding systems. In essence, however, the unshortable electric fence and the herringbone and rotary designs remain the same today as when envisioned by their inventors. Farmers also focused on selecting cows for temperament and milking speed; although both were also, arguably, a large focus of culling when cows were hand-milked, with scale came the requirement for speed and an intolerance for cows that disrupted the routine and increased the risk to milker health and safety.

Summary

If grazing dairy farms were to be financially and socially sustainable, there was a need for scale and for machines to undertake the most laborious tasks, not to mention the need to control feed management. It is said that necessity is the mother of invention, and the inventions that facilitated expansion in grazing systems came, primarily, from farmer ingenuity and the keen observation skills of applied scientists who improved upon their ideas. Furthermore, however, the speed of uptake of these technologies was evidence of the maturity of the industry and the recognition that dairy farms could be expanded to 100 cows and beyond.

PASTURE AND GRAZING RESEARCH

The first principle of a successful grazing system relayed by McMeekan (1960) was to "grow as much pasture as economically practicable." During the 100-year period considered in this review, significant research efforts have focused on defining optimum soil concentrations of required minerals and identifying the factors influencing pasture growth, quality, and utiliza-

tion. The International Grassland Congress was established in 1927 as an outlet for scientists to discuss and debate the results of field experiments. The congress has met 23 times since then and on every continent except Africa. The congress covers a myriad of disciplines underpinning grazing systems. Plant genetics and soil constraints have been a feature of the congress since 1927, representing 30 to 40% of the papers presented at each event, but plant physiology became more important in the 1960s, 1970s, and 1980s, the nutritive value of forages increased in prominence from the 1960s onward, and grazing systems became a significant part of the congress from the mid-1950s onward (Humphreys, 2005). The considerable research efforts undertaken in this time and discussed at length in these conference proceedings have been distilled into practical strategic and tactical grazing management practices that are taken for granted in modern grazing dairy production systems.

Soils and Fertilizers

Taxonomic characterization of soils was undertaken globally through the first half of the 20th century (Blaxter and Robertson, 1995), and this allowed more targeted research toward optimizing soil fertility. In 1917, it was recognized that soil fertility was the key limiting factor in the successful establishment and production of ryegrass and clover pastures (Cockayne, 1912a,b). Elting and Lamaster (1934) reported that lime and phosphorus were "the only profitable fertilizer treatment for pasture." The application of lime alone increased pasture yields by 34%, whereas application of phosphorus increased DM yield by 41%; applying both increased per hectare DM yields by 79% compared with untreated controls. Nevertheless, customized soil nutrient recommendations were still a half-century away.

Activities in soil science and fertilizer increased in universities, colleges of agriculture, and in research stations and government agencies devoted to the subject from the 1930s. In 1933, the Soil Erosion Service (precursor to the Natural Resources Conservation Service) was established in the United States, as a direct response to the soil erosion phenomenon that became known as the "Dust Bowl" and following the establishment of soil erosion experiment stations in 1929. Their very detailed surveys of soil classification and land-use capability remain valuable today in identifying appropriate land use and fertilizer recommendations. Around the same time, the national Soil Survey Departments were established in the United Kingdom (Blaxter and Robertson, 1995).

During the 1920s and 1930s, fertilizer application rates were derived from "local experimentation" and demonstration. Farmyard manure remained an important source of nutrients for grassland pastures, with responses from hundreds of experiments across Britain and Ireland reported by the early 1940s (Boyd and Lessells, 1954): pasture production increased by 58 kg of DM/ha per 1 t of farmyard manure. By the 1940s, a comprehensive series of fertilizer mixtures were being produced, and extra nitrogen, phosphorus, and potassium (N, P, K) were allocated in addition to farmyard manure (Blaxter and Robertson, 1995). By the early 1950s, 22% of grassland area was receiving farmyard manure, whereas 32, 35, and 21% were receiving N-, P-, and K-containing fertilizers, respectively (Boyd and Lessells, 1954). In truth, however, the application rates were low: 34 kg of N, 80 kg of P_2O_5, and 44 kg of K_2O per ha per year, and the majority was applied to meadows for hay and silage crops and not specifically for grazing.

By the 1960s, soil maps were developed for the different pasture-growing regions of the world. With soils classified for the different grassland regions, optimum soil P, K, and S concentrations and soil pH for different soil types were defined with more precision (Roberts and Thomson, 1988; Daly et al., 2001; O'Connor et al., 2001; Dougherty and Gourley, 2014). Nitrogen fertilizer experimentation for grassland farming began, in earnest, in the 1950s and 1960s, with myriad experiments investigating the interaction between nitrogenous fertilizers and grass and legume species, soil temperature, and moisture availability on pasture DM yield and quality (Whitehead, 1995) and on cow health and milk production (de Groot, 1963). The increased understanding of the soil's needs was accompanied by great advancements in the ability to manufacture fertilizers and a large increase in the use of chemical fertilizers; for example, in the 50 yr from 1930, the use of N, P, and K increased 20-, 2.5-, and 6-fold, respectively, in the United Kingdom (Blaxter and Robertson, 1995), although, admittedly, much of this increased use was for growing crops and not for pasture production.

In conclusion, from a dearth of knowledge on soil taxonomy and chemical composition in 1917, we have established a worldwide classification system of soils and an understanding of the slope and inflection points of pasture growth in response to increasing soil mineral concentrations. As a result, we have sensible recommendations in almost all pasture-based industries on economic use of fertilizers on different soil types.

Pasture Breeding and Establishment

After World War I, much land that had been cultivated for cereals during the war was returned to grassland pastures (Lazenby, 1981), and post-war

governments were keen to support research in food production (Blaxter and Robertson, 1995). Following the establishment of the Welsh Plant Breeding Station at Aberystwyth in 1917 under the direction of George Stapledon, plant breeding for grazeable forages became the focus (Lazenby, 1981). The importance of leaf and leaf-to-stem ratio on plant digestibility was quickly recognized (Stapledon, 1924) and became a focus of plant breeding, as did breeding for usage (e.g., upright for haymaking, more prostrate for grazing; Lazenby, 1981; Raymond, 1981). The New Zealand Plant Research Station was set up in 1928, and Bruce Levy began a trial to identify superior strains of ryegrass and white clover for New Zealand conditions. Levy was a champion of intensive grassland management, advocating heavy use of superphosphate and higher stocking rates and recommending pasture improvement by sowing the best grass and clover species.

In the 1920s, the placement of grass seed in furrows alongside fertilizer was tested, mimicking the establishment of cereal crops. Although the practice improved pasture establishment in sandy soils and with low-density pasture species compared with the more commonly applied surface broadcast of seeds, it was not an effective way of establishing high-density pastures, a trait identified as being important for grazing (Stapledon, 1927a,b,c). In 1953, the sod-seeder was developed and patented at the University of Sydney, Australia, which allowed more accurate seeding of pastures in a range of soil types.

A grass seed certification scheme was introduced into New Zealand in 1929 to ensure the purity and germination of pasture seeds (Hunt and Easton, 1989) and to aid in plant breeding efforts. In 1961, plant variety rights legislation was introduced in Europe, following a meeting of plant breeders in Paris, which increased the rate of introduction of new pasture varieties and cultivars (Raymond, 1981); similar legislation was subsequently introduced in the United States (1970), New Zealand (1973), and Australia (1987). As a result of this initiative, merit testing of varieties and cultivars began in earnest in the 1960s, and qualitative and quantitative data were collected to produce a description of a pasture variety or cultivar's relative position against its peers.

In 1965, an influential British farmer claimed that stocking rate and nitrogen fertilizer were more influential than seed mixtures or the age of pastures (Paterson, 1965), highlighting a perceived lack of progress, at least at the farm level, in pasture genetics. This perception was not fully accurate. Research plot growth trial results indicate that annual DM yield increased by 0.52% per year under conservation and 0.35% under simulated grazing, with similar gain levels within maturity groups or ploidies (McDonagh et al., 2016). Furthermore, Woodward et al. (2001), in a novel experiment that compared the effect of 1960s and 1980s ryegrass and clover cultivars on milk production in a multi-year farm systems study, reported significant gains in clover breeding, although newer ryegrass cultivar varieties did not perform as expected. The apparent contradiction between the studies may reflect the timing of the increased pasture DM production, which has largely occurred in spring, when pasture cannot be consumed directly by the cow and converted to milk.

In response to these results, and recognizing that other pasture-level traits are important to profitability, considerable efforts have been made to establish multi-trait genetic improvement indices for grasses during the last decade. In both Ireland (2011; Pasture Profit Index) and New Zealand (2016; Forage Value Index), indices have been developed and national phenotype databases have been established to ensure that seasonal DM production, nutritional profile, and persistency traits are adequately represented and appropriately weighted from an economic perspective. These initiatives are a major step forward in addressing the historical lack of genetic gain in pasture species and cultivars.

Pasture–Endophyte Relationships

Microscopic fungal endophytes live symbiotically within grass hosts where they confer protection againts insect herbivores through the production of alkaloid compounds. Unfortunately, these same compounds can have negative effects on animal health and productivity if eaten in sufficient quantities by dairy cattle.

References to endophyte toxicoses date back to Biblical times (approximately AD50; Bacon, 1995), although their influence must have predated written history. The first ryegrass endophyte was discovered in Australia in 1920 (McLennan, 1920), but it was 1940 before it was reported in New Zealand ryegrass plants (Neill, 1940). Similarly, the true extent of endophyte toxicosis was defined in tall fescue in the 1940s in the United States. In the decade following its release in 1943, millions of hectares of Kentucky-31 tall fescue were established in the southeastern United States (Mueller, 1986). It soon gained a reputation for causing livestock health problems and reduced animal performance (Pratt and Haynes, 1950; Blaser et al., 1956) and was regarded by dairy farmers as an unpalatable grass that was not suitable for milking cows (Mueller, 1986). This affected the growth of dairy grazing systems within much of the eastern United States, because no other cool-season perennial grass was widely adapted and persistent (Hoveland, 2009).

So bad were the toxicoses in some grass species–endophyte symbioses that Cunningham (1948) claimed that tall fescue was poisonous to cattle, although he retracted this claim when he discovered no adverse effects from feeding grass seeds inoculated with endophyte to birds and rodents (Cunningham, 1958). In the 1980s, it was categorically proven that the alkaloid compounds produced by endophytes cause "ryegrass staggers" during spring in dairy cattle (note: this is different from hypomagnesemic grass staggers) and heat stress during summer. In the United States, high endophyte Kentucky-31 was reported to reduce milk production and increase rectal temperature (Strahan et al., 1987), and Baxter et al. (1986) reported a greater milk yield in orchardgrass pastures than in fescue pastures infected with endophyte. In ryegrass, the alkaloid lolitrem B was isolated as the causal factor for ryegrass staggers (Gallagher et al., 1981; Harvey, 1983), whereas ergovaline was isolated as the compound contributing to vasoconstriction and heat stress during summer. Endophyte-free pastures, however, were not an option, as endophytes were reported to provide protection from pasture pests, such as the Argentine stem weevil, and to enhance seedling establishment (Popay and Rowan, 1994).

Although severe when occurring, the effects of endophyte were short-lived and it was unclear what actual effect the alkaloids had on animal production. Clark et al. (1999) reported inconsistent effects of endophyte on milk production in a series of short-term experiments—a 4% reduction in milk and milk fat yield from cows grazing high-endophyte perennial ryegrass pastures was reported during one spring but these effects were not repeated in the subsequent spring and the effect was reversed in autumn, with the cows that grazed high-endophyte pasture producing higher milk yields. Nevertheless, the debilitating effects of the endophyte alkaloids on animals could be severe at times and there was increased pressure to find a solution. Techniques were developed to identify desirable endophytes that conferred protection against insect attack but lacked the debilitating effects on animals. These "novel" endophytes, as they became known, were inoculated onto endophyte-free seed. Bluett et al. (2003) evaluated one of the first of these novel endophytes (AR1) and reported a 9% increase in average milk production over 3 lactations compared with the wild-type endophyte, encouraging scientists to develop other varieties. Multiple varieties of novel endophytes have been delivered since the 1990s that provide significant protection against insect attack, while possessing less of the secondary compounds that cause ryegrass staggers and vasoconstriction-related heat stress (Milne, 2007).

Grazing Management—The Path Toward Rotational Grazing

In 1927, Stapledon reported that increasing the period of rest of a pasture between defoliations results in increased DM production (Stapledon, 1927c) and these findings were subsequently confirmed by others (Woodman and Norman, 1932). At the same time, however, Woodman and colleagues (Woodman et al., 1928, 1929, 1931; Woodman and Norman, 1932) reported that more frequent defoliations resulted in higher quality feed. Nevertheless, they concluded that pasture DM production was positively associated with digestible OM production, suggesting that a suitable rest period might exist that accommodates the increased DM production and maintains feed quality.

Detailed plant physiology experiments further supported the advantage of resting pastures for a period after grazing. In a comprehensive review of the role of stored energy reserves in plants and their mobilization following grazing, Weinmann (1948) established a need for a rest period between defoliations to allow plants to replenish carbohydrate stores used in regrowth. Subsequent experiments in the 1950s confirmed a greater pasture DM yield per hectare with an increase in rest period length between defoliation events (Raymond, 1981). These studies led to the idiom that "grass grows grass," which meant that the increase in pasture growth with increasing rotation length was not linear but exponential. Brougham (1955) confirmed this when he concluded that, following grazing, grass grows in a sigmoidal (S-shaped) pattern, beginning slowly and increasing exponentially to a ceiling yield, above which tissue death equals growth and there is no further increase in green digestible material. This sigmoidal curve was subsequently made famous by Voisin in his textbook *Grass Productivity* (Voisin, 1959). Later studies confirmed that DM yield was depressed under fast grazing rotations and this effect was more marked in summer (Campbell, 1969). In comparison, slower rotations increased DM yield, particularly during autumn and winter, when pasture growth rate was slow.

These experiments led to the hypothesis that animal production should be higher from intermittently grazed swards (i.e., controlled or rotational grazing) than continuously grazed swards (i.e., uncontrolled or set-stocking). Subdivision of larger farm areas into smaller paddocks (i.e., a grazing area) and utilization of rotational grazing were reported as widespread in New Zealand a decade earlier (Holford, 1937). The advancement of rotational grazing was facilitated by the development of an electric fence in the early 1930s and subsequent improvements to facilitate longer

fences and more consistent voltage (Jones, 1988). In 1952, the "New Zealand grazing system" was outlined by McMeekan (1952) as one that employs "controlled rotational grazing," based on alternately grazing and resting the pasture to

- increase total yield;
- maintain pasture in a leafy state; and
- aid utilization by grazing stock.

Despite this clear acceptance of rotational grazing by farmers, researchers in many countries debated whether this investment in technology and resting of pastures would increase productivity.

The first experiments in support of rotational grazing were reported by McMeekan (1947). Calves managed in a rotational grazing system were 27 kg heavier entering their first winter (approximately 9 mo of age) than calves raised under set-stocking and, as a result, had a substantially reduced mortality rate. By the time of their first calving, there was a 63-kg difference in BW in favor of the rotationally grazed heifers.

McMeekan (1947) also reported that rotational grazing resulted in a 26% per year advantage in milk fat yield in lactating dairy cows compared with set-stocking. This early support for rotational grazing was challenged, however, by subsequent research at Ruakura, wherein near identical milk production was reported from cows in either rotational or set-stocking management systems. It was hypothesized that the conflicting experimental results related to an interaction between grazing management strategy and stocking rate (McMeekan, 1960) and, in 1957, McMeekan reported a 13% increase in milk fat production per hectare under rotational grazing at higher stocking rates (McMeekan, 1957). Mott (1960) also concluded that the apparent difference between experiments related to an interaction between stocking rate and grazing management technique. He reported that milk production per hectare continued to increase with stocking rate, well beyond the point at which milk production per cow declined. In fact, maximizing milk production per hectare was associated with a 12% reduction in per cow DMI. The promotion of rotational grazing's superiority over continuous stocking in enabling an increase in stocking rate and milk production per hectare was confirmed in a multi-year experiment in the United States (Bryant et al., 1961a). They reported that rotational grazing increased the stock-carrying capacity by between 20 and 30% and increased milk production per hectare, although the effect was dependent on the grazed pasture species.

The debate, in essence, was settled by the publication of a joint New Zealand-Irish collaboration. McMeekan and Walshe (1963) confirmed the interaction between grazing system and stocking rate in a 4-yr experiment; rotational grazing increased milk fat production per hectare by 7, 6, 22, and 29% (in years 1 to 4, respectively) at the high stocking rate. Interestingly, though, their work also implied an interaction between grazing strategy and time, with the low-stocked rotational grazing system producing 10 to 12% more milk fat/hectare than the set-stocked system in years 3 and 4 of the experiment; it was as though the benefits of rotational grazing at low stocking rates were not achieved for several years. The work also highlighted the necessity of multi-year experiments when evaluating significant system-level changes.

Determining the Appropriate Time to Graze

In conjunction with the acceptance of rotational grazing, between the 1960s and the 1980s, scientists in many countries made significant efforts to define criteria by which farmers could decide the appropriate time to graze and when cows should be removed following grazing. A variety of sward height and mass measurements were evaluated (L'Huillier and Thomson, 1988; O'Donovan et al., 2002):

- compressed sward height, using a disc or plate;
- undisturbed sward surface height, using a ruler;
- extended tiller height, again using a ruler but extending the full leaf before measuring height;
- the capacitance probe to estimate mass; and
- visual estimations.

Compressed pasture height measurements and associated regression equations to estimate pasture mass have been the most commonly used methodology, although O'Donovan et al. (2002) reported that visual estimation was the most accurate method of pasture mass determination, provided the observer was trained using calibration cuts. Nevertheless, all of these methods simply ranked pastures on height or estimated mass; none considered the appropriate time for grazing from a plant morphology or physiology perspective.

Since the 1970s, there has been an increased effort to understand the management of pasture that optimizes pasture DM production, utilization, and animal requirements. This research built on the recognition that plant stores were important in the regrowth of swards after grazing, but recognized that the length of the rest period for pastures must also be managed to maintain nutritive quality. Tainton (1973) reported that ryegrass-white clover pastures should not be grazed beyond the development of a full canopy (termed canopy closure), as growth rates decline rapidly at this point,

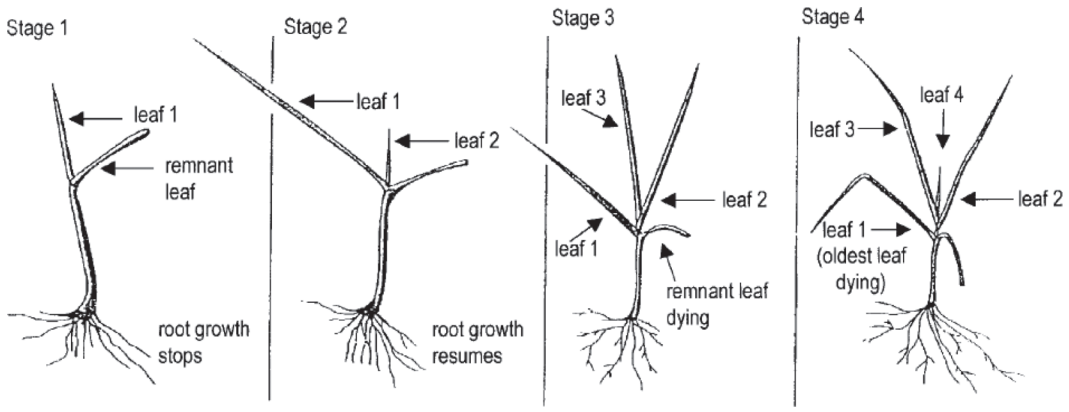

Figure 2. Regrowth of a ryegrass tiller following defoliation (source: Donaghy, 1998).

the rate of leaf senescence increases, and tiller density declines. Having defined that ryegrass maintains only 3 live leaves (i.e., it is a 3-leaf plant; Figure 2; Donaghy, 1998), Davies (1977) proposed that a ceiling yield is reached after emergence of the third new leaf. This provided a morphological basis for grazing management; the optimum point for grazing is between the adequate replenishment of plant stores and canopy closure. However, there was very little uptake of this management strategy for a further 15 yr. Fulkerson et al. (1993) first applied "leaf regrowth stage" as a grazing management strategy and, after a decade of research, recommended that, at least for perennial ryegrass, the "2-leaf stage" of regrowth sets a lower limit to grazing, because it facilitates the replenishment of the plant's carbohydrate stores, whereas senescence of the oldest leaf (i.e., >3-leaf stage of regrowth) sets the upper limit. These recommendations have been widely accepted as the way to maximize the yield of digestible pasture per hectare (Fulkerson and Donaghy, 2001).

Postgrazing Residual

The other principle identified by McMeekan (1960) as being important to the success of a grazing system that relates to grazing management was "to use all the grass grown," which in management terms refers to optimizing the postgrazing residual. This is a fine balance between harvesting the pasture grown but not undermining future growth potential by grazing too severely (i.e., over-grazing) and not underfeeding the cow to the detriment of milk production. Brougham (1957) reported that defoliation events that removed all of the leaf resulted in slow regrowth and Lee et al. (2008a) confirmed that consistent low postgrazing residuals (~2 cm) reduce annual DM production. It is unlikely, therefore, that McMeekan meant his advice literally. He more than likely meant that the astute grassland manager should use as much of the grass at each grazing as is consistent with maximizing long-term total pasture consumed. To this end, Lee et al. (2008a) identified that there was little effect of post-defoliation residual heights between 40 and 80 mm on subsequent pasture DM production, but indicators of nutritive value (i.e., CP, ADF, ADF as a % of NDF, DM digestibility, and ME) changed through time and reflected a general decline in feed quality with increasing postgrazing residual.

Because pasture DM production is regarded as largely insensitive to postgrazing residual within the normal ranges in a dairy production system, the focus of grazing residual research and discussion has largely revolved around effects on animal production. Individual cow DMI (Cohen et al., 2000) and milk yield (Wales et al., 1999) are influenced primarily by the amount of pasture offered per cow on a daily basis; the higher the allowance, the greater the pasture DMI and milk yield, but the size of the increase declines with increasing allowance.

Because the increase in DMI with increasing allowance is less than unity, however, postgrazing residual height and mass also increase with allowance. Stakelum and Dillon (1991) highlighted that high postgrazing residuals in spring resulted in a subsequent reduction in OM digestibility and the proportion of green leaf in the sward. Because of the reduction in pasture quality, subsequent DMI and milk production declined, despite cows being offered the same green leaf allowance. In subsequent work, they reported that milk yield was 1 to 3 kg/d less from cows grazing pastures that had previously had high residuals, unless pastures were mown and grazing pressure was reduced (Stakelum and Dillon, 1991).

These results are consistent with US research that investigated the difference in milk production when cows were allowed to graze either the top or the bottom of the sward reported by Bryant et al. (1961a); cows grazing the top of the sward produced 30% more milk per day than those grazing the bottom of the sward, highlighting the difference in nutritive value between the leaf and the stem. It was, therefore, imperative to avoid stem elongation through excessive postgrazing residuals. Stakelum and Dillon (1991) concluded that low residuals were very important in spring but less important from summer on. Lee et al. (2008b) reported that consistent postgrazing residuals were the key. They reported that milk yield and presumably DMI were not affected by postgrazing residuals between 41 and 59 mm during a single rotation in spring, as long as the target residual was the same as that achieved previously. These results are consistent with Norton (2014), who reported no effect of grazing residuals between 35 and 50 mm on either milk production or the marginal milk production response to supplementary feeds. Nevertheless, these previous studies focused on animal production, with scant regard for per hectare production. Ganche et al. (2013a,b), in contrast, concluded that consistent postgrazing residuals of 35 mm maximized pasture DM yield and milk production per hectare. In summary, there is general agreement that postgrazing residual is important for subsequent pasture quality and milk production/cow. The optimum postgrazing residual is, however, poorly defined; nevertheless, it is likely between 35 and 50 mm (Ganche et al., 2013a,b; Norton, 2014).

The research investigating optimum postgrazing residual height and mass forms yet another argument in favor of rotational grazing over set-stocking. In set-stocking, it is not possible to establish a consistently low average postgrazing residual (i.e., high utilization), because some areas of the pasture become overgrazed or grazed excessively frequently (Lee et al., 2010), whereas other areas are not adequately utilized. The move to rotational grazing allowed, for the first time, the ability to have low average postgrazing residuals without overgrazing certain areas, because the area offered/animal was controlled (i.e., choice was removed) and the animals were moved after a short duration (e.g., a day), ensuring that the grazed pasture had time to fully recover between grazing episodes.

Practical Outcomes of Grazing Research

The grazing research undertaken over the last 100 yr has had a significant influence in defining the modern system and in achieving 2 of the principles outlined by McMeekan (1960): "grow as much pasture as is practicable" and "use all of the pasture grown."

The need for a rest period resulted in the acceptance of rotational grazing as being superior to set-stocking; this has facilitated various productivity improvements:

- greater pasture DM production per hectare, through exploiting the sigmoidal nature of pasture growth and the replenishment of pasture plant carbohydrate stores;
- greater ability to manage higher stocking rates by avoiding the "feast and famine" that would occur if pasture could not be rested between grazing events, particularly when pasture growth rates are low;
- more targeted and efficient use of N fertilizer because applications can closely follow grazing events.

The research into "ceiling yield" allowed the extension of research-derived recommendations for rotation length and grazing management decisions from any country to all temperate grazing areas.

Furthermore, it led to the refinement of the "autumn rotation planner" (Macdonald and Roche, 2016). In the 1960s, very long rotations were recommended in autumn (>120 d) to ensure sufficient feed was available in the winter. This did not increase the availability of digestible feed, as senescence and new growth were equivalent, but it led to a reduction in the quality of the pasture being offered to early lactation cows and reduced the spring pasture growth rate. It was subsequently recognized that rotation lengths longer than the duration required to produce 3 leaves resulted in no further accumulation of digestible OM and, in fact, could reduce plant viability and DM production the following spring. This, eventually, led to the recommendation that pasture could be accumulated in situ for grazing during the winter (i.e., the autumn rotation planner), but that the length of the inter-grazing interval should not be longer than the time taken to produce 4 leaves (the extension to 4 leaves was because senescence and the reduction in pasture nutritive value were slowed in winter by low minimum temperatures). As a result, autumn rotations were reduced to between 60 and 100 d, depending on location, and feed quality for early lactation cows was greatly improved.

This research and the resultant understanding of pasture growth profiles led to the development of what is arguably the simplest but most effective decision support resource available for grazing farmers: the spring rotation planner (**SRP**; Macdonald and Roche, 2016). As pasture growth is significantly less than the

herd's DM demand during the winter in the majority of places where grazing is practiced, the SRP "rations" the pasture accumulated during the autumn so that winter pasture growth is maximized and cows are well fed through winter and early spring (i.e., from calving to the day that pasture DM growth is equal to the herd's DM demand: referred to by farmers as "balance day" or "magic day"). By using this decision support resource, the pasture remains in the exponential phase of growth for as long as possible without significant loss of OM digestibility and DM production is maximized. The SRP (Figure 3) provides the farmer with the rotation length required through winter and early spring and the amount of land area that can be allocated on any given day during winter and spring. In an important study outlining the system-level benefits of the SRP (Bryant and L'Huillier, 1986), pasture DM production was 1,775 kg of DM/ha greater during the first 6 mo of the grazing season when a farm adhered to the SRP during winter than if they had grazed on a faster rotation; this was approximately equivalent to an 18% increase in DM yield during that period and an 11% increase in the annual DM yield.

Summary

In 100 yr, research into soil improvement, pasture breeding, and grazing management have moved grazing systems from cows being offered low yielding, low digestibility, unimproved pastures with limited grazing infrastructure to highly specialized systems in which cows calve seasonally (i.e., in spring) and graze productive pastures. Instead of free access to large areas of the farm at all times (set-stocking), cows rotate around the farm regularly (i.e., rotational grazing). To allow this, the farm is divided into a network of paddocks, facilitated by the development and refinement of the electric fence, that are interconnected by a series of farm tracks or lanes (see Figure 1). Grazing management has moved from allocating a certain amount of pasture area each day to a better understanding of the needs of the pasture plant and the interaction between the animal and the plant. As a result, systems have evolved to produce 780 kg of 4% FCM and 58 kg of milk fat and protein from every tonne (DM) of pasture grown (approximately equivalent of 1,000 kg of 4% FCM and 74 kg of fat and protein/t of DM pasture utilized), with virtually no mechanization other than the milking parlor and the electricity required for the electric fence (Macdonald et al., 2008a).

COW FERTILITY

The success of seasonal grazing systems is dependent on each cow recalving every 365 d, in theory, and the herd calving within a short timeframe (i.e., ~60 d,

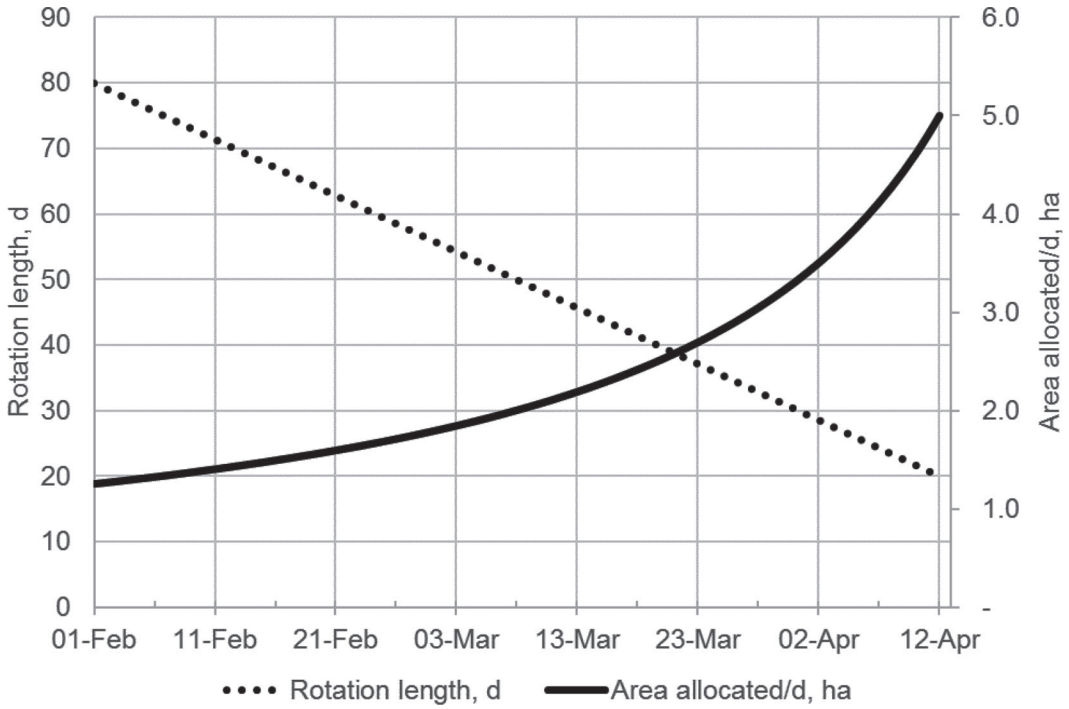

Figure 3. An example of the spring rotation planner for a seasonal-calving, grazing herd. Rotation length (dotted line) declines and the area available to be grazed per day (solid line) increases as the farm progresses through winter and into spring and pasture growth rates increase.

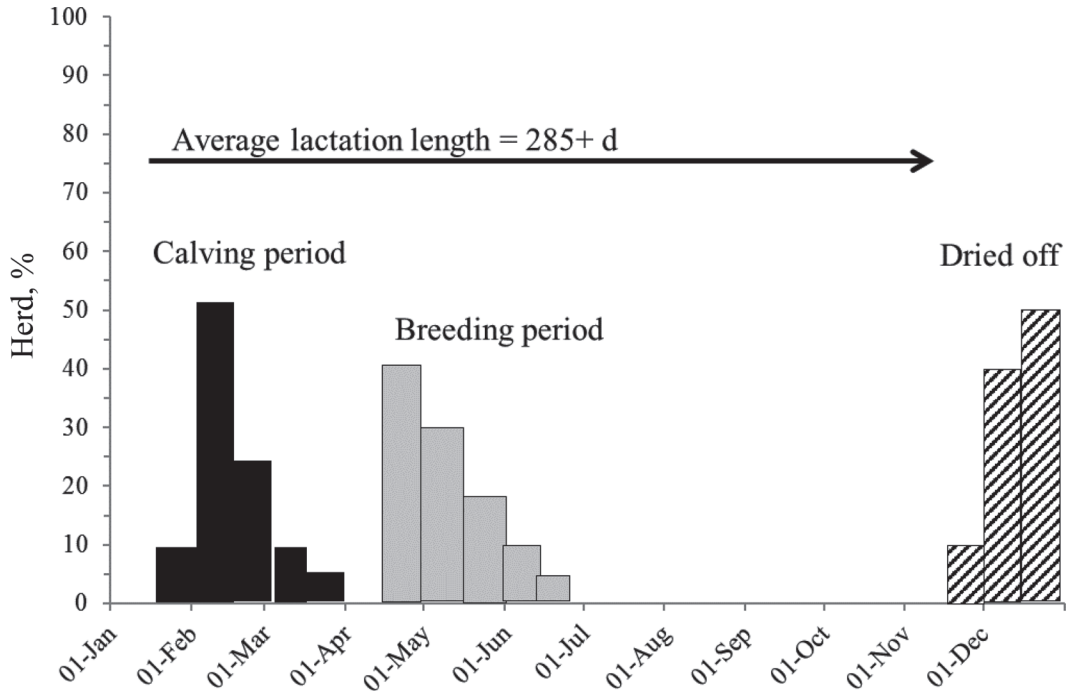

Figure 4. Seasonality of calving, breeding, and drying off (i.e., cease lactation) for cows to ensure the supply and demand curves for pasture are synchronized.

including replacement heifers; Holmes, 1995; Roche et al., 2017b). This logically requires the herd to become pregnant in an equally short timeframe (~70–80 d, with the difference being the allowance for later calving, cull cows being replaced by heifers). Although this "seasonal peculiarity" in intensive grazing systems is generally accepted, little thought is given to the reproductive technologies that have facilitated the evolution of the large-scale, intensive grazing system. For example, the consequence of a seasonal mating pattern is that a significant amount of semen from the top sires is required during this intensive mating period to satisfy demand.

To ensure success, the planned start of mating must begin approximately 80 d after the planned start of calving (Figure 4). Cows, therefore, have to return to estrus quickly after calving, be identified by the farmer as being in estrus, and be highly fertile when inseminated, either artificially (AI) or naturally (with a bull). Until the 1930s—when average herd sizes were small and the requirement for bulls was minimal—this was easily manageable. However, the advancement in electric fence technology, milking parlor technologies, and improved pasture management facilitated the shift to large grazing herds. To remain seasonal, however, large herds would have required large numbers of bulls on each farm, with the logistical and safety issues that this would bring. High fertility semen delivered by AI was the answer to this problem, but there were many logistical hurdles to clear before this technology could be delivered. Overcoming the difficulties of seasonal breeding on expanding grazing dairy farms was the quandary that faced researchers as they entered the 1940s.

Optimizing Semen Supply

Liquid Semen Versus Deep Frozen Semen. Grazing systems needed an AI service suited to the unusual requirements of highly seasonal demand (James, 1941). Although deep frozen semen technology was being developed elsewhere, it was not suitable for grazing systems because of the high capital costs associated with medium-term storage for a short period of demand. The early use of AI in research scenarios was very successful (James, 1941), with conception rates (**CR**) similar to those in achieved natural mating. Low semen dilution rates, short semen shelf life, limited availability of sires of positive genetic merit, and the concentrated peak demand for the insemination service, however, meant that this initial success was not translated into acceptable field results (James, 1946).

The decision was made to develop a room temperature semen extender to reduce the number of sperm cells required per insemination and increase the number of insemination straws from each bull ejaculate. The semen extender was based, initially, on the addition of egg yolk to the Cornell University Extender (**CUE**;

Foote, 1970). The initial service used chilled semen with a dose rate of 25×10^6 sperm per insemination deposited in the posterior body of the uterus (instead of deep cervical insemination as practiced elsewhere; Moller et al., 1972). This dose rate had been reduced to 5×10^6 sperm by 1963 through trialing modifications to the CUE, such as adding glycine, glycerol (Shannon, 1964), and caproic acid (Shannon, 1962), as well as saturating the diluent with nitrogen (Shannon, 1965a), and varying the egg yolk concentrations (Shannon and Cursons, 1983). These modifications were necessary to inactivate a toxic heat-labile protein in seminal plasma (Shannon, 1965b) and to neutralize the toxic effect of the hydrogen peroxide produced by an amino acid oxidase released by dead sperm (Shannon and Curson, 1972). The result was a room temperature diluent called Caprogen.

The success of the Caprogen diluent was subsequently demonstrated by achieving high CR relative to the deep frozen semen used in similar systems. Conception rate, measured as the percentage of pregnancies confirmed after insemination, is a key performance indicator of fertility and is usually estimated from the nonreturn rate (**NR**). The 49-d NR in most of the studies associated with the development of Caprogen have exceeded 60%. This 49-d NR was reported to overestimate final CR by only 2.9 percentage units (66.9 vs. 64.0%) in 959 cows across 2 field studies involving seasonally calving herds, where each pregnancy was tested to confirm conception (Macmillan et al., 1977b). A subsequent study that included progesterone monitoring reported a CR to AI of 65% when tail-painting was used as an aid to improve efficiency of estrus detection (Xu et al., 1998). A novel variation involving the use of Caprogen was the development of rediluted deep frozen semen, in which semen was glycerolated before being frozen at a concentration of around 800×10^6 sperm (Shannon and Vishwanath, 1995). It could then be rediluted with Caprogen when thawed before use at a dose of 20×10^6 sperm/insemination. When rediluted deep frozen semen at this dose rate was compared with normal "liquid" semen in Caprogen at 2.5×10^6 from the same 11 sires, the 49-d NR were 68.1 and 67.6%, respectively (Shannon and Vishwanath, 1995). In contrast, pregnancy tested cows in seasonally calving herds in Victoria and Tasmania (Australia) that used deep frozen semen and included some herds with DIY inseminating had conception rates of only 49% (Morton, 2010) and 40% (Morton et al., 2016).

Sperm Dose Rates. The variations to the CUE diluent mentioned earlier and further developments of the Caprogen diluent between 1958 and 1988 facilitated a 96% reduction in the sperm required per successful insemination (i.e., from 25×10^6 to 1×10^6). This meant that fewer sires were needed to meet the ever-increasing demand for inseminations from an expanding dairy industry and facilitated the expansion in herd size on individual farms. Two additional factors contributed to meeting this demand in New Zealand:

- most herd owners were prepared to accept a nominated bull service, often referred to as "bull-of-the-day," rather than selecting semen from specific sires to inseminate specific cows within a herd; and
- the peak in demand for inseminating was accommodated by individuals within the rural community being prepared to accept employment for as little as 6 wk as inseminators, and frequently inseminated over 100 cows in a single day.

This was preferable to individual herd owners practicing "DIY" inseminating, as was occurring in many other dairy industries. The combination of these factors has contributed to the evolution of an AI service particularly suited to the pasture-based seasonal dairying system.

Sexed Semen. Conventional semen contains approximately equal numbers of sperm bearing X and Y chromosomes; hence, the likelihood of a female or male calf is approximately equal (Roche et al., 2006c). Sexed semen refers to semen that has gone through the process of sorting the X and Y chromosome sperm, and most commercially available sexed semen used in the dairy industry contains ~90% X-bearing sperm. Initially, sexed semen products had markedly inferior fertility compared with conventional semen (Butler et al., 2014), but the gap has closed in recent years with iterative improvements in the sorting technology.

The role for sexed semen in seasonally calving herds has focused on its potential to accelerate herd expansion or to facilitate surplus heifer production for sale, allow shorter periods of dairy semen usage and increase the beef output from the dairy herd, and increase rates of genetic progress (Hutchinson et al., 2013). The best fertility results were obtained when sexed semen was used with well-grown heifers or cows with longer intervals from calving to insemination and better BCS (Butler et al., 2014). Extensive studies in New Zealand herds indicate that doses as low as 1×10^6 sperm per insemination with sexed sperm or normally processed sperm stored in a Caprogen diluent could be used with lactating cows, with the calving rates being only 3 to 4 percentage units lower with sexed sperm (Xu, 2014).

Modeling studies relevant to seasonal-calving, pasture-based systems indicated that the economic returns from the intensive use of sexed semen to accelerate genetic progress were not always positive (Hutchinson

et al., 2013; Murphy et al., 2015, 2016). This was partly because this pattern of use of sexed semen reduced a herd's ability to maintain a seasonally concentrated calving pattern, through a lower CR in lactating cows (Hutchinson et al., 2013). A follow-up modeling study indicated that targeted usage of sexed semen (i.e., for highest fertility cows only) plus one of either conventional dairy semen, beef semen, or short-gestation-length semen for any cows that returned to estrus was more profitable than use of conventional semen only (Murphy et al., 2015, 2016). Further improvements with diluents used in processing sexed semen to reduce between-sire variation (as happened with the development of room temperature diluents, such as Caprogen) may allow sexed semen to be used without compromising reproductive performance in seasonally calving herds in the future.

Insemination Management on Farm

Submission Rates and Estrus Detection. Submission rate (**SR**) is a key performance indicator used in seasonally calving herds to monitor the rate of progress of the insemination program, particularly during the first 3 wk. It was derived initially by recovering calving and insemination data from 97 herds that used AI exclusively for at least 7 wk in the 1971 season to show that herds with a high 4-wk SR (>90%) had the highest herd in-calf rates at 4 and 7 wk, even though they had lower average CR (Macmillan and Watson, 1973). This was because more cows in the high-SR herds had short return intervals through errors in detection preceding first insemination (Macmillan et al., 1977b) and genuine short cycles (Macmillan and Watson, 1971). The use of a 3-wk SR has now become a standard key performance indicator in breeding management programs in seasonal-calving, pasture-based herds.

With increasing herd size came a reduction in rates of estrus detection and in the occurrence of genuine short cycles (Macmillan and Watson, 1973). The solution proved to be the simple and inexpensive technique called "tail-painting" or "chalking" (Macmillan and Curnow, 1977). The technique was adopted widely in grazing systems all over the world and the efficiency and accuracy were subsequently reported as 98.4 and 97.6%, respectively (Xu et al., 1998).

One consequence of high detection efficiency, however, was that it revealed the extent and severity of anestrus during the postpartum period in many seasonal-calving herds (Fielden and Macmillan, 1973). For example, 85% of 1,028 primiparous cows that had not been inseminated during the first 4 wk of the seasonal-breeding period and had calved at least 6 wk before the planned start of breeding were diagnosed as having small "inactive" ovaries (Fielden et al., 1976). The incidence was associated with differences in age, breed, interval from calving date to the first day of AI, and herd size (Macmillan et al., 1975; Macmillan, 2002). The influence of low BCS has also been recognized in subsequent studies (see Roche et al., 2009a). Most studies conducted since 1990 have diagnosed anestrous cows during the month preceding the onset of the seasonal-breeding period, or within the 10 d after the start of the seasonal-breeding period; using these timing criteria, 20 to 30% of cows within several multi-herd studies were classified as anestrous (Rhodes et al., 2003; Shephard, 2005), and up to 44% in a recent study in Victorian herds (Plozza et al., 2016). The classification in the early studies of inactive ovaries was subsequently reported to be incorrect. Ovarian follicle waves emerged every 8 to 10 d from about 10 d postpartum, but the follicle failed to ovulate (McDougall et al., 1995; Nation et al., 1999).

Estrus Synchronization and Development of the CIDR. Although simple single-injection programs that utilize prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were developed for use in the milking herd (Macmillan et al., 1977a; Macmillan, 2002), these programs had to be used in conjunction with high standards of estrus detection because of the post-injection variation in the interval to estrus (Macmillan and Henderson, 1984). Furthermore, because the $PGF_{2\alpha}$ injections were ineffective with anestrous cows (Macmillan et al., 1977a), they failed to address a major problem in seasonal-calving herds. Although the effectiveness of whole-herd synchronization programs has been demonstrated under grazing conditions, they have not been widely adopted. This is, at least in part, because the resulting concentration in calvings can overwhelm limited labor and feed resources.

The greatest changes in the application of synchronization in seasonally calving herds followed the development of the controlled internal drug-releasing (**CIDR**) insert for the vaginal administration of progesterone for periods of 5 to 14 d in dairy cows and heifers that were anestrous (Macmillan and Peterson, 1993). The insert has been used in combinations with injected $PGF_{2\alpha}$, GnRH, and estradiol benzoate. Its use has become the standard treatment for anestrous cows in seasonally bred dairy herds.

Reproductive Management of the Herd—Getting Cows in Calf

Recent industry investment in intensive monitoring programs has produced standardized sets of management recommendations in combination with publications for use by herd owners, advisers, and veterinar-

ians. The first of 2 Australian studies (Morton, 2010) derived records for a single season from 124 seasonally calving herds containing over 31,000 cows. The second study was a longitudinal study with records derived from 74 herds during the period from 2000 to 2009, covering 481 mating periods and reproductive data for over 100,000 cows (Morton et al., 2016). The results in both studies measured the effects of a wide range of factors that influenced 2 primary performance indicators (6-wk in-calf rate and 21-wk not-in-calf rate) at the herd and cow levels, as well as 2 secondary performance indicators (3-wk submission rate and confirmed CR to first insemination; Morton, 2010). The 2 primary indicators were identified as being the critical outcomes for a breeding program in a seasonal-calving herd, whereas the secondary indicators were the key drivers to achieve any goals set for the primary indicators. A similar study based on records from over 100,000 cows was conducted in New Zealand (Xu and Burton, 2003) except that the 21-wk period was reduced to 12-wk not-in-calf rate to reflect the more intensive breeding policy in a strict seasonal-calving system. None of the studies included calving interval, which is a key indicator of reproductive performance in herds where cows calve throughout most of the year but is of limited relevance to seasonal-calving systems because it cannot be allowed to vary beyond 365 d.

Summary

Although the complexities of seasonal breeding are often acknowledged, the need for a service that could provide sufficient volumes of semen from high-genetic-merit bulls for AI is often understated. The pivotal work of Pat Shannon and his team in developing diluents that allowed a 96% reduction in the quantity of sperm per insemination and the extended viability of "fresh" semen facilitated the increased scale of grazing farms while maintaining the seasonality of calving in-line with pasture supply. The simple technology of tail-painting facilitated accurate estrus detection in large herds and high submission rates of cows for AI. Synchronization program developments, other than the use of CIDRs to kick-start estrus in anestrous cows, have largely been ignored, most likely because of the pressure they put on a limited labor force at calving and the limited feed resources that are available in grazing systems at the onset of a seasonal calving period (i.e., winter). The development of the CIDR, however, facilitated the breeding of cows that have a delayed return to estrus after calving and increased the likelihood of pregnancy within the defined breeding period. Significant industry investment has resulted in a successful extension effort that helps farmers identify the metrics of reproductive failure (and success) on their farm and provides potential solutions to overcome the managerial failings.

COW BREEDING FOR GRAZING SYSTEMS

An old Irish adage states, "an ounce of breeding is worth a pound of feeding," implying that animal quality and performance is born, not made. This is consistent with McMeekan's principle for running a successful grazing system: "use animals that will process the grass efficiently" (McMeekan, 1960). Although milk recording services became available in many countries at the beginning of the 20th century, most "dairy cattle" possessed dual-purpose characteristics in 1917 to supply both replacement female calves for milk production and bull calves with good beef characteristics. This was identified as "the one factor wherein the charge that farmers are careless in business was justified" (Anonymous, 1924); for example, just before the report cited, 50% of champion milk producers in New Zealand had been purchased for a fraction of their value because the previous owner had no knowledge of the yields of individuals within his herd (Anonymous, 1924).

In the early 20th century, farmers were encouraged to "milk record" to identify the "top-notch" and "boarder" cows (the term "boarder" was used for a cow that boarded but provided very little value in their herd; Anonymous, 1924). Following World War I, the numbers of farmers using herd recording services and the number of cows recorded globally greatly increased: for example, in the UK, in 1917, 478 farmers recorded 13,838 cows; by 1938, this had increased about 10-fold to 4,302 farmers recording 161,077 cows (Atkins, 2016). In comparison, in New Zealand, 5% of cows were being milk recorded in 1917, increasing to approximately 25% in 1950 and to a peak of almost 90% of all cows in the country, or 3 million cows, in 2005. Milk recording was a producer-driven initiative in the early years and became an official, national scheme in 1936 in New Zealand and in 1943 in the UK (Atkins, 2016). Milk recording in Ireland began in the 1940s but was undertaken only on herdbook-registered cows. Cows were milked using bucket plants and the milk of each cow was weighed. The first "milk recording jars" appeared in Ireland in the 1950s, facilitating the determination of yields from the graduated scale on the jars; mechanical milk meters were introduced in the 1960s and electronic "do-it-yourself" milk meters (Berry et al., 2006) in the early 2000s. With the increased ease of milk recording/herd testing, the proportion of the Irish herds being milk recorded increased from approximately 10% of the national herd in 1991 to 51% in 2016.

By 1917, herdbook societies had been in operation for approximately 100 yr (Brotherstone and Goddard,

2005), but their focus on improving dairy characteristics was limited because of the concurrent breeding for beef-related traits. The Shorthorn breed had risen to prominence because of its milk yield and "butchering potential." In fact, herds of Shorthorn cattle were identified in every area of England "where milk production was an important part of the agricultural economy," whereas the Ayrshire remained the dairy breed of choice in Scotland (Atkins, 2016). Similarly, in New Zealand, the Shorthorn was the most popular dairy breed in 1920 (~50% of cows), followed by the Jersey breed (~30% of cows). Although relatively rare on dairy farms (≤10% of cows), Holstein cows were popular among urban "cow-keepers" in the early part of the 20th century (Atkins, 2016). Through the concerted efforts of the Friesian and Jersey herdbook societies, these breeds rapidly overtook the Shorthorn breed in popularity. In the UK, the British-Friesian increased to 20% of cows in 1947 and 76% in 1970, whereas the Shorthorn fell from 85% in 1908 to 3% in 1970; this breed change was mirrored in Ireland. In New Zealand, because of the focus on dairy product export, the Jersey breed replaced the Shorthorn; between 1921 and 1949, the Jersey increased from 30% to 86% of the national herd and the Shorthorn decreased from 50% to < 5%.

The development of the BLUP methodology for genetic evaluations (Henderson, 1950) heralded a new era of genetic evaluation, providing a framework that easily ranked animals on traits of importance. Grazing systems until the late 1900s operated similar breeding objectives to most other countries, focusing on yield of milk or yield of milk constituents. In New Zealand, a national breeding objective that included just fat yield was introduced in 1953 and genetic evaluations of sires were published annually. Genetic progress was accelerated with the widespread adoption of AI technologies from 1950. Breeding strategies for increased milk yield in intensive feeding and housed systems in North America and Europe and a reasonably lucrative dairy beef market for Friesian bulls resulted in greater use of Holstein-Friesian semen, initially imported from North America and later from Europe during the 1970s and 1980s (Harris and Kolver, 2001). Furthermore, the relative price of whole and skim milk powder over butter and cheese, which were declining in popularity due to simplistic and, ultimately, inaccurate health messages, led to an increased economic weighting on milk protein over fat and a further shift from Jerseys to the Holstein-Friesian breed through the 1990s.

The rate of adoption in grazing systems of germplasm identified from nongrazing systems was exceptionally high toward the end of the 20th century. For example, Harris and Kolver (2001) reported that the percentage of North American/Dutch Holstein-Friesian genetics in sires used for AI in New Zealand increased from 22% in 1980 to >70% in 1999. As a result, from 1980 to 1999, the average percentage of North American/Dutch Holstein-Friesian in the New Zealand dairy cow population increased from 2 to 38%, and the percentage of Holstein-Friesian cows with some North American/Dutch Holstein-Friesian increased from 7 to 96%. By the mid-1990s, the breed structure of the New Zealand national herd was 57% Holstein-Friesian, 16% Jersey, 18% Holstein-Friesian × Jersey crossbred, 2% Ayrshire, and 7% other dairy breeds and their crosses. This was a large change from a national herd of 30% purebred Jersey, 11% Holstein-Friesian, and 50% Shorthorn in 1921. A similar holsteinization phenomenon occurred in Ireland; a retrospective analysis reported that the proportion of North American Holstein-Friesian genetics increased from 8% in 1990 to 63% in 2001 (Evans et al., 2006).

In the 1990s, selection indices were further developed to incorporate more traits of economic importance. For example, the national breeding objective in Ireland at the time (i.e., Relative Breeding Index) was expanded to include milk component traits as well as volume, whereas that in New Zealand (i.e., Breeding Worth) was expanded to include positive weightings for protein as well as fat, a negative weighting on cow body weight, and an economic measure of the lifetime value of a cow relative to a set annual DMI (as a proxy for feed conversion efficiency; Harris, 1995). At the same time, New Zealand moved from a within-breed evaluation index to a single genetic evaluation across all breeds. These changes heralded the first signs of the multi-breed and multi-trait genetic evaluation systems and indices that were to follow.

The evolution of the "specialist grazing cow" began in earnest during the 2000s. In the 1990s, producers reported concerns that the modern Holstein-Friesian cow had inferior fertility compared with her predecessor. Much of those protestations were ignored because it was largely thought that breeding for or against fertility traits was not possible due to its low heritability. Numerous experiments were undertaken in New Zealand (Kolver et al., 2002; Roche et al., 2006b; Macdonald et al., 2008b), Australia (Fulkerson et al., 2008, 2001), and Ireland (Buckley et al., 2000; Kennedy et al., 2002; Horan et al., 2005a) to determine whether different genetic strains performed differently under different feeding systems (i.e., genotype × environment interaction). Although there was evidence that feeding regimen influenced the marginal milk production response to nonpasture feed and the ability of the dairy cow to exhibit her genetic potential compared with her peers (Kennedy et al., 2002; Fulkerson et al., 2008), the greatest differences between the strains were in the

extent of negative energy balance in early lactation (Buckley et al., 2000; Roche et al., 2006b) and in their pregnancy rates in a seasonal calving system (Horan et al., 2005c; Macdonald et al., 2008b), irrespective of feeding regimen. In brief, 6-wk in-calf rate in North American-derived Holstein-Friesian cows was 15 percentage points lower than that of New Zealand-derived Holstein-Friesian cows (Horan et al., 2005c; Macdonald et al., 2008b) and they were less likely to survive to successive lactations (Harris and Kolver, 2001). Subsequent research concluded that the lower pregnancy rates in the North American-derived Holstein-Friesian was a result of early embryo loss 2 wk after conception and that at least some of this difference may be epigenetically regulated (Walker et al., 2012, 2013).

As evidence accumulated in seasonal-calving grazing systems of the inferior reproductive performance and survival of Holstein-Friesian strains selected in non-seasonal breeding systems, breeding objectives evolved rapidly. In 2001, the Economic Breeding Index (**EBI**) was introduced in Ireland and included calving interval and survival, in addition to the milk yield traits already included in the Relative Breeding Index. Similarly, fertility was included in the New Zealand national breeding goal in 2002. By 2017, the EBI included 18 traits encompassed within 7 sub-indices: (1) milk production, (2) fertility and survival, (3) calving performance, (4) maintenance, (5) beef performance, (6) health, and (7) management. The evolution of the EBI since 2001 is illustrated in Figure 5, and the impact of this breeding objective on genetic trends for milk solids production and fertility is in Figure 6.

Recognition of the success of this multi-trait approach in designing the ideal cow for seasonal calving and grazing systems has led to the development of the US Grazing Merit Index (GM$; Gay et al., 2014) and the UK Seasonal Calving Index in 2014, with both indices putting increased emphasis on functional traits associated with grazing and the need to achieve pregnancy within 80 d of calving. The seminal paper on genome wide-enabled selection by Meuwissen et al. (2001), coupled with the commercial availability of low-cost, high-density genotype panels, revolutionized dairy cow breeding programs in the early part of the 21st century, including in countries like Ireland and New Zealand, the impact of which is described in Spelman et al. (2013).

Summary

The predominant breed in grazing systems shifted from Shorthorn and Jersey to Holstein-Friesian between 1917 and 2017, but there has been a shift away from breed preference in the 21st century because of across-breed genetic evaluation systems and a single breed-agnostic breeding objective. The multi-trait breeding goals for seasonal calving and grazing animals have been very successful in pursuing the optimum cow for grazing systems and, with developments in phenotyping tools and strategies, as well as advances in "-omics" technologies, we expect that grazing cows will be more fertile, longer lived, resilient to changes in feed availability and quality, and produce large yields of fat and protein relative to BW and annual DMI.

NUTRITION RESEARCH IN GRAZING SYSTEMS

At the World Dairy Congress in 1928, Robert Boutflour from Harper-Adams College (Shropshire, UK) identified 4 factors that, in his opinion, were the most

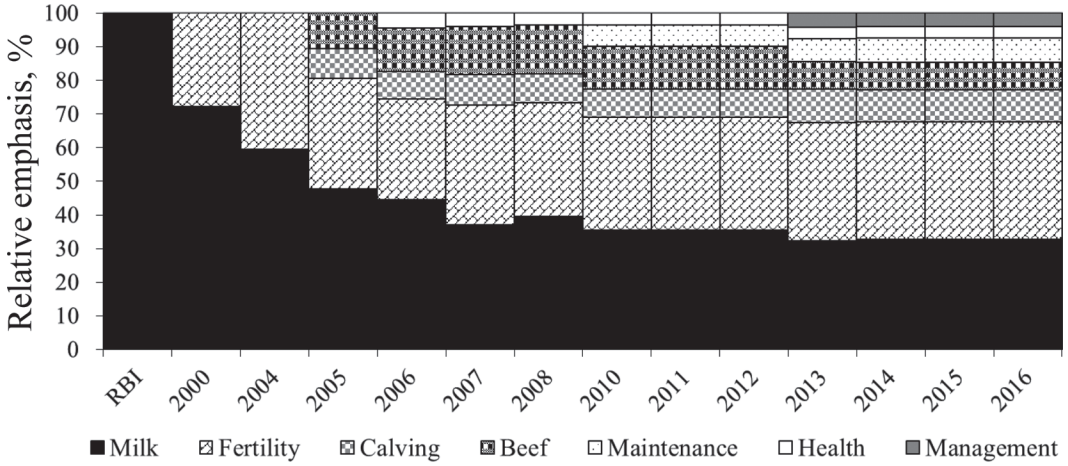

Figure 5. The evolution of the Economic Breeding Index, a multi-trait breeding objective in Ireland that includes traits other than production that are important to profitability. RBI = Relative Breeding Index.

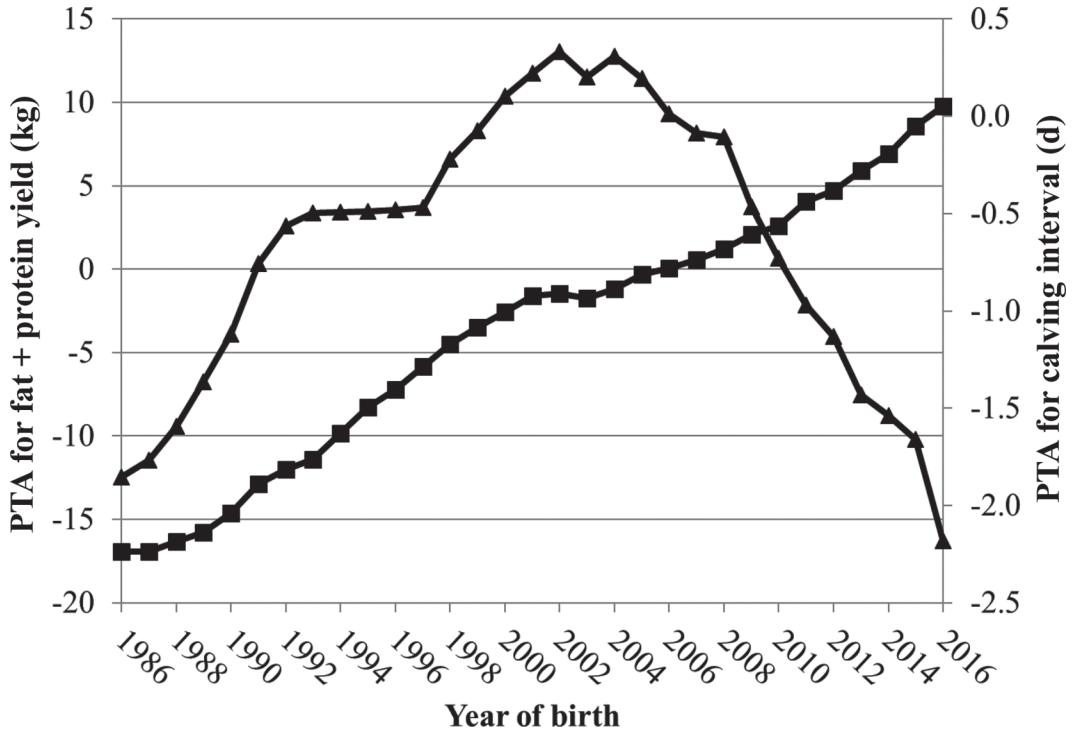

Figure 6. The effect of introducing the Economic Breed Index in 2001 on genetic trends for milk fat and protein production (■) and calving interval, as a measure of fertility (▲). Despite the reduced weighting on milk production and an increased weighting on fertility, the genetic trends for increased milk production did not diminish.

limiting for milk production: (1) "lack of control of indigestible fiber"; (2) "lack of control of the total amount fed"; (3) "the neglect of the preparation of the cow for her lactation period"; and (4) "the over-stocking of the udder." The first 3 referred to dairy cow nutrition. At the time, cows grazed unimproved pastures and were supplemented with "undecorticated cotton cakes and inferior fodders" (Boutflour, 1928). Boutflour recommended that cows should be "steamed up" before calving, offered only a limited amount of crude fiber, and the forage-to-concentrate ratio should be reduced gradually through early lactation, always ensuring that the cow is "fed for one gallon more milk than she is giving" (i.e., individually fed relative to her milk yield). The requirements of close-up transition dairy cows, the need to maximize DMI, the requirement for individualized feeding of supplementary feeds, advantages of concentrate feeds over pasture, and potential advantages for individualized feeding of grazing dairy cows have been the subject of nutrition research for the last 100 yr.

Transition Cow Nutrition

Pasture-grazed transition dairy cows have very little dietary adjustment to undertake and, for the most part, the diet is well balanced, with the exception of some minerals. Protein quantity and quality is adequate and there is little need to adapt cows to their postcalving diet, as the transition from pasture and silage to pasture is benign. The focus of transition cow nutrition, therefore, has been on energy nutrition and the prevention of metabolic diseases.

Energy Nutrition. Boutflour (1928) introduced the term "steaming up" cows before calving, wherein he attacked the prevailing wisdom that the way to avoid milk fever was to underfeed cows in the weeks preceding calving. He recommended that the cow's DMI be stepped up during the 6 wk before calving and "in the last fortnight before calving a sufficient quantity be given to thoroughly prime the cow." The effect of precalving feeding level was evaluated in grazing cows in the 1940s (Lees et al., 1948) and 1960s (Hutton and Parker, 1973) over multiple seasons; both reported benefits to greater DMI before calving compared with feed-restricted cows. However, in both of these experimental programs, the difference in precalving DMI was extreme and for extended periods. For example, the feeding treatments imposed during the nonlactating period by Lees et al. (1948) resulted in a 57-kg difference in BW between treatments. Considering the expected difference in BCS associated with such extremes in feeding, it is hardly

surprising that the high plane of nutrition resulted in 12 to 29 kg more milk fat/cow and that they were easier to get in calf. It is noteworthy, however, that the incidences of milk fever and grass staggers (i.e., hypomagnesemia) were also greater in this group. Hutton and Parker (1973) similarly compared extreme feeding treatments before calving; their treatments resulted in a difference of 0.7 kg of BW gain/d during the final 4 wk before calving. The high plane of nutrition resulted in a 15 to 21% increase in milk fat yield during the first 8 wk of lactation. Despite the extremes in feeding level and the lack of information on cow BCS, these studies served to support the recommendation of Boutflour (1928) that cows needed to be steamed up before calving. In situations where cows were fed only conserved silage during the winter months, the same problem can arise, because silage quality is often inadequate to meet the cow's precalving requirements for energy and sometimes, protein; as a result, increased feed allowance and even concentrate supplementation of precalving cows is sometimes recommended (Keady et al., 2001).

Such a recommendation can be problematic in a seasonal-calving grazing system, because pasture growth is less than herd demand and, as a result, pasture availability is scarce, and cows are often a distance from equipment used for feeding. In trying to define the optimum precalving feeding level for grazing cows, Roche et al. (2005) reported an interesting finding: there was no difference in milk production between herds of cows offered 75, 100, or 125% of daily ME requirements during the month before calving, and a 50% restriction only marginally reduced milk production (4 kg of fat and protein). Furthermore, BW and blood metabolic profiles indicated that the severity of the postpartum negative energy balance increased with precalving feeding level and that the risk of hypocalcemia increased. Further research confirmed these findings (Roche, 2007). Roche et al. (2015) hypothesized that the discrepancy between the historical and recent studies may relate to calving BCS, because formalized systems to define calving BCS were not available until the 1970s and 1980s (Lowman et al., 1976; Mulvany, 1977; Wildman et al., 1982; Robins et al., 2002; Macdonald and Roche, 2004). Roche et al. (2009a) had previously defined the optimum calving BCS for production, health, and reproduction. Consistent with the hypothesis, Roche et al. (2015) reported an interaction between precalving BCS and precalving feeding level such that optimally conditioned cows benefitted from a 10 to 20% restriction in ME intake during the month before calving, but thinner cows did not. Further research (Roche et al., 2017a) by this group also noted that restricting cows to less than 65% of ME requirements resulted in an exacerbation of the peripartum chronic inflammation and reduced milk production. Therefore, the difference between historical recommendations and those reported recently would appear to relate to the degree of precalving restriction and the BCS state of the cows undergoing the feeding level treatments. Nevertheless, in all cases, increased precalving DMI was associated with a greater risk of milk fever at calving.

Preventing Metabolic Diseases. Although grazing cows can be hyperketonemic, this is primarily of ruminal origin if the cows are not over-conditioned (Roche et al., 2013). The primary metabolic disease affecting grazing cows is hypocalcemia and its clinical manifestation, milk fever. Milk fever was a very serious issue in pasture-based systems until the 1970s (Roche and Berry, 2006), at which point the introduction of precalving magnesium supplementation greatly reduced the risk. However, although prevalence is low in pasture-based systems, up to 30% of cows on some farms can still be affected. Therefore, solutions are always being sought by farmers with problems. Altering the DCAD before calving has been a very successful strategy for preventing milk fever in TMR systems (Goff and Horst, 1997), wherein the base DCAD is low (~20 mEq/100 g of DM) and the diet can be tightly controlled. When evaluated in pasture-fed cows (Roche et al., 2003a,b, 2007a), the acid-base biochemistry and the increase in urinary calcium output were similar to those reported in TMR-fed cows. However, to achieve these biochemical changes, DCAD had to be <0 mEq/100 g of DM (Roche et al., 2003b), and this was a practical problem. In grazing systems, the base DCAD can be 2 to 3 times that reported in TMR systems (Roche et al., 2000) and there is no way to determine the DCAD selected by the cow in the paddock. Therefore, if DCAD were to be used in grazing systems, DMI of pasture would have to be minimized and the cows fed a mixture of low-DCAD feed ingredients and anionic salts. The increased capital requirements for feeding, not to mention the need for non-pasture-based feeds, would unduly complicate the feeding regimen and feeding infrastructure of grazing dairy systems. Although biochemically effective, the DCAD concept has been, essentially, branded impractical for the prevention of milk fever in grazing systems.

Classical milk fever occurs within 24 h of calving (Roche and Berry, 2006) when the drain of blood calcium for colostrogenesis is not replenished sufficiently. The incidence of milk fever–related recumbent cows was estimated at >10% in pasture-based systems until the late 1970s (Roche and Berry, 2006), but, by 2001, the incidence had declined to ~1% in a survey of commercial farms (McDougall, 2001). Even with prepartum magnesium supplementation, however, milk fever incidence on individual farms could still affect ~30% of cows. In general, the problem was solved with the

additional supplementation of finely ground limestone (i.e., calcium carbonate) to cows at each of the first 4 milkings after calving (Roche et al., 2003a). Pragmatic ways to supplement grazing cows were developed by farmers; pasture was "dusted" with magnesium oxide pre- and postpartum and with calcium carbonate in the fresh cow paddock (i.e., the first 4 d after calving). This combination has proved to be a very effective control strategy for milk fever.

Despite these effective control strategies for classical milk fever, a non-classical milk fever became apparent in the 2000s and was associated with over-conditioning and, in many cases, low blood P. Most of the temperate grazing systems are located >35°N or >35°S, which means that very little vitamin D is produced naturally. This means that very low phosphorus diets result in a rapid decline in blood P, which can result in a complicated form of milk fever. Its incidence is generally in systems grazing root crops that are low in P, and it is often associated with over-conditioning and excessive energy intake before calving. Precalving supplementation with dicalcium phosphate and an energy-restricted diet in the weeks preceding calving has addressed the majority of non-classical milk fever, although oral vitamin D supplementation is necessary in certain situations (Goff, 2006).

In summary, the incidence of periparturient health disorders in grazing systems is low (McDougall, 2001) and, over the last 50 yr in particular, an awareness of the primary factors involved has led to almost irreducible levels at a national level. Most control strategies involve magnesium supplementation from several weeks before calving (Roche et al., 2002; Roche and Berry, 2006) and calcium supplementation immediately after calving (Roche et al., 2003a), although a minor precalving energy restriction as well as phosphorus and vitamin D supplementation can aid prevention in stubborn cases. For the most part, sufficient manipulation of DCAD for milk fever control is impractical.

Lactating Cow Nutrition

Over the 100 yr considered in this review, research in the nutrition of grazing dairy cows has focused on maximizing milk production per cow by identifying the factor most limiting production. In recent decades, however, the system-level implications of providing supplementary feeds on the milk production of the herd and the cost of this marginal milk produced have been a considerable focus of investigation.

Although Boutflour (1928) recommended a reduction in forage-to-concentrate ratio through early lactation to ensure that the total bulk of the ration did not limit intake, he was referring to poorly digestible fiber sources, such as "undecorticated cotton cakes and inferior fodder sources." It was acknowledged early in the period considered that, in terms of nutrition, pasture could support a reasonable level of milk production. Woodward (1936) noted that the quantity of digestible protein and P consumed by the cows was consistently in excess of requirements for the projected milk production and that Ca intake was twice P intake. He concluded that immature pasture was a relatively well-balanced feed, provided DMI was sufficient for the milk produced: "if a cow will eat enough immature grass to provide the required digestible nutrients and if this grass has a normal content of minerals, her ration is not likely to be deficient in any of the essential food constituents." He concluded that the reason for the mid-season decline in milk production was a lack of feed, not a reduction in the quality of the feed on offer. These figures were in agreement with Melville and Sears (1941), who developed a novel approach to measuring digestibility in grazing animals.

The fact that grazing cows have to "work" more to acquire their feed than housed cows and that this limits their DMI has been well established. In pivotal work to understand the amount of pasture a cow could graze and the likely milk production expected from their pasture DMI, Woodward (1936) reported that cows producing ≤1 lb (0.45 kg) of milk fat could "maintain their production and BW in the spring as well without grain feeding as with grain feeding," suggesting that there was a threshold level of milk production above which grazing cows would produce more milk if supplemented with concentrate feeds. Furthermore, he deduced from feeding experiments that cows could consume enough cut pasture to maintain a milk production of 37 lb/d (17 kg/d), but DMI was often substantially less than this maximum when cows had to graze. Although not expressed specifically, the results imply an interaction between pasture mass, sward structure, and pasture DMI. When pasture was optimally presented, cows could graze 34 lb (15 kg) of DM/d of 69% DM digestibility pasture during mid lactation.

Within a grazing system, however, pasture is not always optimally presented. For example, Cooper (1941) highlighted that peak milk production occurred later in grazing cows than it should, from a physiological standpoint; instead, it coincided with the peak in pasture growth. Therefore, milk production was less because of inadequate availability and feed quality of pasture during early lactation. He also noted that the post-peak decline was not a constant, because of greater variability in both pasture supply and quality during late spring and summer. These limitations of the pasture-based system are identified as a reason to provide cows with non-pasture feeds (i.e., supplementary feeds)

to improve milk production/cow; however, McMeekan (1947) advised that this marginal milk is unlikely to be economic for the dairy farmer.

By the 1940s, evidence had amassed that pasture could sustain reasonable amounts of milk production per cow but that concentrate feeding would increase milk production beyond that achievable on an all-pasture diet. Nevertheless, the perception that pasture was not a well-balanced feed persisted. McMeekan (1947) stated, "from the qualitative angle, it is often assumed and frequently stated that pasture is not capable of supplying the needs of a high producing dairy cow and ideally needs to be supplemented with suitable concentrates." Lancaster (1947) reported on 85 digestibility trials on improved and unimproved temperate pastures and tropical pastures in different regions in New Zealand; OM digestibility in the improved pastures in spring was 78 to 82%. When tropical grasses became dominant, summer OM digestibility declined to 65%, but autumn OM digestibility returned to 80%. Unimproved pastures had OM digestibilities between 73 and 80%. McMeekan (1947) used examples of herds averaging 180 to 230 kg of milk fat/cow per lactation and lifetime production records of almost 2,300 kg of fat/cow on unsupplemented pasture as proof that well-managed rotationally grazed pastures were a high quality feed. In the succeeding decades, better grazing management strategies resulted in the presentation of more vegetative and digestible swards.

Many experiments confirmed the high feed quality of these vegetative pastures (see Roche, 2017) and highlighted that any improvement in milk production from supplementing grazing dairy cows with supplementary feeds could be explained by the increased intake of ME and not a change in any other nutritional factor (Carruthers et al., 1997; Roche et al., 2010; Higgs et al., 2013). In fact, in an extreme example, Kolver and Muller (1998) transferred housed cows consuming TMR onto pastures dominated by perennial ryegrass-white clover; milk production declined almost 20%. However, 90% of the difference in milk production could be explained by DMI, energy expenditure in grazing, and the greater NE_L in milk from grazed cows because of higher milk component percentages; this was without factoring any difference between the ME content of the pasture and the TMR. Collectively, the experimental results provide no argument for substituting another feed for grazed pasture (Roche, 2017). Nevertheless, despite compelling evidence to the contrary, many advisors still believe that there are nutritional advantages to providing cows with sources of NSC, in particular, and even sources of undegradable dietary protein when milk production is not limited by a deficiency of MP or a particular amino acid (Roche, 2017).

Responses to Supplementary Feeds

In the early 1940s, average milk production/cow was equivalent in New Zealand, the United Kingdom, and the United States (Cunningham, 1942). But after World War II, a divergence in production philosophy between systems predominantly based on grazed pasture and those increasingly using cut forages and concentrate feeds became evident. The latter became focused on ensuring the cow was maximally fed, whereas there was a recognition in grazing research that to optimize the utilization of pasture, there may be a "possible cost to the animal" (Melville and Sears, 1941; Mott, 1960). Hamilton (1942) credited the ability of grazing systems to rely almost exclusively on pasture to the development of refrigeration, which has allowed seasonal production of milk; he acknowledged, "the season curve of dairy production follows very closely the curve of pasture production." However, like Cooper (1941), he also acknowledged that milk production was compromised by a lack of available feed during summer and autumn.

The recognition that milk production was compromised by a lack of pasture availability at key times of the year resulted in considerable research efforts into investigating milk production responses to supplementary feeds in grazing systems. Hamilton (1942) referred to the adage that "half the breeding goes down the throat" and noted that high production/cow can only be achieved "when nutrition is kept on a uniform high plane throughout the year." He claimed that the 36% improvement in milk fat production between 1916 and 1940 (79 to 108 kg of milk fat) was a result of the shift to the Jersey breed, superior pasture management, and the increased conservation of hay and silage (the area conserved as silage or hay had increased 400% between 1920 and 1940), acknowledging that farmers were focused on the provision of feed for periods when pasture growth was less than herd demand. In fact, he reported that there was a close relationship between the area saved for hay and silage and production per cow the following season. He also estimated that the amount of hay and silage conserved could be easily doubled. In summary, Hamilton (1942) identified that although vegetative temperate pastures were a well-balanced feed, there were periods of the year where reduced supply compromised milk production, and supplementary feeds could help overcome the deficits and increase milk production/cow.

In addition to the lack of pasture availability, in many situations, it was also expected that the physical bulk of forages and the limited capacity of the ruminant gastrointestinal tract were dominant factors limiting pasture DMI (Boutflour, 1928; Ellis, 1978) and that the provision of a source of highly digestible concentrated

fiber (e.g., sugar beet pulp) or NSC (e.g., corn grain) would increase energy intake by the amount of energy contained in the concentrate. In theory, therefore, 1 kg (DM) of concentrate should result in approximately 2 kg of milk because of the energy contained. In practice, however, the mean response to concentrate supplementation is much less. The discrepancy between theoretical and actual marginal milk production responses is due to (1) a reduction in pasture intake (i.e., substitution) when supplementary feeds are consumed (Leaver, 1985; Stockdale, 2000; Bargo et al., 2003); and (2) a proportion of the consumed energy being partitioned to BCS (Roche et al., 2009a).

Evaluation of concentrate supplementation in grazing systems is reported as far back as the early 1940s (Riddet and Campbell, 1943). Several reviews outline a change in milk production responses to concentrates over time (Journet and Demarquilly, 1979; Gleeson, 1984; Leaver, 1985; Stockdale, 2000; Bargo et al., 2003). Leaver (1985) reported that marginal milk production responses to concentrates in the 1950s and 1960s were 0.4 kg of milk/kg of concentrates. This response is consistent with the response reported by Journet and Demarquilly (1979), and Gleeson (1984) reported similar responses to molasses offered during autumn (0.3 kg of milk/kg of molasses) but no additional milk production to either molasses or barley when cows had an adequate pasture allowance in spring. Stakelum et al. (1988) reported average responses of 0.5 kg of milk/kg of concentrates from a series of experiments undertaken in the early 1980s. However, this response varied from 0.13 to 0.98 and was almost always higher in autumn than in spring. The effect of season on the marginal milk production response to concentrates is well documented (Stockdale, 2000). Reviews by Stockdale (2000) and Bargo et al. (2003) indicate that the response to concentrates increased to 1 kg of milk/kg of DM concentrates (i.e., 0.9 kg of milk/kg of concentrates) during the intervening years, and Horan et al. (2005b) and Roche et al. (2013) reported that total responses were 1.1 to 1.2 kg of milk/kg of concentrate DM (or 1 kg of milk/kg of concentrate). The results indicate that responses to supplements have increased over a half-century of intensive investigation. This is because genetic selection priorities for milk production haves reduced the substitution rate and increased the partitioning of energy directly to milk at the expense of BCS.

The phenomenon whereby cows refuse some pasture following the consumption of an alternative feed is referred to as "substitution" and it has been well researched since the 1970s (Stockdale, 2000). However, evidence linking nutrient intake with feeding behavior was identified much earlier. Atkeson et al. (1942) identified that grazing time declined with increasing pasture digestibility; satiation was acquired more quickly when the energy density of the feed was greater and the cow ceased to expend energy grazing. Confirming these findings, a series of excellent experiments in rodents in the 1940s and 1950s identified regions of the brain responsible for intake regulation (see Roche et al., 2008) and that these regulatory regions were sensitive to nutrient intake. Roche et al. (2007b) provided evidence for such a neuroendocrine basis for substitution in grazing dairy cows when they reported that the concentration of ghrelin, a circulating hormone that signals the hunger status of the animal to the brain, declined after cows consumed concentrates in the milking parlor.

Macdonald et al. (2008b) reported a greater response to supplementary feed in New Zealand Friesian cows from a 1990s genetic strain than a 1970s genetic strain and an even greater response from North American Holstein-Friesian type cows selected exclusively for milk production. These cows were heavier and base ghrelin concentrations had increased, confirming that genetic selection had increased cow BW and levels of hunger, and increased the DMI point at which cows would succumb to satiety signals (Roche et al., 2006d). Consistent with these findings, Linnane et al. (2004) reported less of a reduction in the time spent grazing in Dutch Holstein-Friesian cows selected exclusively for milk production compared with New Zealand Friesian cows selected in a multi-trait index that also considered functional traits.

Another reason for the increase in the marginal milk production response to supplementary feeds was a reduction in the amount of consumed energy being partitioned to BCS gain. With heavy emphasis on genetic selection for milk production, less consumed energy is partitioned to BCS and is used instead for milk production (Roche et al., 2006b; McCarthy et al., 2007; Macdonald et al., 2008b). The physiological basis for this was reported by Lucy et al. (2009), when they reported that genetic selection altered the coupling of the somatotropic axis. Collectively, the results reported since the 1960s imply that the immediate milk production response to supplementary feeds has increased, but the deferred response has probably diminished. In summary, the marginal milk production response to providing grazing cows with concentrate feeds has doubled, from about 0.5 to 1.1 kg of milk/kg of concentrate DM between the 1960s and 2017. Interestingly, Macdonald et al. (2017) noted that this response was not affected by feed type, with the response directly related to the amount of ME in the supplementary feed (i.e., 7.5 g of fat and protein per MJ of ME).

Using Pasture as the Forage Source in a Mixed Ration

In systems with greater variability in pasture supply due to unpredictable rainfall or high summer temperatures (e.g., Australia, United States), a focus of recent research has been on the incorporation of pasture as the forage source to a mixed ration or high concentrate levels "slug-fed" in the milking parlor (Auldist et al., 2013). From a nutrition perspective, cow requirements can be modeled through semi-mechanistic models such as the Cornell Net Carbohydrate and Protein System (Fox et al., 1995). But at a system level, the need to calve or breed seasonally is less important because pasture constitutes such a low proportion of the cow's diet that stocking rates are much higher than would be considered in systems where pasture is the predominant nutrient source. However, the utilization of pasture grown remains paramount to the financial success of these systems and so the agronomic practices that maximize pasture growth and utilization already discussed are equally applicable. Nevertheless, the effects of such high stocking rates in conjunction with the importation of N and P in feed require consideration, particularly in sensitive river catchments and where the population of cows is dense.

Offering Supplementary Feeds on the Basis of Milk Yield

With the greater focus on per cow milk yield after World War II and technology breakthroughs that facilitated individualized feeding of cows, there was a great interest in customizing the pattern of concentrate allocation to grazing dairy cows (Leaver, 1988) as recommended by Boutflour (1928). The general belief was that higher-yielding cows would benefit more from supplementary feed than lower-yielding cows and so cows received incremental increases in concentrate allocation with increasing milk yield. Unfortunately, however, the experimental results over the last 50 yr indicate that there is no advantage in the individualized feeding of cows (i.e., feed to yield) compared with feeding every cow in the herd the same amount of supplement (flat rate feeding; Hills et al., 2015). The lack of effect is believed to be a result of substitution; although high-yielding cows have lower substitution rates than low-yielding cows (Linnane et al., 2004), this tendency results in a greater availability of high quality pasture for the high-yielding cows when all cows receive the same amount of supplement. Herd DMI is not, therefore, increased by individualized feeding and so there is no advantage in milk production relative to flat-rate feeding (Hills et al., 2015).

SYSTEMS RESEARCH

A unique feature of the science undertaken in grazing systems during the last 60 yr in particular has been the use of farmlet comparisons, wherein a particular strategic component of the system has been varied and the system implications of the change investigated (e.g., stocking rate's effect on production, profit, and environmental footprint; Macdonald et al., 2008a, 2011; Roche et al., 2016).

Although, in the strict scientific sense, these experiments are rarely replicated (i.e., the experimental unit is the cow), they are generally undertaken over many production seasons and, as such, are replicated in time and can be analyzed for consistency of response with time. McMeekan, in his landmark publication *Grass to Milk* (McMeekan, 1960), insisted that, in his experience, "it is more valuable to repeat any grassland experiment involving livestock over several years than to have several replications running in the one year." These types of experiments have played a major part in the evolution of the intensive grazing system.

Although agronomical and grazing management research has been undertaken in many universities and research stations worldwide, 2 research stations in particular have become synonymous with farm systems experimentation and the development of the modern grazing systems: Ruakura, in New Zealand, and Moorepark, in Ireland. The Ruakura Experimental Station was established in 1901, but its focus was primarily teaching and instruction until 1939, when a severe outbreak of facial eczema compelled the government to provide £17,000 for equipment and facilities to study the disease (Scott, 1997). [Facial eczema is a disease caused by a toxin (sporidesmin) produced by the spores of the fungus *Pithomyces chartarum* that can grow on pasture during warm and humid conditions, particularly during summer.] In 1943, McMeekan became superintendent and, from then, Ruakura became a name immediately recognized with grazing systems research. A similar government-sponsored research initiative was established in Ireland in the 1950s. Ireland was an economy very much dependent on agriculture but until the 1950s it was reeling from the effects of colonialization (12th to 20th century), World War I, civil war (early 1920s), the Great Depression (late 1920s-early 1930s) and an "economic war" with England (mid-1930s), and the restrictions associated with World War II. An Fóras Talúntais (later Teagasc) was established in 1959 and the Moorepark research station was dedicated to research in dairy production and milk harvesting research. Through the second half of the twentieth century, both Ruakura and Moorepark

became international focal points for the development of pasture-based systems of milk production.

Many strategic components of the farm system have been evaluated, including grazing technique (i.e., rotational grazing vs. set-stocking), stocking rate, cow breed and genetic strain within breed, the system implications of nitrogen fertilizer, and supplementary feeding, and the interactions between many of these variables.

Rotational Grazing

Systems research began in earnest with a 12-yr experiment to evaluate the benefits of "controlled" grazing (i.e., what we now know as rotational grazing) versus "uncontrolled" grazing (i.e., set-stocked) at Ruakura in 1945. This subject was to be the focus of farm systems experimentation in New Zealand and, subsequently, Ireland for nearly 20 yr (McMeekan and Walshe, 1963). Furthermore, variations of it have been investigated in component research in many other countries (Bryant et al., 1961a,b). Initially, what McMeekan (1960) described as the "relatively primitive technique" of set-stocking was compared with "the system characterized by orderly disorder" (controlled rotational grazing) but at the same stocking rate (i.e., number of cows/ha). After 12 yr, McMeekan was forced to concede little if any difference in pasture or animal production between the grazing methods, which was not consistent with the adoption rate of the technique in increasingly intensified grazing systems.

At the 7th International Grassland Congress, which was held at Massey University in New Zealand, his experiment and conclusions were heavily criticized "from all quarters of the world" (McMeekan, 1960). Critics insisted that rotational grazing, which had its basis in the grazing and resting of pasture, must result in the production of more feed and that he had restricted the potential effect by failing to impose an adequate stocking rate that would allow rotational grazing to express its superiority (McMeekan, 1960). Although he argued that no one before that conference had argued a need to increase stocking rate to take advantage of the technique, in collaboration with Michael Joe Walshe from Moorepark, Ireland, he established what was to become the defining farm systems experiment on grazing techniques (McMeekan and Walshe, 1963). Over 4 "complete production seasons," rotational grazing was compared with set-stocking at 2 stocking rates in self-contained farmlets. Although stocking rate had the greatest effect on pasture utilization and milk fat production per hectare, rotational grazing out-performed set-stocking. The effect was greater at the high stocking rate and the effect increased with each successive year of the experiment. They concluded that the optimum stocking rate was 5 to 10% higher for rotational grazing than for set-stocking.

In successive decades, rotational grazing continued to evolve, with component studies that helped researchers better understand the factors influencing timing of grazing, length of the rest period, and optimized post-grazing residual for both the sward and the animal. The foundation for the technique, however, was laid in an intensive 20-yr period of investigation between 1942 and 1962. However, the final nail in the "set-stocking system coffin" was yet to come.

Cow Genetics × Management Interaction

The use of high stocking rates was deemed by many as not being applicable to the industry of the 1960s because it was believed that dairy cows used in the experiments "could hardly be considered representative of the average dairy cow in the industry" (Carter, 1964). Research cows had been bred by AI to superior sires for more than 15 yr. It was suggested that if animals of average productive ability had been used they "would have been dry by Christmas" under the severe treatments imposed (K. A. Macdonald, personal communication). To address this concern, one of the original genotype × environment experiments was established at Ruakura in the early 1960s. The objectives of the experiment were 3-fold (Carter, 1964):

- to continue the comparisons of rotational grazing versus set-stocking under 2 stocking rates;
- to obtain a measure of the actual superiority achieved through AI; and
- to determine whether the results already obtained with the "superior" cows would also apply to the average dairy cow.

The general design of the experiment (Figure 7) was similar to the previous phase [i.e., comparing rotational grazing and set-stocking at either 2.35 (low stocking rate, **LSR**) or 2.95 (high stocking rate, **HSR**) cows/ha], with the added fixed effect of cow genetic merit (i.e., a 2 × 2 × 2 factorial arrangement). Half of the cows in the experiment were sourced from commercial dairy herds that used milk recording, but had not used AI (i.e., low-genetic-merit cows; **LGM**). These cows were compared with cows from the research station (i.e., high-genetic-merit cows; **HGM**). The transfer of an equal number of replacement cows from the experimental station to the contributing farms permitted additional comparisons of HGM and LGM cows in a range of environments.

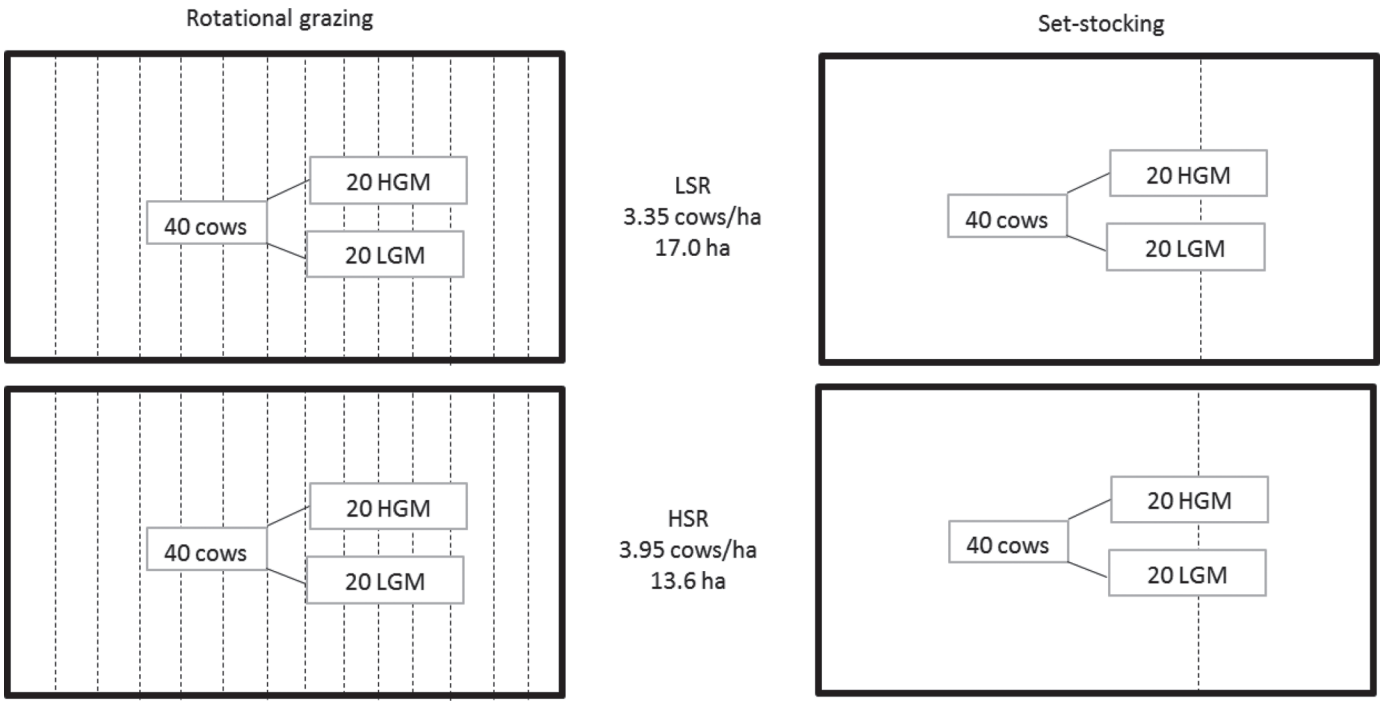

Figure 7. Design of the genotype × environment comparison experiment undertaken at Ruakura (New Zealand) over multiple years during the early 1960s. The experiment investigated the interaction between 2 stocking rates (high and low, HSR and LSR), 2 cow genetic merits (high, which was sourced from a research herd undertaking AI for more than 15 yr, and low, which were sourced from commercial dairy farms that did not use AI; HGM and LGM) under 2 grazing strategies (i.e., rotational grazing and set-stocking).

The first 3 yr of results were presented by Carter (1964). Considering the complexity of the treatment arrangement, the results were groundbreaking. In all potential comparisons, the HGM cows reflected their genetic superiority; on average, they out-produced LGM cows by 24.7 kg of milk fat/cow per year. Interestingly, this effect did not vary with stocking rate, indicating a lack of genotype × feeding level interaction, and that feeding levels/cow did not need to be modified to achieve the advantage of genetic selection. Because of the multiplier effect of stocking rate, the advantage of HGM over LGM increased from between 52 to 71 kg of milk fat/ha per year, with a 0.6 cow/ha increase in stocking rate investigated.

Confirming the previous study (McMeekan and Walshe, 1963), the negative effect of stocking rate on milk fat yield/cow was less and the positive effect on milk fat yield/hectare was greater in the rotational grazing system than in the set-stocked treatment. The increase in milk fat yield/hectare from increasing stocking rate from 3.35 to 3.95 cows/ha was 54 kg of milk fat/ha greater in the rotational grazing system than in the set-stocking system. Furthermore, the advantage of rotational grazing in milk fat production/cow and per hectare was 30% greater in the HGM cows than in their LGM counterparts.

This experiment was to end the debate on whether rotational grazing was superior to set-stocking among all but a few grazing enthusiasts. The advantage of rotational grazing increased under higher stocking rates and with cow genetic improvement, both system-level factors being avidly encouraged and, subsequently adopted. In grazing research, no systems-level modification has come close to equaling the effects of adopting rotational grazing and increasing stocking rate in increasing the production and utilization of pasture/hectare and conversion of that feed to milk.

Stocking Rate

Stocking rate is defined as the number of cows/hectare or as BW/hectare to account for the lower maintenance demands and production capacity of smaller cows compared with larger cows. It is often regarded as the most important strategic decision for a grazing dairy farm, because it influences the amount of pasture consumed and milk produced per hectare (McMeekan, 1960; Macdonald et al., 2008a; McCarthy et al., 2010). In fact, McMeekan (1960) was convinced that "no more powerful force for good and for evil existed than control of the stocking rate in grassland farming." By this, he was acknowledging the existence of an optimum stock-

ing rate, that maximized pasture utilization and milk production/hectare for the long term; too high a stocking rate would result in the "destruction" of pastures, as was often the case with native pastures "in most countries of the world" (McMeekan, 1960).

Nevertheless, McMeekan was a staunch advocate for stocking at "heavy rates" to ensure that animal and pasture productivity were maximized. Subsequent research by many, including C. P. McMeekan, A. R. Bryant, D. Browne, A. Campbell, D. McCarthy, P. McFeeley, C. W. Holmes, D. A. Clark, K. A. Macdonald, W. J. Fulkerson, J. W. Penno, L. Delaby, and P. G. Dillon, led to the development of decision rules that facilitated the dual aims of high stocking rates to optimize pasture utilization and minimizing risk to the cow associated with climatic variability and associated pasture growth (Macdonald and Penno, 1998).

Experiments to evaluate the effect of stocking rate began in Ruakura in the early 1940s and, by 1960, 6 major experiments had been undertaken (McMeekan, 1960). Increasing stocking rate resulted in an 8% lower milk fat yield/cow, for an increase of 0.26 cows/ha, but milk fat production/hectare increased by 17%, on average. It led McMeekan to conclude, "in using stocking rate as a weapon to increase per acre efficiency, we must accept a lower output/animal," a fact independently verified by Mott (1960). Subsequent research confirmed this apparent conflict between per-hectare and per-cow production and acknowledged that at an optimal stocking rate, dairy cows should be able to consume 90% of the pasture they would consume in an unrestricted grazing situation (Macdonald et al., 2008a; McCarthy et al., 2010). It is not clear whether McMeekan did not consider the economic return of the dairy farm in recommending higher stocking rates or whether he thought the increase in variable costs was less than the increased revenue. Nevertheless, a decline production per cow with increasing stocking rate was eventually going to undermine profitability—but at what stocking rate? The answer was to come some 50 yr later (Macdonald et al., 2011).

The need to optimize stocking rate and have a system transferable from the research station to the farm, led to the development of, arguably, the most complete stocking rate experiment ever undertaken (Macdonald et al., 2008a). During the late 1990s, they compared 5 different stocking rates in individual farmlets that were virtually self-contained (i.e., 2.2 to 4.4 cows/ha on a farm averaging pasture production of 18 t of DM/ha). Considering that the type of feed is not important at a system level (Macdonald et al., 2017), the experiment allowed the development of an index to predict the optimum stocking rate for any farm as long as the amount of feed available/hectare (i.e., pasture and supplementary feeds) and the size of the cow (as a proxy for genetic merit) were known. The index was called the comparative stocking rate (**CSR**; kg of BW/t of feed DM available), and subsequent economic modeling (Macdonald et al., 2011) identified the optimum stocking rate at 77 kg of BW/t of feed DM available (i.e., without accounting for any physical wastage of feed). A subsequent reassessment of these results led to the conclusion that the optimum CSR is closer to 85 kg of BW/t of DM (Table 1) and should be higher (~90 kg of BW/t of feed DM) when supplements are used at higher stocking rates (Macdonald et al., 2017).

Table 1. Estimated optimum stocking rate (cows/ha), accounting for size of the cow (i.e., proxy for genetic merit), the amount of pasture DM produced/ha (i.e., primary feed supply), and the amount of supplementary feed imported from off-farm[1]

	400-kg cow					500-kg cow				
	Pasture grown/ha, t of DM/yr					Pasture grown/ha, t of DM/yr				
Supplementary feed, t of DM/cow	12	14	16	18	20	12	14	16	18	20
0.00	2.6 (2.7)	3.0 (3.2)	3.4 (3.6)	3.8 (4.1)	4.3 (4.5)	2.0 (2.2)	2.4 (2.5)	2.7 (2.9)	3.1 (3.2)	3.4 (3.6)
0.25	2.7 (2.9)	3.1 (3.3)	3.6 (3.8)	4.0 (4.3)	4.5 (4.8)	2.1 (2.3)	2.5 (2.6)	2.8 (3.0)	3.2 (3.4)	3.5 (3.8)
0.50	2.8 (3.1)	3.3 (3.6)	3.8 (4.1)	4.3 (4.6)	4.7 (5.1)	2.2 (2.4)	2.6 (2.8)	3.0 (3.2)	3.3 (3.6)	3.7 (4.0)
1.00	3.2 (3.5)	3.7 (4.1)	4.3 (4.7)	4.8 (5.3)	5.4 (5.8)	2.5 (2.7)	2.9 (3.1)	3.3 (3.5)	3.7 (4.0)	4.1 (4.4)
1.50	3.7 (4.1)	4.3 (4.7)	4.9 (5.4)	5.5 (6.1)	6.1 (6.8)	2.7 (3.0)	3.2 (3.5)	3.6 (4.0)	4.1 (4.4)	4.5 (4.9)
2.00	4.3 (4.9)	5.0 (5.7)	5.8 (6.5)	6.5 (7.3)	7.2 (8.2)	3.1 (3.3)	3.6 (3.9)	4.1 (4.5)	4.6 (5.0)	5.1 (5.6)

[1]Optimum stocking rates are based on a comparative stocking rate (CSR) of 85 kg of BW/t of DM of total feed available (Macdonald et al., 2008a, 2011). In a recent publication, Macdonald et al. (2017) concluded that the optimum CSR increased to ≥90 kg of BW/t of DM of total feed available when supplementary feeds are fed; stocking rate at CSR = 90 is presented in parentheses.

During the last 20 yr, the effect of changes to farm management on a grazing dairy farm's environmental footprint has become increasingly important, particularly the role of the farm system in the management of fresh water through the leaching of NO_3 from urine patches. Arguably, the feature most discussed in relation to NO_3 leaching is farm stocking rate. A failure to understand the interacting dynamics of the system has led to the inaccurate conclusion that nitrate leaching per hectare increases with more cows on each hectare because more cows are urinating. However, this is not necessarily true. A greater stocking rate but with the same amount of N being imported results in a greater N export in milk, less surplus N/cow per day, a greater spread of urinary N, a reduction in the number of cows milking during the most sensitive months for nitrate leaching, and a reduction in the size of the positive N balance/cow (Roche et al., 2016). All of these are likely to affect NO_3 leaching/ per hectare.

In support of this, Huebsch et al. (2013) reported a reduction in the amount of NO_3 leached into a vulnerable limestone aquifer over an 11-yr period, despite a 10% increase in stocking rate; this longitudinal study highlighted the complexity of how management factors interact to influence NO_3 leaching. In a more controlled fashion, McCarthy et al. (2015) reported that stocking rate had no significant effect on soil solution concentrations of NO_3, nitrite, ammonia, or total N. An evaluation of their treatments using an N-balance model indicated that the increased grass utilization and milk production per hectare at higher stocking rates resulted in a reduction in N surplus and increased N-use efficiency. In comparison, Roche et al. (2016) reported a linear decline in NO_3 leached/hectare with increasing stocking rate. They hypothesized that this was most likely because lactation length was reduced with increasing stocking rate, thereby reducing the N surplus in each urine patch during the most sensitive time of the year for NO_3 leaching. This would reduce the amount of NO_3 moving below the root zone. However, they also acknowledged the greater export of N in milk through increased pasture utilization and, like McCarthy et al. (2015), suggested that this could influence the N budget away from environmental losses.

In some cases, NO_3 leaching increases with stocking rate in grazing systems. However, this is generally a function of feed importation and an increase in dietary N supply/hectare in association with the increased stocking rate (Ledgard et al., 2006) and not because of the stocking rate per se. With feed importation, the amount of imported N increases and at least some of this will be excreted in urine. More importantly, however, the timing of this feed use, and N excretion, often coincides with the period of greatest risk for surplus NO_3 escaping below the root zone of pasture (Roche et al., 2016). Full system analyses must be undertaken to understand the place for and cost of supplementary feeds in grazing dairy systems and their implications on the farm's environmental footprint.

Breed and Genetic Strain Comparisons

In addition to the comparison of HGM and LGM cows under rotational grazing systems and different stocking rates, several experiments have been undertaken evaluating dairy breeds and genotypes within breed from different countries (i.e., germplasm that developed under different production systems).

Jersey–Friesian Comparisons. Ahlhorn and Bryant (1992) reported that although Jersey cows produce less fat and protein yields/cow than Holstein-Friesian cows, when stocking rate was adjusted to reflect the BW differences (i.e., the same CSR but more Jersey cows/hectare), the Jersey cow produced as much milk, milk fat, and milk protein per hectare at 80 kg of BW/t of feed DM, and significantly more milk, fat, and protein per hectare at a 100 kg of BW/t of feed DM CSR. As the shift in genetics was increasingly toward Holstein-Friesian, the transitional animal (i.e., the Jersey-Holstein-Friesian crossbred) was reported to be 16% heavier and, although they produced 16% more milk than the purebred Jersey comparison, they only produced 5% more fat and 9% more protein (Campbell, 1977). Nevertheless, as has been previously discussed, there was an insatiable drive to use Holstein-Friesian sires to increase milk production/cow.

In recent years, with the increased fertility problems with North American-derived Holstein-Friesian strains, there has been renewed interest in the Jersey cow's role in grazing systems (White et al., 2002; Prendiville et al., 2009; Beecher et al., 2014) and in particular Holstein-Friesian × Jersey crossbred animals (Buckley et al., 2014). Prendiville et al. (2009) reported that Jersey cows required 7 to 8% less total feed for every kilogram of fat and protein produced in a pasture-based system compared with Holstein-Friesian cows. These results are consistent with the reported differences in the mass of the gastrointestinal tract (i.e., 24% lighter in Jersey cows; Beecher et al., 2014), a 2 to 3% greater digestibility of DM and NDF by Jersey cows (Beecher et al., 2014), and greater use of consumed ME for productive purposes by the Jersey cow (L'Huillier et al., 1988) compared with Holstein-Friesian cows. The improvement in the efficiency of ME use, however, was only apparent in a grazing environment with restricted DMI, where Jersey cows produced 20% more milk/kg of DMI (L'Huillier et al., 1988); under ad libitum feeding, this ME conversion advantage disappeared. In ad-

dition, White et al. (2002) and Washburn et al. (2002) reported less mastitis and superior reproduction performance in Jersey cows in the United States compared with Holstein-Friesian cows, but an effect of breed on mastitis or SCS was not reported by Prendiville et al. (2010). Nevertheless, based on their superior feed conversion efficiency, particularly under limited feed availability, the Jersey breed may have an advantage over the Holstein-Friesian in low-input grazing systems. As Arnold Bryant is reputed to have said while summarizing the results of his experiment in the early 1990s, "if you're short of labor, milk Friesians; but, if you're short of land, milk Jersey." (A. M. Bryant, personal communication).

Crossbreeding Crossbreeding of pure Holstein-Friesian cows with bulls from breeds with superior fertility was a farmer-driven initiative in the 1990s to improve reproductive outcomes more quickly than selecting within a single breed. The results, where quantified, have been impressive (Buckley et al., 2014). Research conducted in Ireland during the early 2000s concluded that there was an 8-percentage-point increase in pregnancy to first service and an 11-percentage-point increase in pregnancy after 6-wk breeding in the Norwegian Red × Holstein-Friesian compared with pure Holstein-Friesian. A further study confirmed these results, with pregnancy to first service increasing by 21 percentage points and percentage of cows pregnant after 6-wk breeding increasing by 19 percentage points in Holstein-Friesian × Jersey crossbreds compared with purebred Holstein-Friesian cows. Similarly, in the United States, an analysis of commercial data indicated greater first-service conception rates for Scandinavian Red × Holstein-Friesian (+6 percentage units) and Montbéliarde × Holstein-Friesian (+10 percentage units) compared with purebred Holstein-Friesian cows (23%). In New Zealand, crossbred dairy cattle (primarily Jersey × Holstein-Friesian) are achieving similar rates of genetic gain for farm profit as the purebred populations, but have created additional gain derived from economic heterosis (Buckley et al., 2014). Crossbreeding was yet another technology spearheaded by farmers, with the benefit quantified and subsequently extended by science.

Evaluating Alternative Germplasm—Genotype × Diet Interactions. Until the 1990s, the accepted paradigm, globally, was that the performance of dairy cow genetics was not influenced by environment. This might be the most heavily researched topic in grazing farm systems during the last 30 yr.

A 3-year study in Ireland, completed in 1977, compared 19 successfully imported Canadian Holstein-Friesian heifers against 25 commercial Irish Friesian heifers and 25 pedigree-registered Irish Friesian heifers; the study revealed that the Canadian animals yielded 23 to 28% more than the Irish counterparts (Cunningham and O'Ferrall, 1977). In 1984, the Canadian-New Zealand genotype-by-environment study (CANZ) began (Peterson, 1988). The study involved using 20 Canadian and 20 New Zealand Holstein-Friesian bulls as AI sires for cows in 20 New Zealand and 10 Canadian herds in a 2 × 2 factorial arrangement. Holmes (1995) reported that there was no interaction between the origin of the sires and the daughters' environment, but that there was a significant interaction between individual sires within strain and daughters' environment, such that the correlations between the sire proofs in the 2 countries were approximately half of the expected values.

These results were inconsistent with the previously reported lack of effect of stocking rate on the realization of genetic potential for milk production (Carter, 1964) but in agreement with subsequent Australian research that identified a scaling effect of genetic merit with feeding level (Fulkerson et al., 2008). Fulkerson et al. (2008) reported that grazing cows only expressed the milk production difference predicted from their breeding values when offered ≥0.8 t of DM/cow of concentrates and the actual milk production difference when cows were offered ~0.3 t of DM concentrates was only 55% of the difference predicted by their genetic breeding value. Bryant et al. (2007) also predicted this scaling effect using a mechanistic model of the mammary gland; in their simulations, the benefits of increases in genetic merit were greater at high feeding levels and this was a result of a greater total number of mammary alveoli produced from conception until the end of lactation.

Holstein-Friesian germplasm from farm systems that do not involve grazing or seasonal calving were increasingly used through the 1980s and 1990s because of their superior milk production ability, as previously discussed. To evaluate the effects of such radical changes on the cow and the grazing system, several multi-year farm systems experiments were undertaken around the world (Buckley et al., 2000; Kennedy et al., 2002; Kolver et al., 2002; Horan et al., 2005a,b,c; Roche et al., 2006a; Macdonald et al., 2007, 2008b). Without exception, these studies reported reduced reproductive performance in the genetic strain originating from nongrazing systems. On average, the 6-wk in-calf rate, a key metric of reproductive performance in grazing systems, was 15 percentage points less in the genetic strain that had evolved in nonseasonal systems. Furthermore, there was little advantage in feed conversion efficiency to these genetics in grazing systems. Although there were apparent production advantages in systems using large amounts of supplement, due to low levels of substitution (Linnane et al., 2004) and greater immediate marginal milk production responses to imported feeds

(Kennedy et al., 2002; Horan et al., 2005b; Macdonald et al., 2008b), the greater negative energy balance in early lactation and the failure of the cow to partition toward BCS replenishment during lactation (Roche et al., 2006a; McCarthy et al., 2007) resulted in a very similar full lactation feed conversion efficiency. These experiments quantified the effects of the genotype × diet interaction in grazing systems and accelerated the change to multi-trait indices that focus considerable weighting on important functional traits for grazing dairy cows.

Within a Single Germplasm—Exploiting Natural Variation. Although the Canadian-New Zealand genotype × environment trial did not identify a germplasm source × farm system interaction, an interaction between sires within strain and their daughters' environment was detected. This result would support the ability to select within germplasm for desirable traits. In the 1990s, Colin Holmes and his team at Massey University undertook an interesting study comparing cows differing only in BW. They selected 2 lines of Holstein-Friesian cows that were 60 to 80 kg different in their BW and compared them in a rotational grazing system. Over multiple lactations, the high BW strain offered no consistent advantages in production or efficiency and, in fact, had inferior reproduction (Laporde et al., 1998; Lopez-Villalobos et al., 2001; Tolosa et al., 2001). These results supported the negative weighting placed on BW in genetic selection for grazing cows.

Taking advantage of ability to select within germplasm for desirable traits and the substantial genetic variation for fertility traits that existed in the early 2000s in Ireland, a study was initiated at Moorepark to identify fertility phenotypes under genetic control. Cows with similar genetic merit for milk production traits but either very good (Fert+) or very poor (Fert−) genetic merit for fertility traits were identified and assembled as a single herd of animals. With a similar environment (e.g., nutritional management, health protocols, winter housing), the divergence in fertility phenotypes recorded in these 2 groups of animals was very impressive. Despite calving at similar BCS and having approximately similar milk production, Fert+ cows had earlier resumption of cyclicity, more rapid recovery of uterine health after parturition, greater BCS during lactation, more favorable blood indicators of bioenergetic status, stronger estrous expression, and a larger ovulatory follicle (and greater circulating estradiol concentrations) that subsequently resulted in a larger corpus luteum (and greater circulating progesterone concentrations; Cummins et al., 2012; Moore et al., 2014). These phenotypes collectively affected reproductive performance during the breeding period, with the Fert− cows failing to achieve fertility targets and survive in seasonal calving systems. The collective results from this investigation highlighted the importance of selecting for functional traits important to the profitability of grazing systems, such as fertility, and, for the first time, identified the fertility phenotypes under genetic control in lactating dairy cows.

Supplementary Feeding

Many multi-year farm system-level experiments have evaluated the total biological, economic, and environmental responses to intensifying the dairy system through the importation of supplementary feeds (Jonsson et al., 1999; Roche et al., 2000; Kennedy et al., 2002; Horan et al., 2005b; Jensen et al., 2005a,b; Fulkerson et al., 2008; Macdonald et al., 2008b, 2017). As previously discussed, marginal milk production responses to supplementary feeds are greatest when severe feed restrictions relative to demand result in large reductions in the milk yield of a control group (Penno, 2001). Therefore, at a system level, the way to increase the milk production response to supplements might be by increasing stocking rate or through a high stocking rate in the control treatment (Horan et al., 2005b; Macdonald et al., 2017).

In research experiments, in a scenario of low feed availability, response to additional feed is approximately 0.1 kg of milk or 7.5 g of fat and protein for 1 MJ of ME consumed, irrespective of feed type (Macdonald et al., 2017). However, estimates of the marginal production response to supplementary feeds can be much less on commercial farms; Ramsbottom et al. (2015) reported that marginal milk production responses to purchased feeds were approximately two-thirds of those achieved in research experiments. This failure to capture the full benefit of the supplementary feed has implications for the profitability of feed use, with Ramsbottom et al. (2015) reporting a linear drop in profitability with increased use of supplementary feed above 300 kg/cow. From their data set, it would appear that stocking rate was not adequately increased to provide a reasonable pre-supplement feed deficit and pasture utilization decreased.

However, even in situations where stocking rate was dramatically increased and the response to supplementary feeds was as high as expected (Macdonald et al., 2017), the increase in total costs can outweigh the milk production benefit, because most expenses increase with feed use (DairyCo, 2013; Ramsbottom et al., 2015). In grazing systems, for supplementary feeding to be profitable, milk-to-feed price ratio [i.e., milk price ($/kg of milk) relative to feed price ($/kg of DM feed)] must be greater than 1.5 when grazing residuals are 35 mm (assuming a feed containing 11

MJ of ME). Roche and White (2012) hypothesized that farmers could use postgrazing residuals to estimate the relative feed deficit described by Penno (2001). If their hypothesis is true, the milk-to-feed price ratio needs to be approximately 1.1, 1.5, and 4.5 when postgrazing residuals are ~20, 35, or 50 mm, respectively, to ensure a sufficient milk production response to the supplement to cover feed and nonfeed cost increases.

Once-Daily Milking

While much of the dairy world extended milking frequency to 3 or even more times per day (Bar-Peled et al., 1995; Erdman and Varner, 1995), the role of less frequent milking was investigated in grazing systems (Holmes et al., 1992; Clark et al., 2006). Because a high level of labor productivity (i.e., cows/labor unit) is a key objective in grazing systems because labor is one of the top 2 expense items (Ramsbottom et al., 2015) and because milk yield/cow is limited by DMI (Kolver and Muller, 1998), several researchers have questioned the need to milk cows twice daily and whether milking cows once each day or every 18 h would suffice in grazing systems with moderate yielding cows (Stelwagen et al., 2013).

Holmes et al. (1992) concluded that milking cows only once daily reduced milk production/cow but the size of the reduction was cow-dependent. These results indicated a potential opportunity to genetically select cows for suitability for once-daily milking. Clark et al. (2006) undertook a multi-year comparison, wherein they compared Jersey and Holstein-Friesian cattle milked either twice daily or once daily. They also reported a lower milk production/cow from cows milked once daily: Holstein-Friesian cows and Jersey cows produced 31 and 22% less milk and 29 and 19% less milk fat and protein, respectively. The experimental design, however, included an increase in stocking rate associated with the once-daily milking treatments (i.e., stocking rate in the herds milked once daily was 17 percentage points greater than the herds milked twice daily), and it is not possible, therefore, to separate the effect of a higher stocking rate on milk production per cow from the once-daily milking frequency effect. Nevertheless, milk production/hectare was also less, despite the increased stocking rate; Holstein-Friesian and Jersey cows produced 18 and 9% less milk/ha, on average, over the 4-yr period.

The collective results indicate that breeds or cow strains within breed with low milk volume but high component concentrations are negatively affected to a lesser degree by reduced milking frequency than are high-volume breeds. This conclusion is consistent with what we know about the feedback inhibition of milk secretion (Stelwagen et al., 2013); however, in agreement with Holmes et al. (1992), it indicates that there may be potential to select for cows suitable for once-daily milking in situations where milk component yield is a greater priority than milk volume.

WHERE TO FROM HERE? THE NEXT 100 YEARS

In the last 100 yr, we have witnessed almost unbelievable increases in milk production per hectare. Plant breeding and grazing techniques, customized soil nutrition, and the application of N fertilizer increased the amount of pasture harvested by a cow specifically bred to produce almost her own body weight in milk fat and protein from grazed pasture and successfully rebreeding within 83 d of calving. Farmers and applied scientists working in concert provided the infrastructure that facilitated a farm scale that, in terms of hectares farmed and cows per labor unit, could not have been foreseen when the first issue of the *Journal of Dairy Science* was published. However, the landscape has changed and the next 100 yr will be very different. Research in grazing dairy systems must be more than just "tinkering" at the fringes of the system with small incremental gains in productivity. It will have 3 focal points during the next century: (1) solutions to environmental and animal welfare concerns; (2) provision of technologies that increase the number of cows milked/labor unit and reduce the skill requirements of a farm worker through the use of both structured and unstructured data that can assist in decision making; and (3) improvements in the productivity of each of the components of the farm system in an integrated manner.

Social License to Farm

As with all dairy systems in developed countries, arguably the greatest challenge facing farming and agricultural science in the next century is the provision of solutions for, and evidence against, consumer concerns regarding the animal welfare record of ruminant livestock farming and our environmental footprint. As the nutritional status of people in developed countries has increased and stabilized (i.e., year-to-year volatility in the supply of foods is nonexistent in the developed world), consumers have become increasingly concerned about the methods used to produce food. This has both advantages and disadvantages for grazing systems, as consumers in many countries believe that the welfare of cows allowed to graze is superior to those without access to grazing and, increasingly, full grazing is being viewed as superior to part grazing when climatic conditions allow (Arnott et al., 2017). Nevertheless, grazing systems involve inconsistent pasture supply and nutri-

tive value profiles (Roche et al., 2009b) and, increasingly, consumers anthropomorphize their observations of animal farming. This can have negative consequences for grazing systems. The effect of periods of low DMI is a crucial question to determine the animal welfare requirements for supplementary feeding in grazing systems, as is the requirement for shade and shelter in inclement weather conditions and in different environments.

The social license to operate is decided locally and by only a small part of the consumer population in export-oriented dairy industries. This population tends to be more concerned about the environmental footprint of grazing dairies and, in particular, the effect of grazing systems on water quality and climate change. Well-managed grazing systems affect water quality primarily through the high concentration of N in urine deposited on a small area (i.e., the cow's urine patch). When pasture growth slows in autumn, the ability of the plant to withdraw this N from the soil pool is less and some moves below the root zone. When precipitation exceeds evapotranspiration in winter, this N can be lost to ground water in drainage (Roche et al., 2016). Depending on the current state of water in a region or country, this is either an immediately urgent area of research need or one that will become urgent with increasing dairy intensity in sensitive catchments.

Component solutions will include the breeding or feeding of cows for less N output in urine, breeding pastures with lower N content (% of DM), or using alternative forage species that have lower N content or possess diuretic qualities that result in an increased urine volume or a lower N content in urine. Systems-level solutions will include the investigation of interacting components for complementarity or antagonism. However, solutions must consider the achievements to date; the proverbial baby must not be tossed with the dirty bathwater. Pasture-based dairy farming is successful because of its simplicity in daily decision making and its low infrastructure and variable costs. Any change to the base forage cannot unduly complicate the management of the grazing system; changes to animals cannot undermine productivity and resilience; and system-level changes cannot substantially increase operating expenses nor increase the need for capital infrastructure. If any of these are to change greatly, the system will not be economically viable in a world with ever-declining food prices. With these boundaries considered, these challenges are no less than those faced by the men and women of 1917.

From a climate change perspective, grazing dairy farms will be affected in 2 ways. First, the emissions of CH_4 from ruminants is a large part of the greenhouse gas (**GHG**) inventory in New Zealand, Ireland, and Australia, but less of an issue in more industrialized countries. This means that international agreements to reduce GHG will affect dairy systems. Animal breeding is a potential avenue for CH_4 reduction (Pinares-Patiño et al., 2013) but this is a long-term solution and will depend on the value/cost of carbon taxes and alternative strategies for reducing the farms' GHG footprint (e.g., purchasing carbon credits elsewhere). Currently, the only way to reduce CH_4 emissions in grazing cows is to reduce the amount of feed being consumed. Although CH_4 inhibitors are being explored (Hristov et al., 2015), currently they need to be included with the feed and may not be effective in grazing systems. Further research will be required in this space and, potentially, in conjunction with biotechnologists, the breeding of pasture species with a CH_4 inhibitor contained within the cells of the plant. There is also the possibility of developing vaccines that stimulate the cow to produce antibodies against rumen methanogens (Clark, 2009). With the precautionary principle firmly in place in global governance, reducing dairy farming's contribution to GHGs will be a significant research effort in the future.

The second way in which climate change may affect dairy systems is through changes to atmospheric CO_2, which enhances pasture growth, and through projected increased temperatures or altered patterns of rainfall. Considering the absolute immediacy of the dependence (i.e., daily to weekly) of grazing systems on the climate for the provision of the cow's feed supply, research will be needed to ensure the availability of resilient plants, animals, and systems suitable to a changing or more volatile climate.

Automation and the Replacement of Decision Making

Over the last century, grazing systems were renowned for the lack of employed technology, almost as if it were a badge of honor—producing milk as nature intended without the aid of depreciating assets. Milking parlors, simple aids to detect estrus, hormone intervention for anestrus, and simple tools for measuring pasture height were the only technologies that added significant value to the management of the system. This was despite farm size increasing by more than 1,000% in New Zealand, for example, over those 100 yr. However, with scale and with increasing recognition of social responsibility issues, each decision becomes more important and yet there is less time to consider the decision being made.

With scale, fewer farming families, and smaller families, animal husbandry skills are not as expertly or easily acquired as they were in the last century. Therefore, what were simple tasks, such as the early

detection of disease or the accurate identification of cows in estrus, have become less exact. At the same time, the consumer's requirement for animal welfare management has increased and requires that farmers manage herds of cows as individuals, which will require better animal husbandry skills than historically passed down from generation to generation. This means that farmers will need near- to real-time information on each cow at his or her fingertips. Cow activity, rumen function, core temperature, changes in BW or BCS, and milk production are all easily measured with available technology. These will soon be combined with abilities to detect hormones, metabolites, and even nanoparticles in milk, all of which have the ability to contribute to the picture the farmer receives about each cow. For example, Crookenden et al. (2016) reported that the protein cargo contained in circulating exosomes in early lactation provides a picture of the metabolic health status of transition dairy cows. Exosomes are intercellular, nanoparticle messengers that can cross the blood–milk barrier. If used in conjunction with other measurable factors in milk, it will be possible to automatically draft cows for further inspection by the farmer. However, for this to occur, we need to understand the inference that changes in these biomarkers convey and, more importantly, what can be done to prevent or treat the likely animal health problem. The technology is already being developed for human medicine; however, dairy physiologists will have to work closely with researchers in the biomedical sciences to develop algorithms using machine learning technology that utilize the increasing amount of structured and unstructured data being collated about each animal. These data will enable greater phenotyping of animals and more accurate and rapid genetic selection for cow resistance to disease and resilience.

For pastures, technologies that automatically and accurately measure pasture DM yield and quality will enable improved genetic selection for economically important traits, such as non-peak DM production, digestibility, persistence, and, potentially, lower CP content, from an environmental mitigation perspective. Technologies will be developed to accurately, and without human interference, assign an area of pasture commensurate with the daily DMI needs of the cow and the immediate and projected pasture growth rate. Such virtual fencing technology is being considered by several research groups, but at the time of writing is still some way off. In particular, the technologies that will accurately measure pasture available and likely pasture growth to calculate the allowance will need considerable research efforts. But the unstructured data are already being collected on every "smart" device on the planet. This type of technology could automate one of the most time-consuming but important tasks in optimally managing a grazing dairy farm; that is, the assessment of farm pasture cover and the assignment of daily grazing area.

Productivity Improvements

The immense productivity improvements during the past 100 yr have been driven by well-designed component research studies to establish principles and farm systems research that investigated how these primary system components interact. Much of biological systems research has been overtaken by computer simulations (i.e., modeling), which offer the considerable advantage of being able to estimate effects over long periods quickly and inexpensively. However, sometimes they do not accurately represent what is observed in field experimentation or on farm, and they rarely account for the human capability interface. In the future, farm systems research will be an interaction between field experimentation and computer simulation modeling, with the field observations used to parameterize the models and provide confidence of accuracy, whereas the model will facilitate the prediction of effects under many environments and circumstances. One of the most important developments must be the development of a human capability model that will estimate poor to expert decision making and allow this information to be integrated into the biological modeling to provide the range of likely outcomes and to prioritize the actions that need to be undertaken well.

The current grazing model for production of milk will continue to make steady incremental gains in cow and feed genetics, and the rate of gain may even increase with greater knowledge of the effects of production and nonproduction traits on farm profitability. A greater ability to easily measure important phenotypes and develop associations with molecular signatures through an ever-increasing array of "-omics" technologies that decline in unit cost annually will accelerate genetic gain, even without the use of trans- and cisgenic biotechnologies, whose use to date has been curtailed by non-scientific regulations in most grazing regions. The focus in cow breeding will continue to be in quantifying functional traits that are linked to cow survival and health (e.g., fertility, mastitis, metabolic diseases), both because of their importance to profitability and because premature mortality is increasingly viewed as an animal welfare issue in animal production systems, even though losses are estimated to be 50% less in grazing systems than in housed systems (Compton et al., 2017). This will not diminish our ability to make

progress in milk production, as is evidenced in Figure 6, because voluntary removal of animals will make up a greater proportion of the total number of cows replaced and the "boarder" cow will be removed earlier than they are currently when involuntary animal removal is such a large proportion of total cows replaced.

In comparison, focus in pasture breeding will continue to shift toward more complex traits than just annual DM yield. Seasonality of pasture production—with high yields in spring, inadequate growth in summer in areas prone to moisture stress, and in winter due to low temperatures and solar irradiation—is a critical limitation to the current production system. Pasture production away from peak growth, as well as an increased focus on persistence of selected cultivar varieties, improvements in nutritive value, and any traits that could contribute to a reduction in NO_3-N leaching (e.g., increased winter growth) or methane production will increasingly be the focus of pasture breeding in the next century.

CONCLUSIONS

Through the actions of many, grazing systems have evolved from primitive, poorly performing systems that used unimproved pastures and dual-purpose cows in an extensive manner to an intensive system of grazing management with highly digestible pastures and fit-for-purpose cows, and all primarily without a large increase in capital infrastructure. The *Journal of Dairy Science* has played a pivotal role in communicating the advancements of science throughout this period and, in particular, during the last 20 yr. Necessity is truly the "mother of invention" and the history of the modern grazing system is testimony to this. The development of the electric fence, the herringbone and rotary milking parlors, and the diluents that allowed provision of low volumes of fresh semen for AI were all specific to the need for a seasonal-calving system. Multi-year farm systems experiments were unique to grazing research because the components of the systems were more inherently intertwined than in housed systems with a predictable feed supply. These experiments helped cement some of the underlying principles of grazing management and stock husbandry. The future is bright, but a shift in research resources is needed to allay consumer concerns around animal welfare and farming's environmental footprint. This will be associated with increased use of structured and unstructured data sets being automatically collected and technologies that will individualize the care and management of the dairy cow and accelerate incremental, but permanent, gains in animal genetics for functionally important traits.

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APPENDIX

Table A1. Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
1917	Where it all begins: · Herd sizes are <30 cows · Hay is the predominant conservation method for grass · Set-stocking (night and day paddocks) · Dual-purpose breeds (beef and dairy) · Primary dairy breed is Shorthorn	Atkins, 2016; Anonymous, 1924; Blaxter and Robertson, 1995; McCloy, 2014
1919	Welsh plant breeding station is established at Aberystwyth with Sir George Stapledon as director. Work focuses on breeding forages for different purposes (e.g., grazing, hay-making).	Raymond, 1981; Lazenby, 1981
1920s	Inherently low P and pH status of soils is addressed via a major drive to improve soil fertility through the addition of superphosphate and lime to increase pasture and animal productivity.	Blaxter and Robertson, 1995
1920s	A significant uptake of milk recording occurs to enable selection of cows for higher milk yields.	Anonymous, 1924; Atkins, 2016
1924	Stapledon highlighted the importance of leaf proportion for feed value; as intervals between grazings increase, the proportion of green leaf blade is reduced.	Stapledon, 1924
1927	First International Grassland Congress is held in Leipzig, Germany.	McMeekan, 1953
1928	World Dairy Congress, Boutflour proposed that the 4 factors limiting production were (1) lack of control of indigestible fiber; (2) lack of control of DMI; (3) neglect of the transition cow; and (4) infrequent milking.	Boutflour, 1928
1928–1932	Frequent defoliation is reported to result in higher concentrations of nutrients.	Woodman et al., 1928, 1929, 1931; Woodman and Norman, 1932
Early 1930s	The first electric fences are developed in the United States for livestock control.	Jones, 1988
Mid 1930s	Bill Gallagher modifies US electric fence chargers to control his horse and dairy cows.	Jones, 1988
1936	Grass growth is reported to cease when soil temperatures <5°C, and nitrification slows between 5 and 8°C. Pasture growth is increased at lower temperatures by applying N fertilizer.	Lazenby, 1981
1937	The subdivision of larger areas into smaller paddocks and utilization of rotational grazing is widespread as a means to manage growth in New Zealand.	Holford, 1937

Continued

Table A1 (Continued). Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
Late 1930s	"Young Farmers" clubs are established in New Zealand as a way to share learning and experiences.	Holford, 1937
1939	Ruakura Experimental Station converted from teaching to applied experimentation.	Scott, 1997
1930–1940s	Shift from Shorthorn cattle to Friesian in UK and Ireland and to Jersey in New Zealand.	Atkins, 2016
1941	J. F. James reported similar conception rates with AI and natural mating in research.	James, 1941
1943	C. P. McMeekan becomes superintendent (and later director) of Ruakura Experimental Station (Waikato region, New Zealand).	Scott, 1997
1944	"Macra na Feirme" established in Ireland as young farmers clubs.	http://www.macra.ie/
1945	In New Zealand, McMeekan began advocating for rotational grazing in a long-term experiment (12 yr) that compared rotational (controlled) and set-stocking (uncontrolled).	McMeekan, 1960
1946	Extremely seasonal nature of breeding season is viewed as an "insurmountable barrier" to commercial AI.	James, 1946
1948	A comprehensive review of plant carbohydrate metabolism establishes the need for a rest period between grazing events and support for rotational grazing grows.	Weinmann, 1948
1949	A debate at International Grasslands Congress identifies a greater benefit of N fertilizers in Europe than New Zealand because of a shorter growing season for legumes in Europe.	
1950	Annual pasture DM yields increase with inter-grazing interval (i.e., rotation), which leads to the hypothesis that animal production is greater under rotational grazing compared with set-stocking.	McMeekan, 1947, 1957, 1960
1950	Research facilities to investigate digestibility of pasture are established at Hurley, United Kingdom.	Raymond, 1981
1950	Tetraploid ryegrasses are bred in New Zealand.	Hunt and Easton, 1989
1951	Nitrogen fertilizer does not accelerate leaf appearance but strongly influences leaf size and tillering.	Whitehead, 1995
1952	Ron Sharp, a New Zealand dairy farmer, develops the herringbone milking parlor design.	McCloy, 2014
1955	R. W. Brougham is the first to report that regrowth of pasture following defoliation conforms to a sigmoidal or S-shaped curve.	Brougham, 1955

Continued

Table A1 (Continued). Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
1956	R. W. Brougham suggests that control of management of rotational grazing could be achieved using sward height before and after grazing.	Brougham, 1957
1956	McMeekan reports no advantage to strip grazing (offering only enough pasture for a single feed between milkings) or grazing the whole paddock.	McMeekan, 1957, 1960
1957	McMeekan reports an interaction between stocking rate and method of grazing (set-stocked vs. rotational).	McMeekan and Walshe, 1963; Carter, 1964
1959	Voisin publishes his book, *Grass Productivity*, encapsulating his thoughts on rotational grazing.	Voisin, 1959
1959	"An Fóras Talúntais" (later Teagasc) is established in Ireland and the Moorepark Research Station is dedicated to dairy production research.	
1960	McMeekan publishes landmark treatise, *Grass to Milk*.	McMeekan, 1960
1960	First grassland experiments begin at Moorepark (Ireland) comparing rotational grazing with set-stocking.	P. G. Dillon (personal communication)
1960	Autumn-saved pasture as a method of conserving pasture in situ in late autumn comes into vogue.	McMeekan, 1960
1960s	Merit testing of pasture plant varieties is conducted.	Raymond, 1981
1960	Ryegrass tillers are shown to carry an average of 3 live leaves at all spacings and nitrogen levels.	Davies, 1977; Fulkerson and Donaghy, 2001
1963	The interaction between grazing method and stocking rate is confirmed. At low stocking rates, no difference is found between rotational grazing and continuous grazing. At high stocking rates, rotational grazing allows a 10% increase in stocking rate.	McMeekan and Walshe, 1963
1963	The "unshortable" electric fence for livestock control is developed by D. Phillips; it increases the range of fencing and decreases the cost by ~90%.	Jones, 1988
1960s	Cow breeds in use continue to change. By 1970, 76% of cows in the UK are Friesian and 3% Shorthorn.	Atkins, 2016
Late 1960s	White clover is reported to have consistently higher digestibility than ryegrass.	Raymond, 1981
1969	Campbell reports an increase in pasture DM yield with rotation length.	Campbell, 1969

Continued

Table A1 (Continued). Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
1969	Hutton and Parker confirm the need to fully feed transition cows.	Hutton and Parker, 1973
Early 1970s	The substitution of supplements for pasture is quantified in many countries; grazing time declines by 15 to 22 min/kg of DM supplement eaten.	Leaver, 1985; Bargo et al., 2003; Stockdale, 2000
1973	It is established that grazing should not be delayed beyond the development of a full canopy (i.e., ceiling yield).	Davies, 1977
1973	Submission rate during the first 4 wk of seasonal breeding is identified as key management success variable.	Macmillan and Watson, 1973
1977	Ceiling pasture yield is reached after 3 leaf appearance intervals.	Davies, 1977; Fulkerson and Donaghy, 2001
1977	Tail-painting for estrus detection is first introduced.	Macmillan and Curnow, 1977
1970s	Sperm dose rate is reduced from 25 million to 1 million.	Shannon and Vishwanath, 1995
Late 1970s	Magnesium supplementation reduces milk fever in grazing cows.	Roche and Berry, 2006
1979	The spring gate is developed for electric fences, removing the last impediment to their use on farm.	Jones, 1988
1981	Cow DMI is close to maximum with 8- to 10-cm postgrazing residuals.	
1981	Endophyte is reported to cause ryegrass staggers in sheep; Lolitrem B is isolated as causal factor.	Harvey, 1983
1982	Seminal work by Bryant and L'Huillier determines how much feed should be on the farm in spring and how best to achieve this. This work leads to the development of the autumn and spring rotation planners.	Bryant and L'Huillier, 1986
1982	Endophyte is reported to protect ryegrass from insect attack.	Popay and Rowan, 1994
1980s onward	Novel endophytes are developed that offer insect protection but have fewer or no negative effects on dairy cattle.	Bluett et al., 2003; Milne, 2007
1985	Initiation of experiment comparing Canadian and New Zealand dairy sires that investigated genetics × environment interaction.	Peterson, 1988
Late 1980s	A series of experiments by Stakelum and Dillon in Ireland quantifies the longer-term effect of grazing management on pasture quality.	Stakelum and Dillon, 1991
1990	Jersey versus Friesian breed comparison experiment is undertaken.	Ahlhorn and Bryant, 1992

Continued

Table A1 (Continued). Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
Early 1990s	Farmers begin reporting poor reproduction results with modern cows. General feeling that breeding for or against fertility traits was not possible due to its low heritability.	Harris and Kolver, 2001
1991	Controlled internal drug releasing (CIDR) insert for vaginal administration of progesterone is developed.	Macmillan and Peterson, 1993
1994	Irish and Australian experiments begin to compare North American and Dutch genotypes of Holstein-Friesian with local genotypes. Comparisons continue in different formats for 15 yr in Ireland and New Zealand.	Buckley et al., 2000; Fulkerson et al., 2001; Kennedy et al. 2002; Horan et al., 2005a,b,c
1995	The importance of calving date in maximizing milk production from pasture is defined.	Dillon et al., 1995
1996	A set of management decision rules to optimize milk production in seasonal calving dairy systems is developed in New Zealand by Macdonald and Penno.	Macdonald and Penno, 1998
1997	Kolver and Muller report that primary limiting nutritional factor in grazing dairy cows is intake of metabolizable energy.	Kolver and Muller, 1998
1997 onward	The origin of metabolizable energy does not affect milk production in moderate yielding cows. Carbohydrate course (i.e., sugar, starch, or fiber) is unimportant.	Carruthers et al., 1997; Roche et al., 2010; Higgs et al., 2013
2000	Temporal changes in pasture DCAD through the year and its effect on cow urine pH defined.	Roche et al., 2000
2000	Comparative stocking rate is defined to allow optimum stocking rate to be calculated in different places.	Macdonald et al., 2008a
2000	Higher stocking rates do not increase nitrate leaching.	Roche et al., 2016; Huebsch et al., 2013
Late 1990s–2000s	Novel endophytes are developed that have insect protection but very little effect on dairy cow health and heat stress.	Milne, 2007
2003	DCAD is not a practical solution to milk fever in grazing cows because base DCAD is too high.	Roche et al. 2003a,b
2006	Effect of feed intensification on nitrate leaching is defined. Increasing the amount of feed imported from off-farm to increase stocking rate increases nitrate leaching.	Ledgard et al., 2006
2000s	Genotype × diet interaction is identified. Holstein-Friesians of North American and Dutch origin have lower 6-wk in calf rate than New Zealand Friesian cows, but have a lower substitution rate of supplement for pasture and a greater immediate milk production response.	Horan et al., 2005c; Macdonald et al., 2008b

Continued

Table A1 (Continued). Timeline of grazing research and pertinent discoveries between 1917 and 2017

Date	Milestone	Reference
2001	Grazing studies conclude that DM and milk production/hectare are maximized when pastures are grazed between the emergence of the second and third leaf on ryegrass plants and postgrazing residuals are between 3.5 and 4.0 cm. Subsequent studies defined these key criteria for orchard grass, tall fescue, and prairie grass.	Donaghy, 1998; Fulkerson and Donaghy, 2001
2000s	Breeding indices that accounted for important functional traits as well as production are developed in Ireland and New Zealand. Followed by United Kingdom and United States in 2010s.	Gay et al., 2014; Spelman et al., 2013
2005	Transition cow experiments indicate a very small effect of precalving DMI on milk production. Subsequent work concludes that cows should have a BCS of 5.0 at calving (10-point scale) and consume 80 to 90% of requirements during the month before calving.	Roche et al., 2009a, 2013, 2015
2011	Pasture Profit Index is developed in Ireland to provide a breeding index for perennial ryegrass and to allow cultivar comparisons.	
2015	Analyses of economic databases identify an increase in fixed costs when grazing systems are intensified with imported feed; on average, total costs increase by ~150% of the feed cost. Furthermore, marginal responses to imported feeds on farm are ~40% less than those reported in research trials.	Ramsbottom et al., 2015
2016	Forage Value Index is subsequently developed in New Zealand.	

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A 100-Year Review: Microbiology and safety of milk handling[1]

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ABSTRACT

Microbes that may be present in milk can include pathogens, spoilage organisms, organisms that may be conditionally beneficial (e.g., lactic acid bacteria), and those that have not been linked to either beneficial or detrimental effects on product quality or human health. Although milk can contain a full range of organisms classified as microbes (i.e., bacteria, viruses, fungi, and protozoans), with few exceptions (e.g., phages that affect fermentations, fungal spoilage organisms, and, to a lesser extent, the protozoan pathogens *Cryptosporidium* and *Giardia*) dairy microbiology to date has focused predominantly on bacteria. Between 1917 and 2017, our understanding of the microbes present in milk and the tools available for studying those microbes have changed dramatically. Improved microbiological tools have enabled enhanced detection of known microbes in milk and dairy products and have facilitated better identification of pathogens and spoilage organisms that were not known or well recognized in the early 20th century. Starting before 1917, gradual introduction and refinement of pasteurization methods throughout the United States and many other parts of the world have improved the safety and quality of milk and dairy products. In parallel to pasteurization, others strategies for reducing microbial contamination throughout the dairy chain (e.g., improved dairy herd health, raw milk tests, clean-in-place technologies) also played an important role in improving microbial milk quality and safety. Despite tremendous advances in reducing microbial food safety hazards and spoilage issues, the dairy industry still faces important challenges, including but not limited to the need for improved science-based strategies for safety of raw milk cheeses, control of postprocessing contamination, and control of sporeforming pathogens and spoilage organisms.

Key words: dairy food safety, cheese safety, pasteurization

INTRODUCTION

The first issue of the *Journal of Dairy Science* (**JDS**) in 1917 comprised 5 articles, including 1 article on the microbiology of milk authored by Cornell scientists R. S. Breed and W. A. Stocking (Breed and Stocking, 1917). The article reported both agar-based and direct microscopic bacterial counts for "market milk" collected in New York. This article ended with the noteworthy conclusion, "Research men, using technique which differs much in details, may be depended upon to secure much more consistent agar plate counts from ordinary samples of market milk than laboratory assistants working rapidly and using the routine methods of analysis recommended for the purpose." Although we, the authors of the review presented here, are indeed located in Stocking Hall, which was named after the author of the first JDS microbiology paper, today research women and men are using different rapid detection methods and molecular biology tools, including whole-genome sequencing, to ensure the safety and quality of milk throughout the world.

Between 1917 and 2017, our understanding of the microbiology of raw milk and dairy products has undergone tremendous advances (see Appendix Table A1), in no small part because of the development and use of new microbiological techniques and methods, including development of improved selective and differential bacteriological media and development of molecular biology tools (e.g., PCR). In parallel, development and implementation of improved technologies and systems to control microbial food safety hazards and spoilage organisms have significantly improved dairy product quality and reduced public health hazards associated with dairy products to a level that would have been unimaginable in 1917, as illustrated by the modern availability of HTST pasteurized fluid milk with shelf lives of >21 d.

MICROBIOLOGY AND SAFETY OF MILK HANDLING FROM 1917 TO 2017

Transition to Pasteurization

The widespread implementation of pasteurization for raw milk has improved public health by preventing the

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spread of foodborne diseases across the United States. However, pasteurization was initially controversial and slow to be adopted as a common practice. In 1864, Louis Pasteur discovered that gradually heating wine, and then rapidly cooling it, prevented abnormal wine fermentation due to spoilage microorganisms; this process came to be known as pasteurization. Although Pasteur himself did not apply this principle to milk, implementation of milk pasteurization started considerably before 1917. As early as 1873, the American pediatrician Abraham Jacobi advocated boiling cow milk in bottles before feeding it to infants (Jacobi, 1873; Holsinger et al., 1997). Later, in 1886, the German chemist Franz von Soxhlet devised an apparatus for in-home, in-bottle milk sterilization for infants; this procedure involved boiling milk for 40 min (Andrews and Fuchs, 1944). By 1893, under the advice of Jacobi and others, philanthropist Nathan Straus opened milk depots in New York City, providing sterilized milk to infants of impoverished families (North, 1921; Steele, 2000). Despite an observed effect of sterilization on reducing infant mortality rates and growing support for in-home pasteurization of cow milk for infant consumption, implementation of widespread commercial pasteurization faced strong opposition (North, 1921; Andrews and Fuchs, 1944). Many public health officials and doctors in the United States opposed widespread commercial pasteurization of milk, fearing it would provide only a stopgap measure that might create a false sense of security for the processor while distracting farmers from the need to increase on-farm sanitary measures (North, 1921; Andrews and Fuchs, 1944). In addition, although some recognized pasteurization as a useful method for reducing milk spoilage, they remained concerned about its ability to effectively inactivate milkborne pathogenic microorganisms (Andrews and Fuchs, 1944). Nevertheless, in 1907, the first commercial-scale apparatus for pasteurizing milk by the holding method was installed in New York City (Andrews and Fuchs, 1944). A pivotal shift occurred in January 1908, when the US Public Health Service (**USPHS**) and Marine Hospital Service published *Milk and its Relation to the Public Health*, which revealed that the consumption of raw milk was dangerous and was often the cause of tuberculosis, typhoid fever, diphtheria, scarlet fever, and intestinal disorders of babies. In this document, US Surgeon General Walter Wyman famously wrote, "Pasteurization prevents much sickness and saves many lives" (Wyman, 1908). This report prompted states to respond to public health concerns surrounding diseases associated with raw milk. In July 1908, Chicago became the first American city to pass an ordinance requiring the pasteurization of all cow milk entering the city, except for that from tuberculin-tested cows (Czaplicki, 2007). This ordinance was originally intended only as a temporary measure to control the spread of bovine tuberculosis; simultaneously, farmers were expected to bring their herds into compliance with tuberculin testing (Czaplicki, 2007). However, many cities followed suit, releasing similar ordinances regarding pasteurization (Andrews and Fuchs, 1944). As a result, commercial milk processing facilities were constructed throughout the United States to meet compliance needs, thus rapidly spreading the practice of pasteurization (Andrews and Fuchs, 1944). Once pasteurized milk was introduced to the public, pasteurized milk and milk products rapidly penetrated the market. According to Smith-Howard (2013), by 1916, 80 to 90% of the milk sold in Chicago, Boston, Philadelphia, and New York was pasteurized.

To assist in the prevention of milkborne diseases, in 1924 the USPHS published the *Standard Milk Ordinance for Alabama Municipalities*, later referred to as the first Standard Milk Ordinance; this document included standards for pasteurization (USPHS/FDA, 2016). Subsequently, Frank et al. (1927) reported on outcomes from the implementation of the Standard Milk Ordinance in 14 Alabama towns. Significant improvements were described in raw milk quality and USPHS milk sanitation ratings for both farms and processing facilities that were pasteurizing milk. Consequently, in 1926 a slightly modified version of the Standard Milk Ordinance was published (Frank et al., 1927). In 1927, the USPHS released an accompanying code to provide a uniform interpretation of the ordinance and to offer administrative and technical details regarding satisfactory compliance (USPHS/FDA, 2016). The ordinance and accompanying code were the precursors of the current US Grade A Pasteurized Milk Ordinance (**PMO**; USPHS/FDA, 2016).

As scientific research in the areas of milk production, processing, nutrition, and public health progressed and was shared with the public through publications such as JDS, the controversy surrounding pasteurization diminished to a point where it became more broadly accepted in the late 1930s (Steele, 2000). The practice of pasteurization achieved regulatory authority in 1947, when Michigan became the first state to implement a statewide milk pasteurization law, which compelled further expansion of pasteurization from cities to rural areas. Hence, 1947 represented a major landmark in dairy food safety. Since then, all other states have adopted similar requirements, signifying recognition of the importance of pasteurization in ensuring dairy food safety. Although some states still allow the intrastate sale of raw milk, interstate sale of raw milk and raw

milk products for human consumption is banned except for certain raw milk cheeses aged for >60 d (Steele, 2000; Weisbecker, 2007).

The public health impact of nationwide pasteurization requirements and improved dairy industry sanitation has been profound. In 1989, the US Food and Drug Administration (**FDA**) retroactively determined that about 25% of foodborne and waterborne illnesses in 1938 had been caused by consumption of contaminated milk and milk products; it is estimated that today less than 1% of foodborne and waterborne illnesses are caused by milk and milk products (USPHS/FDA, 2016). It is important to note, however, that consumption of raw milk and raw milk dairy products appears to be responsible for a disproportionate fraction of the human illnesses attributed to milk and milk products. Estimates by the Centers for Disease Control and Prevention based on data collected between 1993 and 2006 suggested that nonpasteurized milk and milk products carried 150 times greater risk of causing outbreaks and outbreak-associated illnesses per unit of product consumed relative to consumption of pasteurized products (Langer et al., 2012). A follow-up study on raw milk and cheese data for 2009 to 2014 estimated that consumption of these raw products carried an 840 times greater risk of illness compared with pasteurized products (Costard et al., 2017). Although these estimates illustrate the continued food safety challenges associated with consumption of raw milk (and to a lesser extent raw milk products), they were based on outbreaks linked to both legally produced and illegally sold raw milk and raw milk products. Therefore, one cannot necessarily conclude that consumption of a given raw milk product will carry an 840 times higher risk of foodborne illness compared with consumption of an equivalent pasteurized milk product. These estimates do highlight, though, the need for further research into the factors contributing to increased risk from consumption of raw milk products and novel intervention strategies to control the risk. For example, a joint assessment by the US FDA and Health Canada suggested that increased *Listeria* monitoring of finished product could greatly reduce the risk of contracting listeriosis from consumption of raw milk soft-ripened cheeses in the United States and Canada (FDA, 2015).

Refinement of Pasteurization Equipment, Requirements, and Other Processing Interventions to Control Microbial Contamination of Milk

In the early 1900s, recognized disease-causing microorganisms associated with the consumption of raw milk in the United States included *Mycobacterium tuberculosis*, *Salmonella* Typhi, *Corynebacterium diphtheriae*, *Vibrio cholera*, *Shigella dysenteriae*, and *Brucella* spp. (Rosenau, 1908b); current bacterial nomenclature rather than that in use at earlier points is provided here and throughout the article. As the dairy industry slowly adopted pasteurization practices, numerous studies provided suggestions for pasteurization time–temperature combinations based on the determination of thermal death times for common pathogenic microorganisms found in raw milk (Rosenau, 1908a; North and Park, 1927). However, the scientific understanding of disease transmission and bacteriology was limited, as reflected by the studies performed. As late as 1927, there was no official agreement on effective time–temperature combinations, methods for determining combinations, or the specific organisms targeted for destruction by pasteurization (Rosenau, 1908b; North and Park, 1927).

For a period of time, *M. tuberculosis* cells were thought to be the most heat-resistant vegetative bacterial cells in milk. Therefore, many scientists between 1883 and 1906 developed heat treatment regimens targeting *M. tuberculosis* (Rosenau, 1908a). However, time–temperature combinations selected for these studies were somewhat arbitrary and methods were inconsistent (North and Park, 1927). In 1908, Milton J. Rosenau, then director of the Hygiene Laboratory of the US Public Health and Marine Hospital Service, published a comprehensive review that established 60°C for 20 min as the minimum time and temperature to heat milk to destroy *M. tuberculosis* (Rosenau, 1908b). This work inspired confidence in the use of relatively low temperatures to control milkborne pathogens and served as the foundation for general acceptance of the pasteurization process among health authorities (Andrews and Fuchs, 1944). In 1911, the National Committee on Milk Standards, a New York contingent of leading bacteriologists and public health officials, recommended a time–temperature combination of 62.8°C for 30 min (North and Park, 1927). In 1920, the Committee on Milk Supply officially recommended 62.8°C for 30 min (Westhoff, 1978). In the 1924 Standard Milk Ordinance, officials provided consensus and defined the pasteurization process as a heating process of no less than 61.1°C for 30 min using approved equipment (Frank, 1924). The suggested standard was later verified to provide a sufficient safety margin to destroy *M. tuberculosis* (North and Park, 1927).

Although the holding method was widely used through the 1930s, a new continuous method emerged as plate heat exchangers were developed, resulting in the HTST pasteurization method most commonly used today (Westhoff, 1978). However, HTST pasteurization requirements were difficult to establish from the existing literature, which did not report minimum treatment times for temperatures above 65.5°C. Additionally, pub-

lic health and regulatory officials were wary of accepting this method and associated equipment (Westhoff, 1978; Holsinger et al., 1997). As such, numerous studies were performed to determine time–temperature combinations capable of effectively controlling *M. tuberculosis* at higher temperatures (Westhoff, 1978). The year 1933 represents another landmark, as this was the year when HTST pasteurization equipment and methods were approved and when the first HTST time–temperature standards were included in the USPHS Milk Ordinance Code; these standards were a temperature of at least 160°F (71.1°C) for at least 15 s (Westhoff, 1978). As technology continued to evolve, continuous flash pasteurization methods gained popularity (e.g., higher heat, shorter time), and suitable standards were set accordingly to ensure the destruction of *M. tuberculosis* (Westhoff, 1978).

Although the time–temperature combinations for pasteurization were initially established for *M. tuberculosis*, the pasteurization target organism was redefined when Enright et al. (1957) determined, using animal experiments, that viable *Coxiella burnetii* survived in milk pasteurized at the existing standard conditions of 61.7°C for 30 min (Enright et al., 1957). *Coxiella burnetii* is the organism responsible for Q fever. In response, the USPHS immediately adjusted their recommendation for vat pasteurization to 145°F (62.8°C; typically rounded to 63°C) for 30 min, with the added recommendation that this limit be raised 3°C for products with more fat than that present in whole milk or in products with added sugar (Anderson, 1957). Consequently, parameters for HTST pasteurization were also adjusted to 161°F (71.7°C; often rounded to 72°C) for 15 s (Anderson, 1957; Holsinger et al., 1997). Today, batch (vat) pasteurization parameters of 63°C (145°F) for 30 min and continuous flow HTST pasteurization parameters of 72°C (161°F) for 15 s still represent the minimum pasteurization time–temperature combinations listed in the PMO, and *C. burnetii* remains the target organism for validation of pasteurization requirements for all US dairy products (USPHS/FDA, 2016); this approach also is considered an international standard (FAO/WHO, 2011). Another key date for dairy microbiology was August 10, 1987, when the FDA published a final regulation mandating the pasteurization of all milk and milk products in final package form for direct human consumption. This regulation, which banned the shipping of raw milk in interstate commerce, became effective September 9, 1987.

In parallel to advancement in pasteurization techniques (e.g., development of HTST) and refinement of minimum pasteurization time–temperature parameters, advancement in technologies that supported pasteurization implementation made essential contributions to microbial dairy food safety and quality. For example, in 1935, Kay and Neave (1935) introduced the phosphatase test, a method used to determine relative alkaline phosphatase (**ALP**) activity. Alkaline phosphatase is found naturally in bovine milk and is inactivated once subjected to proper pasteurization conditions; hence, ALP measurement provides a simple test that can be used to verify proper pasteurization, which was particularly important in the early days of pasteurization (Burgwald, 1939). The Scharer method, which was developed in 1940, is an ALP colorimetric test that was applied until it was no longer accepted under the PMO in 2009 (Scharer, 1938; Rankin et al., 2010). Today, the industry commonly uses fluorometric and chemiluminescent tests, such as the Fluorophos ALP Test System (Advanced Instruments Inc., Norwood, MA) and Charm ALP/PasLite (Charm Sciences Inc., Lawrence, MA; Rankin et al., 2010). Currently, the PMO requires ALP testing using electronic methods for grade A pasteurized milk and milk products and bulk-shipped heat-treated milk products (USPHS/FDA, 2016).

One major challenge that has been described since the early days of JDS (Smith, 1919; Brew, 1922) and that remains in today's dairy industry is recontamination of pasteurized milk and dairy products with microbial pathogens and spoilage microbes after the pasteurization step—for example, during postprocessing filling or packaging. Major pathogens of current concern for postprocessing contamination are *Listeria monocytogenes* and to a lesser extent *Salmonella* as well as *Cronobacter*, particularly in infant formula. In the early days, postprocessing contamination was linked to both milk handlers and the processing environment. For example, according to the USPHS, 3 out of 11 disease outbreaks that were linked to pasteurized milk between 1929 and 1934 were traced to typhoid carriers who operated bottling machines or who handled bottles (Chilson et al., 1936). However, today, postprocessing contamination typically occurs from the built environment and equipment. Interestingly, the challenges of postprocessing contamination were recognized as early as 1917, when the International Dairy and Milk Inspectors' Committee published their report "Rules and Regulations Necessary for Securing a Clean and Safe Milk Supply," which suggested rules for sanitary milk control throughout production, transportation, handling, and delivery (Kelly et al., 1917). Among these were recommendations for implementation of strategies that today would be considered good manufacturing practices in addition to pasteurization. Consistent with this, and as a result of a series of comprehensive studies conducted between 1906 and 1921 (Rosenau, 1908a, b; North et al., 1925), the USPHS concluded that sanitary control throughout the dairy supply chain is essential for en-

suring the control of milkborne disease (Faulkner, 1957; USPHS/FDA, 2016). Thereafter, in 1923, the USPHS established an Office of Milk Investigations to assist the states in the development of effective milk-control programs (Faulkner, 1957). However, implementation of regulations was inconsistent across facilities and states for several decades (Dahlberg et al., 1953). As a consequence, in 1950 the US surgeon general requested that state milk sanitation regulatory agencies establish procedures for a voluntary Interstate Milk Shipper certification program, which resulted in the formation of the National Conference on Interstate Milk Shipments (**NCIMS**) and the Cooperative State Public Health Service Program for certification of interstate milk shippers. Responsibilities under this program were divided between state agencies and the Public Health Service Program. In 1969, the Public Health Service Program responsibilities were transferred to the FDA. Currently, all 50 states, the District of Columbia, and the US trust territories participate in the NCIMS. Biennial NCIMS meetings, which include representatives across the dairy spectrum, including producers, processors, and academics, are used to develop recommendations to modify the PMO. The NCIMS recommendations must be approved by the FDA before they are incorporated into the PMO. In addition to these government regulations, worldwide, the introduction of voluntary food safety management systems (e.g., hazard analysis and critical control point, International Organization for Standardization standards, the Global Food Safety Initiative) has contributed to the development of comprehensive systems to ensure dairy food safety. Simultaneously, starting in the 1930s, major advancements were made in sanitation practices. For example, clean-in-place methods were developed in 1950, and Tetra Pak (Pully, Switzerland) introduced UHT milk packaged in a multilayer carton in 1952.

1950: Introduction of the 60-d Holding Period for Raw Milk Cheese, with Subsequent Concerns Raised by Outbreaks and Laboratory Studies

The 60-d rule, which specifies that certain cheeses made from raw milk must be aged for more than 60 d, was introduced in the final years of World War II, when a lack of skilled cheesemakers in the United States coincided with 2 serious outbreaks of typhoid fever linked to cheese consumption (Johnson et al., 1990). In fact, during the decade encompassing the war (1935–1945), 40 foodborne disease outbreaks were attributed to cheese (Fabian, 1947). In response to this public health challenge, the surgeon general issued a letter on June 16, 1944, calling either for the production of cheese from pasteurized milk or for raw milk cheeses to be adequately ripened for safety (Johnson et al., 1990). Many states, including California, Colorado, Indiana, Illinois, Missouri, Minnesota, New Jersey, and New York, responded to the letter by instituting regulations. It was in these local regulations that the first specific holding time requirements for raw milk cheeses ("cured for a minimum of 60 d") can be found (Johnson et al., 1990). The FDA began standard of identity hearings for cheeses in 1947. These discussions included comments on the holding period, but it was not until August 24, 1950, that the final rule (15 FR 5653; US Federal Registrar, 1950) requiring a minimum 60-d holding period at 35°F or higher for specific cheeses was officially published as a national standard.

Much of the early scientific debate over the 60-d rule is lost to time, but the minimum holding time was at least in part based on research on the survival of *Brucella abortus* in Cheddar cheese (Gilman et al., 1946; Johnson et al., 1990). Although the study reported that cheeses intentionally inoculated with *B. abortus* were positive for up to 6 mo postmanufacture, it also reported that no *B. abortus* were found either in commercial Limburger cheeses produced with *B. abortus*-positive milk and held for 57 d or in cheeses made from milk collected from *B. abortus*-positive herds and stored for 41 d (Gilman et al., 1946). These observations, coupled with the absence of epidemiological data that linked Cheddar cheeses aged for more than 60 d to foodborne outbreaks, led the researchers to conclude that the 60-d holding period was a reasonable measure for control of foodborne pathogens (Gilman et al., 1946). While other studies at the time demonstrated long-term survival of *M. tuberculosis*, *Salmonella* Typhi, and hemolytic streptococci in various cheeses, the 60-d holding period was deemed to offer an adequate, though not absolute, protection from potential levels of pathogenic organisms in raw milk cheeses (Johnson et al., 1990; Boor, 2005).

Today the 60-d rule is captured in the US Code of Federal Regulations (**CFR**) under 7CFR§58.439 (US Code of Federal Regulations, 2016). The section states that if a "cheese is labeled as 'heat treated,' 'unpasteurized,' 'raw milk,' or 'for manufacturing,' the milk may be raw or heated at temperatures below pasteurization. Cheese made from unpasteurized milk shall be cured for a period of 60 days at a temperature not less than 35°F." The standards of identity for cheeses can be found in 21CFR§133 (US Code of Federal Regulations, 2017a). These standards include requirements for permissible moisture content, minimum milk fat content, and the acceptability of pasteurized or raw milk in their production. Some standards of identity require holding periods longer than 60 d, but these aging requirements are aimed at ensuring proper development of charac-

teristics particular to the cheese variety and are not related to safety.

Over the past 70 yr, significant strides in cow health, milking hygiene, dairy processing, and disease surveillance across the farm, processor, and regulatory continuum have greatly improved the quality and safety of dairy products. Along with these improvements have come changes in the foodborne pathogens that are currently more frequently associated with dairy products. Nontyphoidal *Salmonella*, *Listeria monocytogenes*, Shiga toxin–producing *Escherichia coli* (**STEC**) and *Campylobacter* have replaced *B. abortus*, *M. tuberculosis*, *Salmonella* Typhi, and hemolytic streptococci as the primary bacterial pathogens of concern (Scallan et al., 2011). Recognition of the importance of these pathogens in dairy foods and their ability to survive in cheese has further challenged the efficacy of the 60-d rule for protecting public health. An early epidemiological example was an outbreak from 1980 to 1982 of *Salmonella* Muenster linked to raw milk Cheddar cheese in Canada (Wood et al., 1984). One of the implicated cheese lots remained positive for *Salmonella* Muenster for 125 d (Wood et al., 1984). A second Canadian outbreak in 1984, also linked to raw milk Cheddar cheese, determined that *Salmonella* Typhimurium persisted in the cheese for 8 mo (D'aoust et al., 1985). In the United States, 3 outbreaks associated with raw milk cheeses that had been aged for at least 60 d were reported from 1998 to 2011; 2 were caused by *E. coli* O157:H7 and the other was caused by *L. monocytogenes* (Gould et al., 2014). In March 2017, listeriosis was deemed responsible for 6 illnesses and 2 confirmed deaths linked to 60-d aged raw milk cheese from New York State (FDA, 2017a). Although environmental contamination sources after cheese making cannot always be excluded in outbreaks linked to raw milk cheeses, in several outbreaks pathogens present in raw milk were likely the root cause. For example, an investigation of a Dutch outbreak of *S.* Typhimurium phage type DT7 in a raw milk hard cheese aged for 9 mo found the rare *Salmonella* phage DT7 in both the farm's cattle and the dairy production room, highlighting the potential for raw milk contamination to carry through aging (Van Duynhoven et al., 2009). In the future, the use of whole-genome sequencing will enhance public health officials' ability to track specific strains and to identify sources of contamination for raw milk cheeses. Application of these tools will further help clarify the contributions of raw milk and the processing environment as pathogen sources.

Research over the past several decades has substantiated the ability of several foodborne pathogens to survive in cheese longer than the 60-d holding period. The ability of *L. monocytogenes* to persist for long periods of time in cheeses such as Cheddar (Ryser and Marth, 1987), raw milk semihard Swiss (Bachmann and Spahr, 1995), and soft cheeses (D'Amico et al., 2008) has been well documented. Similarly, several studies have demonstrated *E. coli* O157:H7 survival past the 60-d holding period for both Cheddar and Gouda (Reitsma and Henning, 1996; D'Amico et al., 2010). When the 60-d rule was originally adopted, it was understood that the ability of pathogens to survive the holding period varied among cheese types (Johnson et al., 1990) due to differences in attributes such as pH, salt, moisture, water activity, and temperature. More recently, researchers from various institutions have collaborated to provide more detail on the range of product parameters that exist in modern raw milk cheeses (Trmčić et al., 2017), but further study is needed to understand the influence of these parameters and ripening conditions on pathogen survival.

In 2016, required compliance to the Food Safety Modernization Act began for many cheese manufacturers in the United States. The act requires manufacturers to implement adequate controls to prevent or significantly reduce any hazards associated with the food being produced. Section 117.135 of 21CFR (US Code of Federal Regulations, 2017b) states that process controls "include procedures, practices, and processes to ensure the control of parameters during operations such as heat processing, acidifying, irradiating, and refrigerating foods. Process controls must include, as appropriate to the nature of the applicable control and its role in the facility's food safety system: (i) parameters associated with the control of the hazard; and (ii) the maximum or minimum value, or combination of values, to which any biological, chemical, or physical parameter must be controlled to significantly minimize or prevent a hazard requiring a process control." Based on epidemiological and challenge study data cited previously (e.g., Ryser and Marth, 1987; Bachmann and Spahr, 1995; Reitsma and Henning, 1996; D'Amico et al., 2008, 2010), the argument for the current 60-d rule as an adequate process control may be called into question. This notion is supported by a recent assessment of the risk of listeriosis from consumption of soft cheeses (using Camembert as an example), which was conducted by Health Canada and the FDA (FDA, 2015). The results from this study suggested that consumption of raw milk soft cheeses, even if aged for >60 d, presents an approximately 50 to 100 times higher risk for listeriosis compared with consumption of pasteurized milk cheeses. In fact, the assessment found that removal of the 60-d aging requirement for soft cheese would, in fact, reduce the risk of listeriosis from these products, albeit the reduction would be less than 2-fold. Specifically, sales of raw milk soft cheeses aged less than 60 d would be expected to reduce the risk of listeriosis because the shorter period

would permit less time for *L. monocytogenes* growth in these types of cheeses, which have high water activity and a high pH that increases during aging due to the metabolic activities of fungi or bacterial surface populations (e.g., *Brevibacterium*). More important, this risk assessment suggested that testing both raw milk and finished product could considerably reduce the risk associated with raw milk soft cheeses. This analysis underpins the decisions in some countries to remove the 60-d aging periods for certain cheeses; for example, in 2008, the provincial government in Québec (Canada) passed new regulations permitting the sale of soft and semisoft cheeses aged for less than 60 d. Further research into the efficacy of aging different cheese types for 60 d and the effect of additional measures, such as testing raw or finished product, will be important for determining the future of the 60-d rule.

1983: Listeria (Re-)Enters the Dairy Industry as a Pathogen of Concern—And Is Still Here in 2017

Listeria monocytogenes has a long history of being linked to milk and dairy products, including the fact that this pathogen causes severe disease (e.g., brain infections, abortions) in both cattle and humans. The first listeriosis outbreak linked to dairy was reported to have occurred in Halle, Germany, from 1949 to 1957; more than 100 stillbirths were suspected to have been caused by raw milk contaminated with *L. monocytogenes* (Seeliger, 1961). A comprehensive review on dairy-related listeriosis outbreaks was published in JDS in 2004 (Lundén et al., 2004). The second reported listeriosis outbreak linked to dairy occurred in Vaud, Switzerland, between 1983 and 1987; this outbreak was linked to Vaucherin Mont d'Or soft-ripened cheese and involved more than 120 cases with 32 associated deaths (Büla et al., 1995). This outbreak in Europe as well as a concurrent large listeriosis outbreak in 1985 in California, which was linked to Hispanic-style soft cheese, and a 1983 listeriosis outbreak in Massachusetts linked to contaminated pasteurized milk brought *L. monocytogenes* to the forefront of the dairy industry's attention. Since then, listeriosis has remained arguably the most important human foodborne pathogen associated with the dairy industry, and a considerable number of additional human listeriosis outbreaks around the world have been linked to dairy products. Different products (e.g., chocolate milk, fluid milk, butter, various cheeses, and, more recently, ice cream) have been implicated as sources of human listeriosis outbreaks (Lundén et al., 2004; Chen et al., 2016); however, Hispanic-style cheeses have been a particular concern with regard to human listeriosis as detailed in a recent review in JDS (Ibarra-Sánchez et al., 2017). In addition, surface ripened and washed rind cheeses have been linked to several human listeriosis outbreaks and present a particular concern as potential sources of human listeriosis cases and outbreaks. For example, in 2008, a listeriosis outbreak in Chile with 165 reported cases and 14 deaths was associated with the consumption of Brie and Camembert (Montero et al., 2015). Raw milk consumption also is linked to human listeriosis cases and outbreaks; in 2016, a small listeriosis outbreak with 2 cases was linked to organic raw milk produced by a farm in Pennsylvania (CDC, 2016).

Our understanding of the sources of *L. monocytogenes* in dairy products also has evolved over the years. Although contaminated raw milk has been the likely source of some outbreaks (including outbreaks linked to raw milk cheeses and raw milk), studies conducted over the years suggest that *L. monocytogenes* contamination of dairy products typically occurs from the processing plant environment, where *Listeria* spp. and *L. monocytogenes* strains can survive for prolonged time periods and up to decades (for a review see Ferreira et al., 2014). For example, Kabuki et al. (2004) reported evidence for environmental *Listeria* persistence in 2 of 3 Latin-style cheese processing facilities enrolled in their study. In one facility, the persistent *L. monocytogenes* subtype was also identified in the finished products, supporting cross-contamination from the processing plant environment as a root cause of contamination of the finished product. Similarly, Beno et al. (2016) reported the persistence of *Listeria* spp. in 7 out of 9 small cheese making facilities included in a study on the development of pathogen environmental monitoring programs. The importance of environmental *L. monocytogenes* sources and cross-contamination is further illustrated by results from an investigation of a large listeriosis outbreak with 38 human cases in Québec, Canada; this outbreak was linked to a pasteurized soft-textured cheese, and postpasteurization contamination from the processing plant was the likely source of this outbreak. Importantly, investigation of this outbreak also suggested that extensive cross-contamination of different cheeses in retail establishments is a contributing factor (Gaulin et al., 2012). The recognition of the importance of postprocessing contamination with *L. monocytogenes* as a public health hazard has led to evaluation of different methods, including high-pressure processing, to inactivate *L. monocytogenes* in packaged products and in cheeses (e.g., Tomasula et al., 2014). Increased recognition has emerged regarding the essential nature of stringent pathogen environmental monitoring programs targeting *L. monocytogenes* as a means of reducing *L. monocytogenes* contamination of cheese and other dairy products, as illustrated by the FDA's recent publication of a draft guidance titled "Control of *Listeria monocy-*

togenes in Ready-To-Eat Foods: Guidance for Industry" (FDA, 2017b).

Since 1997, identification of human listeriosis outbreaks in general, including outbreaks linked to dairy products, has been facilitated by increasingly worldwide application of molecular subtyping (i.e., DNA fingerprinting) tools. Since 1999, routine subtyping of all *L. monocytogenes* isolates from human clinical cases in the United States has been performed using pulse field gel electrophoresis. This approach has been adopted for subtyping foodborne pathogens in many other countries through a system called PulseNet International, and this adoption will likely aid in the detection of more listeriosis outbreaks linked to dairy products in different parts of the world. Whereas large outbreaks may be detected without the use of subtyping methods, because pulse field gel electrophoresis and other subtyping methods have been increasingly used for routine surveillance of foodborne disease and, specifically, for listeriosis (Jackson et al., 2016), these methods have enabled the detection of a larger number of smaller outbreaks linked to cheese and dairy products. Improved detection of human listeriosis outbreaks will be further facilitated by routine implementation of whole-genome sequencing for surveillance and characterization of *L. monocytogenes* isolates from human clinical cases, foods, and food-associated environments. In the United States, routine characterization of *L. monocytogenes* through whole-genome sequencing was initiated in September 2013 (Jackson et al., 2016); this change in surveillance strategies enabled increased detection of listeriosis clusters and helped solve listeriosis outbreaks (5 and 9 in yr 1 and 2 of whole-genome sequencing implementation, respectively). Importantly, the outbreaks detected after implementation of whole-genome sequencing included 3 multistate outbreaks linked to different styles of soft cheeses as well as 2 outbreaks linked to ice cream (Jackson et al., 2016), illustrating the effect of this technology on the dairy industry. Whole-genome sequencing is also rapidly being implemented for foodborne disease surveillance in other countries (Deng et al., 2016).

1986 to 1992: Enterobacter sakazakii and STEC Are Recognized as Causes of Dairy-Associated Foodborne Disease Outbreaks—A Reminder that We Still Do Not Know All Dairy-Associated Pathogens

Several key pathogens were recognized as concerns to the dairy industry before 1917, including organisms such as *M. tuberculosis*, *Brucella* spp., and *Salmonella*. *Listeria monocytogenes* was added to the list of dairy-associated pathogens in the mid-1980s after it was linked to 2 large listeriosis outbreaks that were caused by consumption of contaminated cheeses in both Europe and the United States. Two key pathogens of concern to the dairy industry (*Cronobacter* and STEC) were only recently recognized as dairy-related pathogens.

Cronobacter spp., which were originally designated as *Enterobacter sakazakii*, are a specific concern for the powdered infant formula industry (for a comprehensive review, including a summary of outbreaks, see Norberg et al., 2012). The first reported cases of *E. sakazakii* infections in neonates that were unambiguously linked to infant formula occurred in 1986 and 1987. An article published in 1989 (Biering et al., 1989) reported 3 cases of neonatal *E. sakazakii* infections that occurred in a hospital in Reykjavík, Iceland, over a 9-mo period in 1986 and 1987. As part of the outbreak investigation, *E. sakazakii* was isolated from several lots of the powdered milk formula used in the hospital, and *E. sakazakii* isolates obtained from the neonates were found to be indistinguishable (by biotypes, plasmid DNA profiles, and antibiograms) from 22 strains grown from the formula. Whereas Muytjens et al. (1983) had previously reported an association between *Cronobacter* spp. infections and consumption of infant milk formula, this group did not isolate *Cronobacter* spp. from the powered infant formula despite its isolation from prepared formula and environmental samples. Since then, several *Cronobacter* outbreaks have been linked to contaminated infant formula, including some that have been linked to intrinsically contaminated powdered infant formula (i.e., formula contaminated in the processing facility) as well as several other outbreaks linked to infant formula that appears to have been contaminated from environmental sources during or after preparation. For example, one outbreak in Belgium in 1998 involved 12 neonates with necrotizing enterocolitis; in this outbreak, considerable evidence suggested that intrinsically contaminated powdered infant formula was a factor that contributed to this outbreak (van Acker et al., 2001). However, in this outbreak as well as in others, cross-contamination during formula preparation (e.g., either from intrinsically contaminated formula or from the environment in which preparation was conducted) may have been a contributing or sole factor responsible for transmission and infection. In addition, investigations of outbreaks and sporadic cases have also shown that problematic practices with regard to storage of prepared infant formula can be an important contributing factor to infection. For example, one study showed that some hospitals in France stored reconstituted infant formula for up to 24 h, which could allow for considerable growth of *Cronobacter* (Caubilla-Barron et al., 2007; Norberg et al., 2012). Consequently, important measures for prevention of *Cronobacter* infections include practices to prevent contamination of powdered

infant formula in processing facilities as well as compliance with guidelines for preparation and storage of reconstituted powdered infant formula (Norberg et al., 2012). Importantly, a paper published in JDS in 2011 detailed potential reservoirs and routes of transmission of *E. sakazakii* based on a study in a plant that produced milk powder (Jacobs et al., 2011), providing important guidance that can inform preventative strategies.

The *E. coli* strains causing enterohemorrhagic colitis (enterohemorrhagic *E. coli*; **EHEC**), including *E. coli* O157:H7, are another more recently recognized pathogen of concern for the dairy industry. Although EHEC are clinically characterized by their ability to cause hemolytic uremic syndrome (**HUS**), genetically these strains are characterized by the presence of *stx1* or *stx2* genes as well as the *eaeA* gene; *stx1* and *stx2* encode Shiga toxins (sometimes also referred to as verotoxins), whereas *eaeA* encodes intimin, which facilitates attachment of *E. coli* to intestinal epithelial cells. Because of the key importance of Shiga toxins in the pathogenesis of HUS, these *E. coli* are sometimes also referred to as STEC. Whereas EHEC were initially linked to cases and outbreaks caused by consumption of raw or undercooked beef products, it is now apparent that many different types of products that are produced without a bacterial control step (e.g., heat treatment) can transmit this pathogen, including produce, raw apple cider, raw milk, and certain raw milk dairy products (for a comprehensive review of STEC in dairy foods, see Farrokh et al., 2013). In 1986, 2 cases of *E. coli* O157:H7 infections were described in young children who consumed raw milk on farms where *E. coli* O157:H7 was subsequently also isolated from fecal samples from dairy cows, suggesting raw milk as a potential vehicle responsible for human O157:H7 infections (Martin et al., 1986). An early report of a linkage between EHEC and dairy products was also provided by Deschênes et al. (1996), who reported 4 HUS cases in a French village between March 1992 and May 1993 that were associated with consumption of a raw milk cheese. Since the first descriptions of EHEC infections linked to raw milk and dairy products, a considerable number of EHEC outbreaks around the world have been linked to consumption of raw milk. Although most of these outbreaks have been linked to isolates representing *E. coli* serotype O157:H7, some of them also have been caused by other serotypes (e.g., O22, O26; Farrokh et al., 2013). Enterohemorrhagic *E. coli* strains also have been responsible for some outbreaks linked to raw milk fermented dairy products and particularly raw milk cheeses. For example, between 1998 and 2011, 4 *E. coli* O157:H7 outbreaks in the United States were linked to consumption of raw milk cheeses (Gould et al., 2014). These findings are consistent with different studies (Reitsma and Henning, 1996; D'Amico et al., 2010) that indicate that *E. coli* O157:H7 can survive in cheeses for more than 60 d. Interestingly, a few EHEC outbreaks linked to dairy products have been linked to pasteurized products, including one large outbreak in Scotland in 1994 linked to pasteurized fluid milk where *E. coli* O157:H7 was isolated from food contact surfaces located after the pasteurizer (Upton and Coia, 1994). These outbreaks further support that postpasteurization contamination with pathogens other than *L. monocytogenes* remains an issue even in the modern dairy industry.

1990: PCR Enters the Dairy Microbiology World and Changes it Forever

The advent of molecular methods for detection and characterization of dairy-associated microbes has had a major effect on dairy microbiology. Although some molecular methods, such as nucleic hybridization-based assays, were commercially available as early as 1985 (for a review, see Vasavada, 1993), the invention of PCR in 1985 opened the door to mainstream application of molecular methods. Application and use of PCR for detection of foodborne pathogens and other microorganisms started in the early 1990s. For example, the first PCR assay for *Listeria monocytogenes* was reported in 1990 (Border et al., 1990), and the first reports of using a PCR assay to detect *L. monocytogenes* in dairy products (soft cheese, milk) were published in 1991 (Furrer et al., 1991; Thomas et al., 1991; Wernars et al., 1991). Since then, PCR assays for virtually all foodborne pathogens of relevance to the dairy industry have been developed, are commercially available, and are increasingly routinely used for detecting pathogens in finished products as well as environmental samples. More slowly, PCR methods are also being developed for spoilage organisms of relevance to the dairy industry (e.g., Ranieri et al., 2012). However, developing molecular assays for spoilage organisms is more challenging than developing assays for pathogens because it is difficult to identify specific, well-defined molecular targets that yield high levels of sensitivity and specificity for spoilage organism detection. For pathogen detection, there is emerging consensus that molecular methods can provide more rapid, more specific, and more sensitive detection compared with many traditional or immunoassay-based methods (e.g., Abubakar et al., 2007). With continued improvements in ease of use and performance of molecular methods, including the development of easy-to-use isothermal methods for DNA amplification, molecular methods will likely continue to expand their penetration in the dairy pathogen testing industry. Increasing efforts to better characterize

spoilage organisms, including through whole-genome sequencing (e.g., Moreno Switt et al., 2014), may also provide new opportunities to develop better assays for detection of specific targeted spoilage organisms of relevance in the dairy industry.

1998: Paenibacillus spp. and B. weihenstephanensis Are Recognized as Psychrotolerant Spoilage Organisms of Concern in HTST-Pasteurized Fluid Milk

Similar to the changes in our understanding of foodborne pathogens associated with milk and dairy products, our understanding of spoilage organisms of concern has also changed considerably over time. Conceptually, spoilage organisms in products made from pasteurized milk may represent (1) organisms introduced from environmental sources or personnel after heat treatment or (2) organisms that survive pasteurization. In addition, thermosensitive bacteria that can grow in refrigerated milk and that produce thermostable spoilage enzymes (e.g., proteases) that are not inactivated by pasteurization are of concern (Murphy et al., 2016). For much of the 20th century, spoilage organisms introduced after pasteurization (e.g., *Pseudomonas* spp. and many types of coliforms, which can cause off flavors and defects) represented the major recognized spoilage concerns. For example, in the first volume of JDS in 1917, Harding et al. (1917) wrote, "The milk coolers and the bottling machines require special watching . . . not only because they frequently add large numbers of germs, but especially because they add them after the milk has been pasteurized." Although spoilage organisms introduced after pasteurization still remain a major concern and challenge throughout the world, many dairy processors have effectively reduced postprocessing contamination. For example, data from Cornell's Milk Quality Improvement program collected from 1991 to 2010 suggest that for many fluid milk processing plants in the northeastern United States, the ability to control postpasteurization contamination has significantly improved over time (Carey et al., 2005; Martin et al., 2012).

Bacterial spoilage organisms that survive commercial pasteurization include both thermotolerant nonsporeformers (e.g., *Microbacterium*, *Micrococcus*, *Streptococcus*, and *Lactobacillus*) and sporeforming bacteria (e.g., members of the order *Bacillales* and *Clostridiales*; Murphy et al., 2016). Sporeformers have long been recognized as spoilage organisms of concern in the dairy industry. For example, a 1981 review article in JDS (Collins, 1981) discussed that psychrotolerant bacteria in the genus *Bacillus* represent the most important heat-resistant psychrotolerant spoilage organisms in fluid milk; common defects associated with these organisms were described as sweet curdling as well as bitter, fruity, rancid, and yeasty flavors. The frequent occurrence of these organisms in raw milk also was described more than 45 yr ago; for example, Chung and Cannon (1971) reported the detection of psychrotrophic sporeforming bacteria in 83% of the raw milk samples obtained from 18 individual producers. Early efforts to classify psychrotolerant *Bacillus* spp. isolates from milk (e.g., Shehata and Collins, 1971) indicated that these isolates had characteristics similar to the *Bacillus* species *brevis*, *circulans*, *cereus*, *coagulans*, *laterosporus*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis*, although the isolates showed growth at temperatures lower than the growth temperature ranges reported for these species. In 1998, *Bacillus weihenstephanensis* was reported as a new species that belonged to the *B. cereus* group but was differentiated by its ability to grow at refrigeration temperatures (Lechner et al., 1998). Subsequent studies indicated that not all psychrotolerant strains in the *B. cereus* group necessarily represented *B. weihenstephanensis* (Stenfors and Granum, 2001), suggesting the existence of a distinct *Bacillus* species (*B. wiedmannii*) that is both psychrotolerant and able to cause disease (Miller et al., 2016). Further clarification of the classification of psychrotolerant sporeformers that cause milk spoilage started with the 1993 proposal to create a new genus *Paenibacillus* within the order *Bacillales* (Ash et al., 1993–1994); several psychrotolerant sporeformers obtained from pasteurized milk either were reclassified as *Paenibacillus* (e.g., *Bacillus polymyxa* became *Paenibacillus polymyxa*) or were identified and classified after description of the genus *Paenibacillus*. Improved definition and taxonomic classification of psychrotolerant *Bacillus* spp. following the description of *B. weihenstephanensis* in 1998 provided an improved ability to detect and define causes of HTST fluid milk spoilage due to the presence of psychrotolerant sporeformers (e.g., Huck et al., 2007a, b) and has led to the recognition that psychrotolerant sporeformers are the current biological limitation for HTST shelf-life extension of fluid milk past 24 d (Fromm and Boor, 2004; Ranieri and Boor, 2009).

1917 to 2017: Changing Microbiological Methods Affect Our Understanding of Dairy Microbiology

A key change that occurred between 1917 and 2017 is the evolution of methods for detection of microbes associated with milk and dairy products. We illustrate this with 3 examples, including (1) detection of the pathogen *L. monocytogenes*, (2) quantification of total bacterial numbers in raw milk, and (3) microbial indicator tests used for finished products.

A comprehensive review and summary of the development of different methods for detecting *L. monocytogenes* can be found in the book *Listeria, Listeriosis, and Food Safety*, particularly its 2 chapters dedicated to conventional and rapid methods for detection of *Listeria* (Brehm-Stecher and Johnson, 2007; Donnelly and Nyachuba, 2007). Direct plating was the initial method of choice for *L. monocytogenes* detection; however, often it was not successful in detecting the microbe. In 1948, the cold enrichment method was introduced by Gray et al. (1948), which essentially represented a nonchemical selective enrichment procedure. Starting in 1950, different selective chemical enrichment procedures that allowed for enrichment at 30 to 37°C were introduced and evaluated. Even following the introduction of selective enrichment and plating media, differentiation of the pathogenic *L. monocytogenes* from nonpathogenic *Listeria* spp. on plating media remained a challenge, particularly because product samples often can be contaminated with both *L. monocytogenes* and other nonpathogenic *Listeria* spp. Classical selective and differential plating media (e.g., Oxford agar, polymyxin-acriflavine-LiCl-ceftazidime-esculin-mannitol agar) do not differentiate between *L. monocytogenes* and nonpathogenic *Listeria* spp. Major breakthroughs that enabled improved detection of *L. monocytogenes* are represented by both molecular methods and chromogenic media that allowed differentiation between *L. monocytogenes* and nonpathogenic species. Today's standard methods for *L. monocytogenes* (or *Listeria* spp.) detection in dairy foods or processing plant environments typically involve a 24- to 48-h selective enrichment followed by a DNA amplification or antibody-based screening test or by plating on appropriate selective and differential media. Detection methods for other foodborne pathogens relevant to dairy (e.g., *Salmonella*, *Cronobacter*) have undergone similar improvements and evolution over the years.

Tests assessing total microbial loads in raw milk have played an important role in ensuring the safety and quality of raw milk for >100 yr. Even for pasteurized milk and dairy products, accurate methods for determining bacterial loads in raw milk are important for both quality and safety because the risk of pathogens and spoilage organisms surviving pasteurization increases with higher bacterial numbers in raw milk. Further, spoilage-associated enzymes that can be produced by bacteria present in raw milk may not be inactivated by pasteurization (Boor, 2001; Murphy et al., 2016). Consequently, federal and state regulatory agencies have developed standards for acceptable total bacteria counts in raw and pasteurized milk, whereas dairy industry organizations typically set more stringent standards. In 1924, the first version of the Standard Milk Ordinance required that grade A raw milk have an average bacterial count of <50,000 cfu/mL (with standards of <200,000, <1 million, and <5 million cfu/mL for grade B, C, and D milk, respectively). The most recent version of the PMO (which covers only grade A milk) requires <100,000 cfu/mL for individual producer grade A milk in the United States (USPHS/FDA, 2016). Today, minimum standards for grade B milk ("milk for manufacturing purposes") are recommended by the Dairy Division of the USDA Agricultural Marketing Service. These were first published in 1972; the current minimum standard for grade B milk was published in 2011 and is 500,000 cfu/mL (USDA Agricultural Marketing Service, 2011). Historically, the PMO has required that laboratory procedures be compliant with the *Standard Methods for the Examination of Dairy Products* (**SMEDP**; Jezeski, 1956; USPHS/FDA, 2016). The SMEDP was first published in 1910 and since then has been updated 16 times to reflect changes to accepted methods of assessing the bacterial qualities of milk and milk products. In the 1910 SMEDP, the SPC was included as the accepted method for determining the total number of viable aerobic bacteria in raw milk; this method is still widely used. In 1916, the direct microscopic count was introduced as an alternative to SPC; this method is no longer deemed acceptable in the PMO because it is not sufficiently sensitive or accurate for regulatory or quality purposes (Jezeski, 1956; Laird et al., 2004). In 1929, the methylene blue test was introduced and was immediately controversial because results were not consistent with SPC data (Thornton and Hastings, 1930); this test was removed from acceptable methods in the PMO in 1961 (Luchterhand et al., 2009). In the 1980s, 3M Petrifilm aerobic count and plate loop count were introduced as alternatives to SPC (Thompson et al., 1960; Ginn et al., 1984). By the 1990s, 3M Petrifilm aerobic count was determined acceptable and is still considered equivalent to SPC (USPHS/FDA, 2016). In the 2000s, BactoScan (flow cytometry) and spiral plate count were introduced as alternative methods (Donnelly et al., 1976; Gunasekera et al., 2000; USPHS/FDA, 2001, 2005). For more detail on method development and procedures between 1905 and 1955, refer to Jezeski (1956); changes between 1956 and 2004 are detailed in previous editions of SMEDP, whereas current methods are detailed in the 17th edition of SMEDP (Laird et al., 2004).

In addition to specific tests for pathogens or spoilage organisms, the dairy industry has been using microbial hygiene indicator testing to detect lapses in sanitation and postprocessing contamination at the processing level for almost 100 yr. Hygiene indicator organisms are microbial markers whose presence relates to the hygienic quality of the food or environment (Chapin

et al., 2014). Advances both in our understanding of different bacteria associated with dairy products and in the development of specific methods for rapidly and reliably detecting specific bacteria associated with milk, dairy products, and dairy-associated environments have changed the types of indicator organisms used today compared with those used in the early 20th century. The use of coliforms as hygiene indicator organisms in milk was first suggested in 1919, although methods were not developed until 1927 (Finkelstein, 1919; Kessler and Swenarton, 1927). Although initially introduced for raw milk testing, coliform testing was later applied to evaluating pasteurized products (McCrady and Langevin, 1932; Chilson et al., 1936). Currently, the PMO limits coliforms to <10 cfu/mL in pasteurized milk and milk products (USPHS/FDA, 2016). Although coliforms historically have been used as hygiene indicator organisms that indicate fecal contamination, several studies have shown that the majority of coliforms originate from environmental sources and that coliform detection in milk and dairy products rarely indicates actual fecal contamination (for a review see Martin et al., 2016). Recent studies also show that coliforms represent only 7.6 to 26.6% of bacteria introduced into fluid milk by postpasteurization contamination (Martin et al., 2012). Most important, coliform tests do not detect *Pseudomonas* spp., which have been shown to represent the majority of postprocessing contaminants in fluid milk (Cousin, 1982; Sørhaug and Stepaniak, 1997). Because coliforms are imperfect indicators of postprocessing contamination, other hygiene indicators have been proposed in the dairy industry and are increasingly being used (Hervert et al., 2016; Martin et al., 2016). Specifically, testing for *Enterobacteriaceae* provides a better indicator for postprocessing contamination because it detects a wider set of organisms that represent a taxonomically consistent group, including *Salmonella* and *Yersinia*, 2 pathogens of concern in dairy products that are not detected with the coliform test (Hervert et al., 2016). Although coliform standards are still included in the PMO and hence coliform testing is still frequently used in the US dairy industry as well as in some other countries (e.g., Japan), *Enterobacteriaceae* testing is the microbial indicator test of choice for most dairy products in many countries in the world, particularly in most of Europe. However, *Enterobacteriaceae* tests do not detect *Pseudomonas* spp. As such, tests that provide for quantification of total gram-negatives are increasingly recommended as an alternative hygiene indicator, particularly for dairy products in which postprocessing contamination with *Pseudomonas* is a concern (e.g., fluid milk, fresh cheeses; Van Tassell et al., 2012; Hervert et al., 2016; Machado et al., 2017). Although total bacterial counts can also be used as a hygiene indicator for some dairy products, the fact that both heat-resistant sporeformers and lactic acid bacteria (all of which represent gram-positive organisms) are also detected with these tests limits their value in many cases.

SUMMARY AND FUTURE DIRECTIONS

Despite the considerable advances that have been made in both our understanding of dairy microbiology and the application of this knowledge to improve dairy food safety (to a point where pasteurized milk and dairy products represent some of the safest foods available) and reduce food spoilage issues, considerable needs and opportunities remain for further advances in dairy microbiology. The emergence of new scientific technologies and tools such as whole-genome sequencing will provide new insight into currently unrecognized microbes in dairy products that can affect quality or that may present public health risks. On the other hand, changes in dairy production and processing, including new and improved processing technologies, will change the microbial ecology of dairy products, leading to recognition of different organisms not of previous concern to the dairy industry. For example, with increasing success of strategies for reducing raw milk contamination with vegetative bacterial cells as well as improved prevention of postpasteurization contamination, pathogenic and spoilage-associated sporeformers that survive pasteurization and heat treatments are likely to become an increasing concern for the dairy industry. In addition, parts of the dairy industry still rely on approaches and methods that have been used for >100 yr (e.g., coliform testing) and use knowledge that was created >60 yr ago (e.g., data on the time–temperature combinations used for HTST pasteurization; the 60-d holding period). Upgrades to both historical knowledge and procedures will thus also be essential for the dairy industry, particularly as requirements for science-based food safety practices are becoming more stringent around the world. Specific challenges and opportunities for different categories of dairy products are briefly discussed below.

Microbiological Challenges in Fluid Milk

Microbial food safety and quality issues will continue to evolve around (1) prevention of postpasteurization contamination and (2) control and reduction of thermotolerant and sporeforming organisms that can survive pasteurization, particularly HTST pasteurization. The specific challenges will depend on the type of pasteurization (HTST vs. UHT) used for production of fluid milk. Although UHT typically refers to a process that

includes aseptic packaging, microbiological challenges in this process still involve postpasteurization contamination, although mainly in facilities that may not have sophisticated quality management systems. There also are sporeformers that survive UHT treatment—in particular *Bacillus sporothermodurans*, which also has been identified in dairy farm environments (e.g., Scheldeman et al., 2006)—even though UHT spoilage by sporeformers occurs rarely. However, control of sporeformers that survive UHT treatment may be of some importance. Bacterial spoilage and food safety issues represent a much larger challenge for HTST milk. Postpasteurization contamination with psychrotolerant spoilage organisms (e.g., *Pseudomonas*) is not uncommon in many facilities around the world, and the development and consistent implementation of improved strategies for controlling these microbes will be important for this industry. It is likely that improved tools for source tracking (including whole-genome sequencing and metagenomics tools) will allow for an improved understanding of contamination sources and hence will facilitate improved control strategies for the prevention of postpasteurization contamination, which will also reduce the risk of pathogen introduction (in particular *L. monocytogenes*). In HTST processing facilities that effectively control postpasteurization contamination, sporeforming pathogens—for example, *B. cereus* and the cold-growing species *B. wiedmannii* (Miller et al., 2016)—and spoilage sporeformers that can grow at refrigeration temperatures (e.g., *Paenibacillus* spp.) will become major challenges for the industry. Control of sporeformers will require new systems approaches that will need to include efforts to minimize introduction of these organisms at the farm level as well as optimization and development of processing strategies that allow for spore removal (e.g., microfiltration).

Microbiological Challenges in Dairy Powders

Dairy powders (e.g., whey powders, milk powders) are becoming an increasingly important product for many countries (e.g., New Zealand, United States) and dairy processors. Because milk powders are an important ingredient in infant formula, control of pathogens (including but not limited to sporeformers such as *B. cereus* and *C. perfringens*) will be of particular importance for this group. For dairy powders used to produce foods for highly susceptible population subgroups, such as infants, control of opportunistic pathogens (including but potentially not limited to *Cronobacter* spp.) that may not be of concern in other products will also become increasingly important. In these products, nonpathogens that carry antimicrobial resistance genes in transferrable genetic elements, as well as the presence of antimicrobial resistance elements in general, may also emerge as a concern. With regard to spoilage organisms, control of a wide range of sporeformers, including thermophilic, mesophilic, and psychrotolerant sporeformers as well as *Clostridium* spp., will be of continued importance, particularly because dairy powders can be used as ingredients in a wide range of products, including reconstituted milk that then will be used to manufacture a range of dairy products (e.g., cheese, UHT milk). Hence, use of a systems approach to reduce sporeformers will be of particular importance for dairy powders, particularly because there is convincing evidence that for some thermophilic sporeformers contamination sources typically are located in the processing equipment (i.e., at locations that feature high temperatures that facilitate the growth of these organisms, such as regeneration sections of HTST units). Development and implementation of improved source tracking tools and better understanding of the ecology and diversity of powder-associated sporeformers will hence also be of particular importance for the control of sporeformers in the dairy powder production chain. Importantly, although specifications for sporeformer levels play an important role in dairy powders, methods for detection and enumeration of sporeformers in dairy powders show limited standardization and often involve different heat inactivation time–temperature combinations and media types. Standardization of spore detection methods for different sporeformer groups (e.g., thermophilic, mesophilic, and psychrotolerant sporeformers) thus will also be important.

Microbiological Challenges in Cheese

Cheeses represent a broad category, ranging from fresh cheeses with near-neutral pH to cheeses with low water activity and low pH that have been aged for 2 yr and beyond. Consequently, microbiological challenges associated with cheeses are equally diverse. With regard to food safety, rational, science-based approaches to ensuring the safety of raw milk cheeses, which are culturally and economically important products in many parts of the world, remain a major challenge that will need to be addressed. Improved control strategies for *L. monocytogenes* in cheese also remain a major challenge, particularly with several human listeriosis outbreaks associated with cheese worldwide linked to this high-fatality pathogen. Finally, there is a need for science-based information and data that support how sporeforming pathogens that can survive pasteurization (e.g., *B. cereus*) are controlled in different cheese types. With regard to spoilage, in certain types of hard cheeses, control of *Clostridium tyrobutyricum* is likely to continue to be a concern, particularly because the trend

toward "clean" labels makes addition of compounds that control growth of this organism less desirable. A systems approach that includes premium programs for raw milk that shows low numbers of this sporeformer may become more common in different countries of the world. Elimination and control of postpasteurization contamination with spoilage organisms will also remain an important area in cheese. Although bacterial spoilage organisms that lead to defects that are easily detected by consumers (e.g., blue color formation by *Pseudomonas*; Martin et al., 2011) are predominantly of concern in fresh and high pH cheeses, mold growth is a concern for a wide range of products. Development and implementation of improved tools for source tracking and for identification of spoilage organisms in cheese thus likely will be an important area of emphasis. Although cheese is the main dairy product in which production of mycotoxins may present a concern (Sengun et al., 2008), no foodborne illness cases due to mycotoxin contamination of cheese have been reported (Hymery et al., 2014). In addition to mycotoxin production due to mold growth on products, mycotoxins can be introduced into all dairy products through indirect contamination, which results when dairy cows ingest feed that contains mycotoxins that pass into the milk, such as aflatoxin M1 (Sengun et al., 2008).

Microbiological Challenges in Other Fermented Dairy Products

Microbial food safety and spoilage issues are generally of limited concern with fermented dairy products other than cheese because these products (e.g., yogurt) typically are characterized by low pH. However, fungal (i.e., yeast and mold) contamination is a related challenge; similar to cheese, we were not able to find records of mycotoxin-related illnesses attributable to mold-contaminated yogurt (mold growth is also less common in yogurt). Fungal related concerns in yogurt are increasing with the clean label trend, which is driving the need to eliminate antifungal compounds from these products. Thus, development and implementation of improved tools for source tracking and identification of fungal spoilage organisms will be important. In addition, development of alternative approaches to control yeast and mold (e.g., protective cultures that prevent or reduce growth of yeast and mold) will also be important for this sector of the dairy industry.

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APPENDIX

Table A1. Major milestones in milk and dairy food safety

Date	Milestone	Reference
1924	The US Public Health Service releases the Standard Milk Ordinance (precursor to today's Pasteurized Milk Ordinance), which defined pasteurization conditions targeting destruction of *Mycobacterium tuberculosis*.	Frank, 1924
1947	Michigan becomes the first US state to require pasteurization of milk.	Steele, 2000
1950	A 60-d holding period requirement for raw milk cheese is published in the United States.	Boor, 2005
1957	Minimum pasteurization time and temperature conditions are increased to ensure the destruction of *Coxiella burnetii*.	Enright et al., 1957
1980–1982	A series of *Salmonella* Muenster outbreaks in Canada is linked to survival of *Salmonella* in raw milk Cheddar that had been aged for at least 125 d.	Wood et al., 1984
1985	Jalisco cheese *Listeria monocytogenes* outbreak occurs in California.	Linnan et al., 1988

Continued

Table A1 (Continued). Major milestones in milk and dairy food safety

Date	Milestone	Reference
1986	Two cases of *Escherichia coli* O157:H7 infections are described in children who consumed raw milk on farms where *E. coli* O157:H7 was subsequently isolated from fecal samples from dairy cows, suggesting raw milk as a potential vehicle for human O157:H7 infections.	Martin et al., 1986
1986–1987	Three cases of neonatal infection caused by *Enterobacter sakazakii* (now named *Cronobacter*) are identified in a hospital in Reykjavík, Iceland; these appear to be the first *Cronobacter* cases definitively linked to infant formula.	Biering et al., 1989
1987	The US Food and Drug Administration (FDA) publishes a final regulation mandating the pasteurization of all milk and milk products in final package form for direct human consumption; this regulation banned the shipping of raw milk in interstate commerce.	US Federal Registrar, 1987
1990	After the invention of PCR in 1985, the first PCR assay for *Listeria monocytogenes* is reported.	Border et al., 1990
1996	PulseNet is launched by the Centers for Disease Control and Prevention (CDC).	Swaminathan et al., 2001
1998	*Bacillus weihenstephanensis* is described as a new psychrotolerant species of the *Bacillus cereus* group, which is found in heat-treated milk.	Lechner et al., 1998
2004	Psychrotolerant *Paenibacillus* is indicated as an important cause of HTST fluid-milk spoilage.	Fromm and Boor, 2004
2008	A large listeriosis outbreak with 38 human cases in Québec, Canada, is linked to pasteurized soft-textured cheese; cross-contamination of different cheeses in retail establishments is identified as a contributing factor.	Gaulin et al., 2012
2013	The FDA and CDC start routine use of whole-genome sequencing to characterize *Listeria monocytogenes* obtained from human cases and from foods.	Jackson et al., 2016
2015	Whole-genome sequencing links listeriosis outbreak to ice cream in the United States.	CDC, 2015

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The use of national-level data to describe trends in intramammary antimicrobial usage on Irish dairy farms from 2003 to 2015

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ABSTRACT

In this study, we used national-level data to describe trends in on-farm intramammary antimicrobial usage in Ireland from 2003 to 2015. We calculated actual sales of intramammary tubes and the quantity of active substance sold, by year, product type [lactation or dry cow therapy (DCT)], antimicrobial group, World Health Organization antimicrobial classification, and from 2009 to 2015, prescribing route. We also estimated on-farm usage of lactation and dry cow intramammary antimicrobials using defined daily dose (DDDvet) and defined course dose (DCDvet) calculations, and dry cow coverage. Sales of tubes of antimicrobial for DCT have increased, and the estimated national dry cow coverage in 2015 was 1,022 DCDvet per 1,000 cows per year. An increase has also occurred in sales of teat sealant (2015 sales: 66.7 tubes with teat sealant for every 100 tubes with antimicrobial for DCT). In contrast, the number of tubes of antimicrobial sold for lactation use has decreased to 1,398 DDDvet and 466 DCDvet per 1,000 animals per year. Sales in intramammary tubes with at least one critically important antimicrobial (CIA) have either risen since 2007 (DCT) or fallen (lactation therapy). Increases were observed in both the number of dry cow and lactation tubes containing CIA considered of highest priority for human health. Differences between prescribing routes with respect to CIA usage were observed. This study provides detailed insight into on-farm usage of intramammary antimicrobials in Ireland. It demonstrates positive national progress but also highlights areas for review. In particular, blanket dry cow treatment in Ireland should be reconsidered. It is not possible to investigate farm-level variation in antimicrobial usage from national sales data. In several countries, measurement and benchmarking have been critical to progress in reducing antimicrobial usage in farm animal production. Central collation of data on farm-level antimicrobial use is also needed in Ireland to allow objective measurement and benchmarking of on-farm usage. More generally, standardized indicators to quantify antimicrobial usage in farm animals are urgently needed to allow country-level comparisons.
Key words: intramammary, antimicrobial, critically important, mastitis, Ireland

INTRODUCTION

Mastitis is an important challenge to dairy production internationally. In Ireland, the problem is well understood (More et al., 2012), and considerable work has been undertaken to quantify the economic losses associated with mastitis, both to farmers (Geary et al., 2012) and the processing industry (Geary et al., 2013). In addition to direct monetary concerns, mastitis can adversely affect cow welfare (Medrano-Galarza et al., 2012) and farm management (Jansen et al., 2010), increase the risk of antimicrobial residues (van Schaik et al., 2002), and adversely affect product quality and the international reputation of milk and milk products (More, 2009). A national mastitis control program, CellCheck, was established by Animal Health Ireland (http://animalhealthireland.ie) in late 2010, and farmers now have considerable resources to assist with on-farm mastitis control. In this time, bulk tank somatic cell counts (**BTSCC**), a key measure of udder health, have had a substantial national improvement (Animal Health Ireland, 2016).

In recent years, concern about the use of antimicrobials in animal production has increased. Antimicrobials are a global common good, and prudent use, both in humans and animals, is critical to their long-term effectiveness. In dairy production, antimicrobials are used for a range of animal diseases, but most frequently for either the prevention or treatment of mastitis (Oliver et al., 2011), either lactation or dry cow therapy (**DCT**), as part of a broader mastitis control strategy. Intramammary antimicrobials represent a small proportion of the total quantity of antimicrobials used in farm animal production. In Ireland, for example, 3.8% of veterinary

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antimicrobials sold in 2014 were for intramammary use, either for lactation (0.6%) or dry cow (3.2%) therapy (Health Products Regulatory Authority, 2015).

Methods to quantify on-farm antimicrobial usage are recognized as central to the broader discussion about prudent antimicrobial usage in food production. With such information, it is possible to evaluate temporal trends in usage and to facilitate within-country benchmarking and between-country comparison and studies on drivers for on-farm antimicrobial usage. Considerable progress in this regard has been made in several European countries, including Denmark (Wielinga et al., 2014; DANMAP, 2015) and the Netherlands (Speksnijder et al., 2015; Autoriteit Diergeneesmiddelen, 2016), where farm-level usage data are collected routinely. This is not the case in Ireland, where farm-level usage data are only available as part of defined studies with a primary focus on antimicrobial resistance (Gibbons et al., 2016). However, national sales data are available and have previously provided some insights into farm-level usage (More et al., 2012; Health Products Regulatory Authority, 2015).

Building on earlier work (More et al., 2012), in this study we used national-level data to describe trends in intramammary antimicrobial usage on Irish dairy farms from 2003 to 2015. The results are presented using agreed indicators of on-farm antimicrobial usage to allow inter-country comparison.

MATERIALS AND METHODS

The Data

National Intramammary Antimicrobial Sales Data. Kynetec (Newbury, Berkshire, UK), an international market research company specializing in agriculture and animal health, gathers data on all intramammary sales conducted through each of the 5 main veterinary wholesalers in Ireland. According to Kynetec, this is likely to represent an estimated 85% of all sales of these products in Ireland. We obtained data from Kynetec, summarized for each year from 2003 to 2015 inclusive, of national sales of intramammary products for cows during lactation and at drying off.

In this data set, the reference population was all dairy herds in Ireland, and the unit of interest was the quantity of intramammary antimicrobial product used, expressed either as a quantity of active substance (kg) or number of intramammary tubes. The number of intramammary tubes containing teat sealant was also of interest. The period of interest was 2003 to 2015.

Schedule 8 Prescribing Data. Under national legislation (European Communities, 2007), antimicrobial veterinary medicinal products may only be supplied in Ireland on the basis of a prescription from a registered veterinary practitioner. Further, the animal(s) to which the prescription relates must be under the care of the practitioner. This requires that the practitioner has sufficient knowledge of the animal(s) to form an opinion of the condition of the animal(s) and has visited the animal(s) sufficiently often and recently enough, and at least once in a 12-mo period, to have acquired an accurate picture of the current health, welfare, and disease status of the animal(s). The above-mentioned 12-mo period does not apply to the prescribing of an intramammary antimicrobial agent if the animal belongs to a herd covered by a program meeting the requirements of schedule 8 (within this legislation). Schedule 8, which is unique to Ireland, outlines the requirement of such a program and states that the primary purpose of the program is the prevention and treatment of clinical and subclinical mastitis. The roles and responsibilities of the milk purchaser, the milk supplier (the farmer), and the veterinary practitioner under whose direction the program operates are outlined in schedule 8. Intramammary antimicrobial agents may be prescribed to farmers under either the routine or schedule 8 prescribing routes.

We obtained national data for 2009 to 2015 from the national Department of Agriculture, Food and the Marine on the number and type of intramammary antimicrobial tubes supplied through the schedule 8 prescribing route, both for lactation and DCT. However, anomalies were identified in the data from 2010 for reasons that are not clear. In this study, only the 2011 to 2015 data were used.

Dairy Cow Numbers. Data on the number of dairy cows in Ireland each year between 2003 and 2015 were obtained from Eurostat, the statistical office of the European Union, either directly from their website (data for 2004–2015; http://ec.europa.eu/eurostat) or from the website of the Agricultural and Horticultural Development Board (2003; http://dairy.ahdb.org.uk). These data had been derived from the Irish Animal Identification and Movement database (https://www.agriculture.gov.ie) and collected under Regulation 1165/2008 (European Council, 2008), with dairy cows being defined as "cows kept exclusively or principally for the production of milk for human consumption and/or for processing into dairy products, including cull cows for slaughter (whether fattened or not between last lactation and slaughter)."

Data Analysis

Actual National Sales. The national sales data from Kynetec were analyzed to determine the number of tubes sold, by year and product type (lactation, dry

cow), the number of dry cow intramammary tubes sold, with either active antimicrobial substance or teat sealant, and the quantity of active substance (kg) sold annually, by year, product type, and antibiotic group.

WHO Antimicrobial Classification. The World Health Organization (**WHO**) has classified antimicrobials with respect to importance for human medicine (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, 2012). Under this system, an antimicrobial that meets both of the following criteria is considered critically important to human health (**CIA**), or highly important if only one criterion is met:

- An antimicrobial agent that is the sole, or one of limited available therapies, to treat serious human disease,
- Antimicrobial agent is used to treat diseases caused by either (1) organisms that may be transmitted to humans from nonhuman sources or (2) human diseases caused by organisms that may acquire resistance genes from nonhuman sources.

The following are relevant to the current study:

- CIA include aminoglycosides (including dihydrostreptomycin, framycetin sulfate, kanamycin, neomycin, and streptomycin), 3rd and 4th generation cephalosporins [including cefoperazone (3rd) and cefquinome (4th)], macrolides (including erythromycin), and penicillins (natural, aminopenicillins, antipseudomonal, including amoxicillin, ampicillin, benethamine penicillin, penethamate hydriodide, procaine benzylpenicillin),
- Highly important antimicrobials for human medicine include 1st and 2nd generation cephalosporins [including cefacetrile, cefapirin, cephalexin, cephalonium (1st)], lincosamides (including lincomycin and pirlimycin hydrochloride), penicillins (antistaphylococcal, including cloxacillin and nafcillin), sulfonamides, dihydrofolate reductase inhibitors and combinations (including sulfadiazine, trimethaprim), and tetracyclines (including oxytetracyclines).

Novobiocin sodium (an aminocoumarin; classified as important for human medicine) was the only other intramammary antimicrobial used in Ireland during the period of interest.

A subgroup of CIA has recently been defined by the WHO, termed highest priority CIA (**HP CIA**), which includes 3rd and 4th generation cephalosporins, glycopeptides, macrolides, quinolones, and potentially carbapenems (Collignon et al., 2016). In this study, only the 3rd and 4th generation cephalosporins and macrolides were used as intramammary antimicrobial products during the study period.

We first classified all intramammary antimicrobials sold in Ireland according to these 2 WHO systems of classification. We then calculated the quantity and percentage of active substance (kg) and the number of tubes, by product type (lactation, dry cow) and WHO antimicrobial classification (CIA, HP CIA).

Prescribing Route. The aforementioned calculations were also conducted on the schedule 8 prescribing data. With the schedule 8 prescribing data, the total number of tubes supplied for lactation therapy and for DCT were available for all milk purchasers (13 milk purchasers in 2011, 12 from 2012 to 2015), whereas finer detail of the number of each type of tube sold (the number of tubes by product) was not always available (these data were not available from milk purchaser A in 2011 to 2014, milk purchaser B in 2011 to 2015, and milk purchaser C in 2014; accounting for between 0.8 and 6.5% of lactation tubes and between 1.5 and 6.2% of dry cow tubes). We assumed that the relative distribution, by WHO classification, of antimicrobials among lactation tubes and among dry cow tubes was the same among milk purchasers for which these finer data were and were not available.

Using both the total sales and schedule 8 prescribing data, we then calculated the quantity and percentage of active substance (kg) and the number of tubes, by product type (lactation, dry cow), WHO antimicrobial classification (CIA, HP CIA), and prescribing route. Separately for each WHO classification, 2 logistic regression models, one for tubes and one for quantity of antimicrobial agents, were developed in SAS version 9.3 (SAS Institute Inc., Cary, NC) to model the proportion of tubes with at least one CIA or HP CIA, or agents that were CIA or HP CIA. The models included year, product type, and prescribing route. Multiple comparisons of the prescribing route by year and product type were accounted for using a Bonferroni correction. Differences were considered to be statistically significant if $P > 0.05$.

Estimated On-Farm Usage. All subsequent calculations were conducted using the national sales data, using indicators of antimicrobial usage as recommended by Collineau et al. (2017). The European Medicines Agency has recently proposed a defined daily dose for animals (**DDDvet**) and the defined course dose for animals (**DCDvet**) for intramammary antimicrobial products in cattle (European Medicines Agency, 2016), drawing on principles presented previously (European Medicines Agency, 2013, 2015). All lactation intramammary products are assigned a DDDvet of 1 unit dose (**UD**, equivalent to an intramammary tube)/teat. All of these products are also assigned a DCDvet of 3 UD/

teat, except for products containing pirlimycin, which have a DCDvet of 8 UD/teat because the number of treatment days is substantially higher than for other lactation intramammary products. For dry cow intramammary products, a DCDvet of 4 UD/udder is assigned.

Therefore, for lactation products, we calculated the number of DDDvet per 1,000 animals per year as

(total number of tubes sold for lactation usage/ assigned DDDvet for each tube)/number of lactating cows at risk of clinical mastitis each year × 1,000,

where total number of tubes sold for lactation usage was assumed to equal the number of lactation tubes recorded by Kynetec/0.85, the assigned DDDvet for each tube was as stated by the European Medicines Agency (2016), and the total number of lactating cows at risk of clinical mastitis each year was considered to be the total number of adult dairy cows. To allow comparison with other studies, this indicator was converted to DDDvet per 1,000 cow-days by dividing DDDvet per 1,000 animals per year by [the mean intercalving interval/365 × (365 – mean length of the dry period)]. From 2008 to 2015 in dairy herds with more than 30 calvings (the only herds and years with available data), the mean intercalving interval was 398 d (Irish Cattle Breeding Federation, 2016). Data on the mean length of the dry period in Ireland were not available, and it was assumed to be 60 d.

The number of DCDvet per 1,000 animals per year was

(total number of tubes sold for lactation usage/ assigned DCDvet for each tube)/number of cows at risk of clinical mastitis each year × 1,000,

where total number of tubes sold for lactation usage was assumed to equal 1/0.85 × the number of lactation tubes recorded by Kynetec, the assigned DCDvet for each tube was as stated by the European Medicines Agency (2016), and the total number of lactating cows at risk of clinical mastitis each year was considered to be the total number of adult dairy cows. To allow comparison with other studies, this indicator was converted to DCDvet per 1,000 cow-days by dividing DCDvet per 1,000 animals per year by [the mean intercalving interval/365 × (365 – mean length of the dry period)].

For dry cow products, we calculated:

The number of DCDvet per 1,000 animals per year as

(total number of dry cow tubes sold/assigned DCDvet value for each tube)/total number of lactating dairy cows eligible for DCT each year × 1,000,

where total number of dry cow tubes sold was assumed to equal 1/0.85 × the number of dry cow tubes recorded by Kynetec, the assigned DCDvet for each tube was as stated by the European Medicines Agency (2016), and the total number of lactating dairy cows eligible for DCT each year was calculated as the total number of adult dairy cows × (1 − the annual replacement rate) × 365/mean intercalving interval. We assumed that DCT was not administered to nulliparous heifers or to cows at the end of their final lactation before culling. From 2008 to 2015 in dairy herds with more than 30 calvings (the only herds and years with available data), the mean annual replacement rate was 20.4% and the mean intercalving interval was 398 d (Irish Cattle Breeding Federation, 2016).

We estimated national DCT coverage (the percentage of lactating cows receiving dry cow intramammary antimicrobial therapy at drying off) after considering the number of lactating dairy cows eligible for DCT (as above), the total number of DCT tubes sold, and varying assumptions about the relationship between Kynetec sales data and on-farm usage.

Data management and analyses were conducted using MS Excel (Microsoft Corp., Redmond, WA). For each measurement over time, a linear regression model was fitted using SAS version 9.3 (SAS Institute Inc.) to test whether there was a linear trend, a quadratic trend, or no change over time. Initially a model that included a linear term (time) and a quadratic term ($time^2$) was tested; however, the quadratic term was dropped if $P > 0.05$ and similarly the linear term (time) was also dropped if $P > 0.05$.

RESULTS

Actual National Sales

Number of Tubes and Quantity of Active Substance. From 2003 to 2015, a decrease ($P = 0.005$, of ~26,000 tubes per year) occurred in the number of tubes of intramammary antimicrobials sold for lactation therapy (Figure 1). During the same period, an increase ($P < 0.001$, of ~106,000 tubes per year) occurred in the number of tubes of intramammary antimicrobials sold in Ireland for DCT, based on sales data collated by Kynetec (Figure 1). An increase also occurred in the number of tubes of teat sealant sold ($P < 0.001$, of ~211,000 tubes per year; Figure 2). From 2011 to 2015,

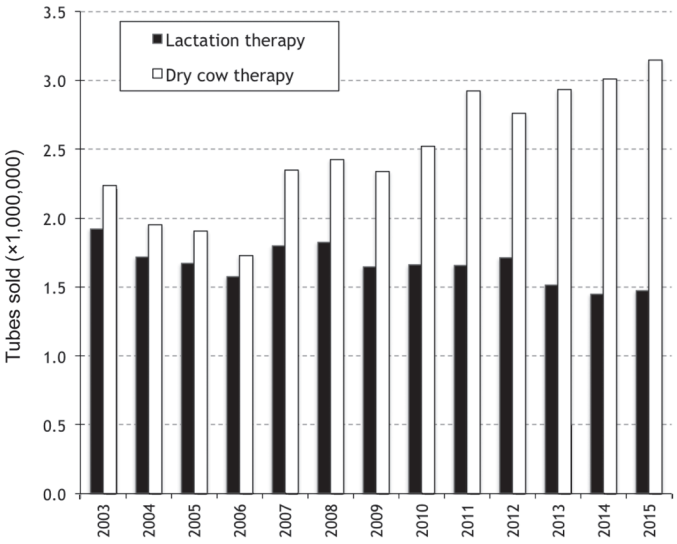

Figure 1. The number of tubes of lactation and dry cow intramammary antimicrobials sold in Ireland from 2003 to 2015, based on sales data collated by Kynetec (Newbury, UK). These sales data represent approximately 85% of actual on-farm usage.

the number of tubes of teat sealant sold each year was 64 to 67% of the total number of tubes of intramammary antimicrobials sold for DCT in the same year.

The quantity of active substance (kg) in intramammary antimicrobial tubes sold annually in Ireland from 2003 to 2015, by product type (lactation and DCT) and antimicrobial group, is shown in Table 1.

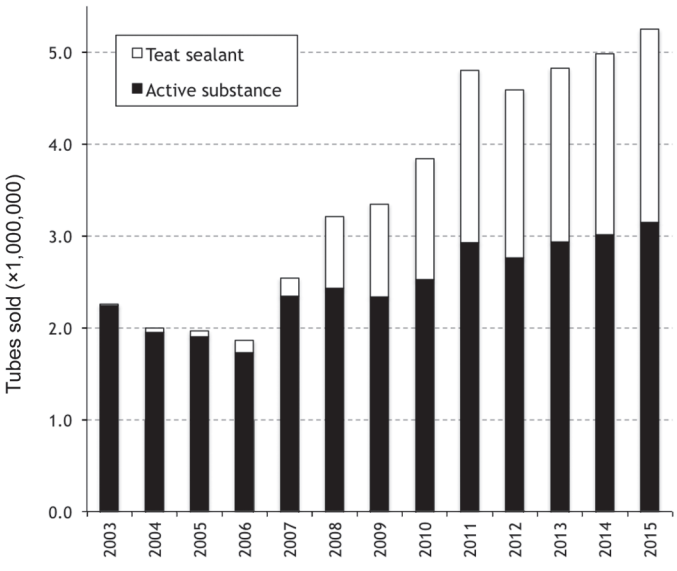

Figure 2. The number of dry cow intramammary tubes sold in Ireland from 2003 to 2015 containing either active antimicrobial substance or teat sealant. These sales data, collated by Kynetec (Newbury, UK), are estimated to represent approximately 85% of actual on-farm usage.

WHO Antimicrobial Classification. All antimicrobials sold were classified as either CIA or highly important for human medicine, except novobiocin (an aminocoumarin), which accounted for 10 and 0% by weight of active substance sold in 2015 for lactation and DCT, respectively (Table 1, Figure 3). In 2015, 6.9 and 5.4% of lactation and DCT tubes contained a HP CIA, respectively. Of the HP CIA, erythromycin (a macrolide) was used from 2003 to 2005 only, whereas cefoperazone (a 3rd generation cephalosporin) was used throughout the study period (2003–2015) for lactation therapy, and cefquinome (a 4th generation cephalosporin) throughout the study period and from 2009 in increasing quantities, for lactation and DCT, respectively (Table 1).

From 2003 to 2015, a decrease ($P = 0.002$, by ~28,000 tubes per annum) occurred in the number of lactation tubes sold with a CIA (Figure 3a), but no change ($P = 0.240$) occurred in the number of lactation tubes sold with no CIA (Figure 3a). During the same period, an increase ($P < 0.001$) was observed in the number of lactation tubes (an increase of ~8,000 per year) sold containing HP CIA (Figure 3b). From 2003 to 2015, the number of tubes sold annually in Ireland of dry cow antimicrobials with at least one CIA initially fell but has been increasing from 2007 (a quadratic curve, with an annual increase from 2007 of ~31,000 tubes per year, $P = 0.036$). The number of dry cow tubes with no CIA increased ($P < 0.001$) from 2003 to 2015, by ~87,000 per year (Figure 3a). From 2003 to 2015, an increase ($P < 0.001$) was observed in the number of dry cow tubes (an increase of ~18,000 per annum) sold containing HP CIA (Figure 3b).

Generally similar patterns were observed when considering the quantity of active substance sold annually. With respect to the quantity of active substance sold for lactation therapy, a decrease ($P < 0.001$) occurred in the sale of CIA from 2003 to 2015, but no change was observed among either highly important ($P = 0.508$) or other ($P = 0.632$) antimicrobials (Supplemental Figure S1a; https://doi.org/10.3168/jds.2016-12068). During the same period, an increase ($P < 0.001$) occurred in the quantity of antimicrobials with HP CIA that were sold (Supplemental Figure S1b; https://doi.org/10.3168/jds.2016-12068). With respect to the quantity of active substance sold for DCT, an initial fall occurred, then an increase from 2007 (a quadratic curve, with an annual increase from 2007 of ~12,000 tubes per year, $P = 0.012$) in CIA (Supplemental Figure S1a; https://doi.org/10.3168/jds.2016-12068). From 2003 to 2015, an increase ($P = 0.001$) occurred in the sales of highly important antimicrobials, but no change ($P = 0.111$) for other antimicrobials. From 2003 to 2015, an increase ($P < 0.001$) occurred in the quantity of active substance

Table 1. The quantity of active substance (kg) in intramammary antimicrobial tubes sold annually in Ireland from 2003 to 2015, by product type (lactation and dry cow therapy) and antibiotic group

Item	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Lactation therapy													
Aminocoumarin	56.5	50.3	45.4	40.6	48.7	43.0	41.5	51.6	51.1	50.4	52.9	43.3	44.5
Aminoglycosides	269	238	227	206	230	260	219	169	171	183	191	163	172
Cephalosporins													
1st generation	85.5	80.0	73.8	70.9	68.5	77.7	74.7	89.0	89.4	90.4	85.7	76.8	79.1
3rd generation	1.0	1.3	1.8	1.4	2.2	2.5	1.8	2.0	1.6	1.5	1.3	1.1	1.2
4th generation	2.0	2.1	3.6	3.6	4.0	4.5	4.4	8.3	9.2	9.8	10.1	7.9	7.3
Lincosamides	10.5	5.4	4.6	3.6	3.4	4.7	3.4	3.9	3.3	2.7	3.8	4.3	4.2
Macrolides	0.9	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillins													
Narrow spectrum													
β-Lactamase sensitive	120	104	99.8	89.2	102	108	91.6	55.2	55.2	63.1	70.2	60.6	64.0
Penicillinase-resistant	3.1	2.7	3.1	3.8	2.3	1.7	1.6	2.9	3.1	2.8	2.4	1.6	1.7
Moderate spectrum	1.1	1.0	1.2	1.4	0.9	0.6	0.6	1.1	1.2	1.1	0.9	0.6	0.7
Broad spectrum	77.7	69.5	71.2	73.4	84.4	81.6	81.6	99.8	96.3	95.7	47.8	72.5	70.7
Sulfonamides	1.7	1.5	1.2	0.6	17.1	5.0	3.1	3.7	3.3	2.1	3.3	1.7	1.4
Tetracyclines	3.6	3.5	4.1	3.8	4.6	11.9	4.4	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal	632	560	537	498	567	601	528	487	485	503	470	434	447
Dry cow therapy													
Aminocoumarin	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aminoglycosides	55.7	45.4	40.3	34.9	35.8	92.6	67.6	39.9	41.5	105	127	147	148
Cephalosporins													
1st generation	147	155	164	170	213	271	276	297	327	415	426	478	481
3rd generation													
4th generation	0.0	0.0	0.0	0.0	0.0	0.0	1.1	5.7	22.8	22.7	25.6	22.1	25.3
Lincosamides													
Macrolides													
Penicillins													
Narrow spectrum													
β-Lactamase sensitive	203	162	144	115	128	138	137	167	177	106	134	132	160
Penicillinase-resistant	652	529	510	445	675	637	539	507	580	482	524	483	500
Moderate spectrum	160	146	144	132	205	145	119	137	140	135	123	126	128
Broad spectrum													
Sulfonamides													
Tetracyclines													
Subtotal	1,218	1,038	1,003	896	1,256	1,284	1,139	1,153	1,288	1,266	1,359	1,388	1,443
Total	1,850	1,598	1,540	1,395	1,823	1,885	1,667	1,640	1,773	1,769	1,829	1,821	1,889

with HP CIA sold for DCT (Supplemental Figure S1b; https://doi.org/10.3168/jds.2016-12068).

Estimated On-Farm Usage

Usage by Prescribing Route. The estimated percentage of tubes supplied through the schedule 8 prescribing route did not change between 2011 and 2015, either for lactation ($P = 0.252$) or dry cow ($P = 0.139$) therapy (Figure 4). In 2015, the schedule 8 prescribing route was used extensively, representing 45 and 51% of lactation and dry cow tubes, respectively.

Different patterns were observed when comparing prescribing routes with respect to CIA (Table 2) and HP CIA (Table 3). With DCT, the odds of prescribing CIA was greater, and of HP CIA much greater, through the routine compared with the schedule 8 prescribing route. In 2015, for example, the odds of prescribing a tube with at least one CIA or at least one HP CIA by the routine route was 2.09 and 19.3 times greater, respectively, compared with those prescribed by schedule 8. For lactation therapy, significantly more CIA were prescribed through schedule 8 compared with the routine prescribing route, whereas the converse was true

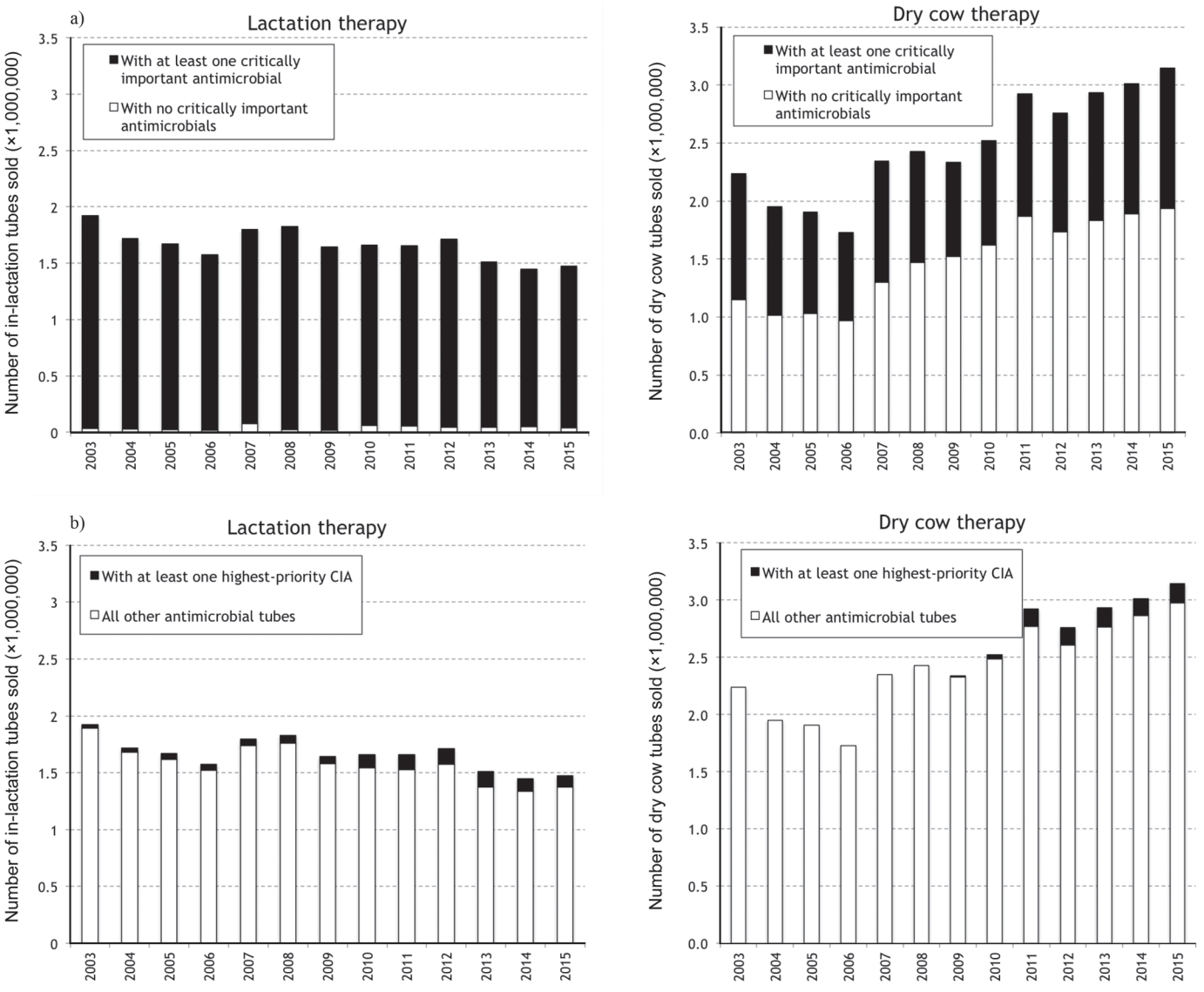

Figure 3. Number of tubes of dry cow and lactation intramammary antimicrobials for lactation (left) and dry cow (right) therapy, sold annually in Ireland from 2003 to 2015, containing either (a) at least one or no critically important antimicrobials (CIA) for human medicine and (b) at least one or no highest priority critically important antimicrobials for human medicine. The figures are based on sales data collated by Kynetec (Newbury, UK).

Table 2. Comparison of prescribing route for critically important antimicrobials in Ireland from 2011 to 2015, by method of measurement (number of tubes, kg of active compound), product type (dry cow and lactation therapy), and year

Item	Year	Prescribing route		Odds ratio[1]	95% Confidence limits		*P*-value[2]
		Routine	Schedule 8		Lower	Upper	
Number of tubes, % of tubes with at least one critically important antimicrobial							
Dry cow therapy							
	2011	45.5	28.8	2.07	2.06	2.07	<0.001
	2012	46.4	31.2	1.90	1.90	1.91	<0.001
	2013	46.0	29.8	2.01	2.00	2.02	<0.001
	2014	44.7	29.7	1.92	1.91	1.93	<0.001
	2015	47.3	30.0	2.09	2.09	2.10	<0.001
Lactation therapy							
	2011	96.2	97.8	0.57	0.56	0.58	<0.001
	2012	97.4	97.9	0.82	0.80	0.83	<0.001
	2013	97.1	97.4	0.90	0.88	0.92	<0.001
	2014	96.0	98.1	0.46	0.45	0.47	<0.001
	2015	96.0	99.2	0.21	0.20	0.21	<0.001
Quantity of antimicrobials, % of critically important among all antimicrobials							
Dry cow therapy							
	2011	50.7	18.2	4.63	4.60	4.67	<0.001
	2012	52.4	19.2	4.62	4.59	4.66	<0.001
	2013	46.5	18.3	3.87	3.85	3.90	<0.001
	2014	44.5	18.9	3.45	3.42	3.47	<0.001
	2015	47.9	19.3	3.84	3.81	3.87	<0.001
Lactation therapy							
	2011	72.4	75.8	0.84	0.83	0.85	<0.001
	2012	74.8	75.6	0.96	0.94	0.97	<0.001
	2013	73.5	74.6	0.95	0.94	0.96	<0.001
	2014	74.5	76.2	0.92	0.90	0.93	<0.001
	2015	69.1	80.6	0.54	0.53	0.54	<0.001

[1]The reference group is the schedule 8 prescribing route. Therefore, an odds ratio of 2 is interpreted as the routine prescribing route having double the odds of prescribing a critically important antimicrobial compared with the schedule 8 route.

[2]Adjusted using the Bonferroni method.

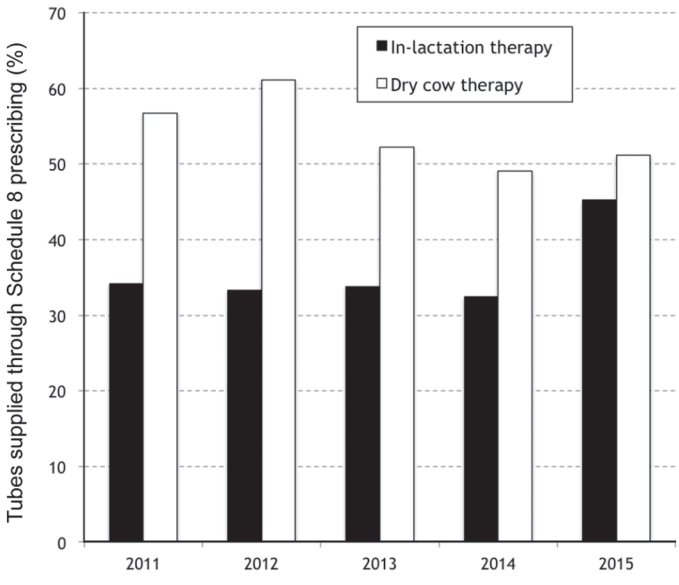

Figure 4. Estimated percentage of tubes of intramammary antimicrobials supplied through the schedule 8 prescribing route, by year and purpose. The Kynetec (Newbury, UK) data were assumed to represent 85% of actual on-farm usage.

with HP CIA. Again using 2015 as an example, the odds of prescribing a tube with at least one CIA or at least one HP CIA by the routine route was 0.21 and 2.81 times as likely, respectively, compared with those prescribed by schedule 8.

Estimated Defined Daily Dose for Animals, Defined Daily Course for Animals. The estimated on-farm antimicrobial usage of lactation and dry cow intramammary antimicrobials, using defined daily dose and defined course dose calculations, is presented in Table 4. The estimated usage of lactation antimicrobials has fallen from 1,993 to 1,398 DDDvet per 1,000 animals per year from 2003 to 2015, respectively (a quadratic curve, with an annual decrease from 2008 of ~92 DDDvet per 1,000 cows per year, $P < 0.001$), and from 663 to 466 DCDvet per 1,000 animals per year from 2003 to 2015, respectively (a quadratic curve, with an annual decrease from 2008 of ~30 DCDvet per 1,000 cows per year, $P < 0.001$). From 2003 to 2015, an increase ($P < 0.001$) occurred in the estimated usage of antimicrobials in DCT, rising from 794 to 1,022 DCDvet per 1,000 animals per year from 2003 to 2015, respectively.

Estimated Dry Cow Coverage. The estimated national coverage of DCT in Ireland from 2003 to 2015, based on varying assumptions about the relationship between Kynetec sales data and on-farm usage of intramammary antimicrobials, is presented in Figure 5. The estimated dry cow coverage has increased ($P < 0.001$), by between 2.9% (sales data assumed to represent 90% of all on-farm usage) and 3.2% (sales data assumed to represent 80% of all on-farm usage) for each year between 2003 and 2015, reaching approximately 100% coverage during at least the last 6 yr of the study period.

DISCUSSION

This study provides important insights into intramammary antimicrobial usage in Ireland, relating to both lactation and dry cow therapies. Farm-level data are not currently available in Ireland, and therefore this study was conducted solely using national-level sales and prescribing data.

These results highlight reducing usage of lactation therapies in Ireland in recent years (Table 1), from a high of 2,099 DDDvet per 1,000 animals per year (equivalent to 6.0 DDDvet per 1,000 cow-days and 2.0 DCDvet per 1,000 cow-days) in 2008 to 1,398 DDDvet per 1,000 animals per year (4.2 DDDvet per 1,000 cow-days, 1.4 DCDvet per 1,000 cow-days) in 2015 (Table 4). The 2015 figures compare favorably to those reported in other important dairying countries. In the Netherlands, the estimated mean usage of lactation therapy on 94 study farms was 1.45 animal-defined daily doses (**ADDD**; synonymous with DDDvet) per cow, or 1,450 DDDvet per 1,000 cows per year (Kuipers et al., 2016). The estimated mean usage of these therapies on study farms was 6.30 defined daily dose animals (synonymous with DDDvet) per 1,000 cow-days, or 1,922 DDDvet per 1,000 cows per year in Belgium (Stevens et al., 2016b). In Canada, the antimicrobial drug use rate (synonymous with DDDvet) during lactation was 3.52 animal defined daily doses per 1,000 cow-days or 1,074 DDDvet per 1,000 cows (Saini et al., 2012). On conventional Wisconsin farms, the treatment of clinical mastitis contributed 2.02 defined daily doses (synonymous with DDDvet) per cow per year, equivalent to 2,013 DDDvet per 1,000 cows per year (Pol and Ruegg, 2007). We can only speculate at the reasons for the observed fall in on-farm antimicrobial usage during lactation in Ireland. It should be noted that the national BTSCC has improved substantially in recent years, coincident with the introduction of CellCheck, which has considerably raised awareness of appropri-

Table 3. Comparison of prescribing route for highest priority critically important antimicrobials in Ireland from 2011 to 2015, by method of measurement (number of tubes, kg of active compound), product type (dry cow, lactation), and year

Item	Year	Prescribing route		Odds ratio[1]	95% Confidence limits		P-value[2]
		Routine	Schedule 8		Lower	Upper	
Number of tubes, % of tubes with at least one highest priority critically important antimicrobial							
Dry cow therapy[3]							
	2014	9.5	0.1	85.5	81.91	89.23	<0.001
	2015	10.4	0.6	19.3	18.89	19.62	<0.001
Lactation therapy							
	2011	8.4	6.6	1.30	1.29	1.32	<0.001
	2012	8.6	6.7	1.32	1.30	1.33	<0.001
	2013	10.5	6.9	1.57	1.55	1.59	<0.001
	2014	8.0	6.6	1.23	1.22	1.25	<0.001
	2015	9.6	3.6	2.81	2.77	2.85	<0.001
Quantity of antimicrobials, % of highest priority critically important among all antimicrobials							
Dry cow therapy[3]							
	2014	3.5	0.03	107.3	95.78	120.3	<0.001
	2015	3.8	0.2	23.8	22.67	25.08	<0.001
Lactation therapy							
	2011	2.5	1.8	1.44	1.38	1.49	<0.001
	2012	2.7	1.5	1.80	1.73	1.87	<0.001
	2013	2.8	1.7	1.66	1.59	1.72	<0.001
	2014	2.3	1.6	1.45	1.39	1.51	<0.001
	2015	3.0	0.9	3.48	3.32	3.64	<0.001

[1]The reference group is the schedule 8 prescribing route. Therefore, an odds ratio of 2 is interpreted as the routine prescribing route having double the odds of prescribing a high priority critically important antimicrobial compared with the schedule 8 route.

[2]Adjusted using the Bonferroni method.

[3]In 2011 to 2013, there was either little or no usage of highest priority critically important antimicrobials among dry cow therapy prescribed under schedule 8.

Table 4. Estimated on-farm antimicrobial usage of lactation and dry cow intramammary antimicrobials in Ireland from 2003 to 2015, using defined daily dose (DDDvet) and defined course dose (DCDvet) calculations[1]

Item	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Lactation antimicrobials													
Defined daily dose													
DDDvet per 1,000 animals per year	1,993	1,800	1,976	1,812	2,081	2,099	1,893	1,940	1,882	1,900	1,644	1,511	1,398
DDDvet per animal per year	2.0	1.8	2.0	1.8	2.1	2.1	1.9	1.9	1.9	1.9	1.6	1.5	1.4
DDDvet per 1,000 cow-days	6.0	5.4	5.9	5.4	6.3	6.3	5.7	5.8	5.7	5.7	4.9	4.5	4.2
Defined course dose													
DCDvet per 1,000 animals per year	663	600	658	604	693	699	631	646	627	633	548	503	466
DCDvet per 1,000 cow-days	2.0	1.8	2.0	1.8	2.1	2.1	1.9	1.9	1.9	1.9	1.6	1.5	1.4
Dry cow antimicrobials													
Defined course dose													
DCDvet per 1,000 animals per year	794	700	771	681	930	955	921	1,009	1,138	1,048	1,092	1,076	1,022

[1]It is assumed that the Kynetec sales data represent 85% of all on-farm usage.

ate mastitis control strategies. It is possible, therefore, that the observed decrease in usage may be related to both a reducing incidence in clinical and subclinical mastitis and to more prudent on-farm antimicrobial usage. Further investigation of this issue is warranted. It is important to note that *Staphylococcus aureus* is generally the pathogen most commonly associated with IMI in Ireland (More et al., 2012), although subsequent work has shown that environmental pathogens, such as *Streptococcus uberis* and *Escherichia coli* also present a considerable challenge (Keane et al., 2013). The efficacy of mastitis therapy for chronic *S. aureus* infection during lactation is extremely low, leading to very low cure rates following treatment (Oliver et al., 2011). Therefore, despite ongoing reductions in usage, it is possible that there remains an overreliance on the use of antimicrobials in Ireland in situations where the efficacy of treatment is low (More et al., 2012).

Over the last 6 yr at least, the estimated national coverage of DCT has been close to 100% (Figure 5). This follows a substantial rise since 2007 both in the number of dry cow tubes (Figure 1) and the quantity of active substance (Table 1) sold. Concurrently, a significant increase has also occurred over time in the use of teat sealant (Figure 2). It is important to note that teat sealants are being used in addition to (rather than as a substitute for) antimicrobial therapy, given

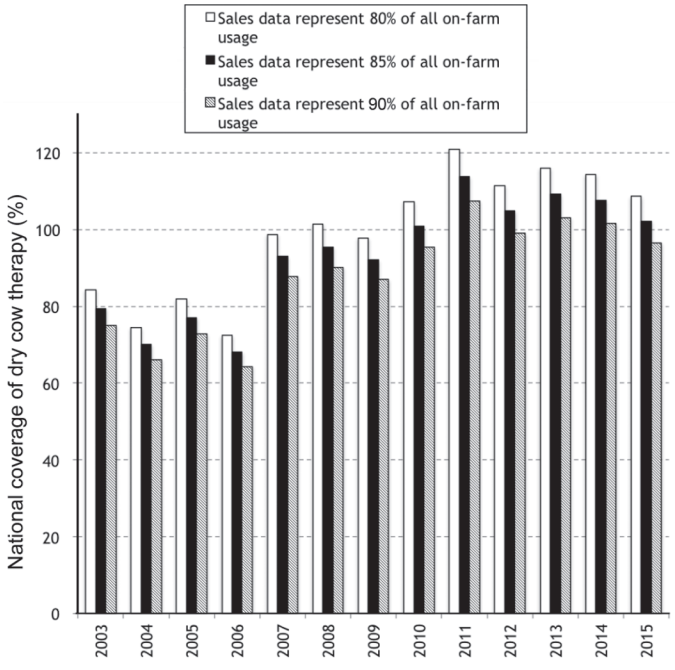

Figure 5. Estimated national coverage of antimicrobial dry cow therapy in Ireland from 2003 to 2015, using different assumptions about the relationship between Kynetec (Newbury, UK) sales data and on-farm usage of dry cow intramammary antimicrobials.

the near universal use of antimicrobial products for DCT observed. In several years, the estimated national coverage exceeded 100%, for reasons that are unclear. We caution, as highlighted below, that all estimates of on-farm usage need to be interpreted with care given the assumptions made during these calculations. The on-farm usage of dry cow antimicrobial therapy is substantially greater than other countries with recently published data. In the current study, the estimated DCDvet in Ireland varied between 681 (in 2006) and 1,138 (2011) per 1,000 animals per year, and was 1,022 per 1,000 animals per year in 2015 (Table 4). In a recent Dutch study, the estimated mean usage of DCT on 94 study farms from 2005 to 2012 was 2.57 ADDD per cow per year, equivalent to 643 DCDvet per 1,000 cows per year (Kuipers et al., 2016). Considerable farm-level variation was present, with almost 40% of farms using less than this.

Although the adoption of DCT in Ireland was encouraged over the last decade for the purpose of improving udder health, blanket DCT should be reconsidered, both in light of these results and for several other reasons. In recent years, substantial national progress has been made in reducing BTSCC, and consequently there is an increasing number of farms where selective DCT would be an option. Further, the unjustified use of blanket DCT is at odds with growing concerns about on-farm antimicrobial usage (Biggs et al., 2016). Nonetheless, recent studies have highlighted some of the challenges related to selective DCT. Selection based on SCC at the last milk recording before drying off gives a substantial reduction in antimicrobial use, but leads to an increase in clinical mastitis, subclinical mastitis, and culture-positive quarters (Scherpenzeel et al., 2014). In contrast, no adverse effect was observed on postcalving IMI or clinical mastitis (Cameron et al., 2014) or on milk production or SCC (Cameron et al., 2015) in the subsequent lactation when Petrifilm-based on-farm culture systems were used to allow targeting of selective DCT. The use of teat sealant, in place of an antimicrobial, is also an option for selective DCT. The incidence of IMI and clinical mastitis in dairy cows during early lactation was considerably reduced following the application of internal teat sealants at drying off either alone or with the addition of antimicrobials, based on a recent meta-analysis by Rabiee and Lean (2013). Industry-agreed guidelines for selective dry cow treatment under Irish conditions are available (CellCheck, 2011). In Ireland, key challenges, relevant to selective dry cow treatment, include milk recording (to generate individual cow data) and hygienic practices at drying off and subsequently. Whole-herd milk recording was conducted on 39% of herds (comprising 52% of dairy cows) in 2015 (ICAR, 2016); however, the average number of tests per lactation was relatively low (4.40 and 4.49 in 2014 and 2015, respectively). This presents a challenge for the adoption of selective dry cow treatment, where limited (or no) individual cow data are available to inform decision making.

This work highlights the widespread use of CIA (Figure 3a, Supplemental Figure S1a; https://doi.org/10.3168/jds.2016-12068) both to treat and prevent mastitis in dairy cows in Ireland. Although the use of HP CIA is limited, a significant increase has occurred from 2003 to 2015 in the number of tubes for both lactation and DCT that contain at least one HP CIA (Figure 3b, Supplemental Figure S1b; https://doi.org/10.3168/jds.2016-12068). In 2015, 38.5 and 5.4% of tubes for DCT contained a CIA or a HP CIA, respectively. The equivalent figures in 2015 for lactation therapy were 97.4 and 6.9%, respectively. Focus should primarily be placed on HP CIA, which are of highest priority for human health, and were identified specifically "to allow stakeholders in the agriculture sector and regulatory agencies to focus risk management efforts on drugs used in food animals that are the most important to human medicine" (Collignon et al., 2016). Macrolides are classified as HP CIA by the WHO (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, 2012), but not by the European Medicines Agency based on their degree of risk to people due to resistance development following use in animals (European Medicines Agency, 2014). If the classification of macrolides were changed, this would not affect the results of the current study, noting that all HP CIA in intramammary antimicrobial products in Ireland since 2006 have been 3rd and 4th generation cephalosporins (Table 1). Widespread use of HP CIA in dairy cattle has been reported elsewhere. For example, Stevens et al. (2016b) recently reported widespread use of 3rd and 4th generation cephalosporins and fluoroquinolones in dairy farms in Belgium, but with considerable farm-level variation. However, there are also examples of national action to successfully restrict the use of HP CIA in cattle production, specifically as a consequence of public health concerns. In Denmark, the use of 3rd and 4th generation cephalosporins in intramammary applications has fallen over 10-fold between 2007 and 2014, to 21,000 defined animal daily doses (conversion to DDDvet from aggregated data is not possible). This was partly a consequence of public concern, particularly with respect to a broader debate and information about the development of extended-spectrum β-lactamases, and the introduction of legislation requiring testing for antimicrobial resistance in cases where antimicrobial agents other than simple penicillins are prescribed for

mastitis (DANMAP, 2011). Denmark also has differential taxes on the sales of antimicrobials and other medicines for veterinary use (vaccines: no tax; penicillins, simple and narrow spectrum: 0.8% tax; 3rd and 4th generation cephalosporins: 10.8% tax; DANMAP, 2014). In the Netherlands, a combination of compulsory and voluntary actions with clear reduction goals were introduced in recent years, including substantial restriction in the use of 3rd and 4th generation cephalosporins and fluoroquinolones in farm animals (Speksnijder et al., 2015).

In Ireland, antimicrobial agents are available only by veterinary prescription. With intramammary products, 2 prescribing routes are allowed; the routine and schedule 8 prescribing routes. With this latter route, there is no requirement for a herd visit by the prescribing veterinarian at least every 12 mo if the herd is covered by a mastitis prevention and treatment program as outlined in the relevant legislation (European Communities, 2007). In this study, we highlight the similar importance of both of these 2 routes, in terms of the number of tubes sold (Figure 4). Further, differences between prescribing routes with respect to CIA usage were observed. In general, CIA and HP CIA were less likely to be prescribed through schedule 8 prescribing compared with the routine prescribing route, the exception being CIA in lactation therapy. The difference between the 2 prescribing routes was most marked with HP CIA in DCT: during 2014 and 2015, HP CIA were present in between 9.5 and 10.4% of these tubes through the routine prescribing route compared with 0.1 to 0.6% of tubes through schedule 8 prescribing (Table 3). Given the importance of schedule 8 prescribing in Ireland, as evident from this study, further work is justified to better understand factors associated with veterinary prescribing under these 2 routes.

This study was mainly conducted using national sales data. Although such data are centralized and readily available, they generally do not allow for the distribution of consumption in different animal species, weight groups, or production types (European Medicines Agency, 2013). In contrast to most antimicrobial products, however, intramammary tubes are generally used as intended (that is, for intramammary application), except for occasional use for the treatment of pink eye in cattle. Therefore, sales can be reasonably extrapolated to on-farm usage as intended. We caution, however, that the on-farm usage estimates should be interpreted with caution. Several assumptions were needed when estimating on-farm usage from national sales data, including the number of lactating dairy cows at risk of clinical mastitis, the number of lactating dairy cows eligible for DCT, and the relationship between Kynetec sales data and on-farm usage. Concerning the number of animals at risk of clinical mastitis or eligible for DCT, we relied on available national data, but noting that data about the annual replacement rate and intercalving interval were limited to 2008 to 2015 and to herds with at least 30 calvings. We conducted a sensitivity analysis, as presented in Figure 5, to highlight the effect on national coverage of DCT of variations in the relationship between Kynetec sales data and on-farm usage. We had limited access to the remaining sales data that were not captured by Kynetec, specifically the sales of lactation and dry cow tubes available in the Irish market during the study period, and within this the percentage of tubes that contained an HP CIA. The Kynetec and non-Kynetec data are similar in this regard, providing confidence that the Kynetec data are representative of all intramammary antimicrobials sold in Ireland.

There has been considerable confusion in the literature concerning measurement of on-farm antimicrobial usage, with both methodology and terminology. This issue is less problematic for intramammary antimicrobial usage in comparison to other administration routes. Nonetheless, as illustrated previously, the measurements used to quantify lactation usage in Belgium (defined daily dose animals), Canada (antimicrobial drug use rate), the Netherlands (ADDD), and the United States (defined daily doses) can be compared, whereas those from Denmark (defined animal daily doses) cannot. Further, differing approaches have been used when quantifying DCT usage. In this study, we measured on-farm usage of antimicrobials for DCT using defined course dose for animals (DCDvet), noting that a single application provides long-term action during the dry period and represents a therapeutic course. This approach is logical, but at odds with recent publications where the levels of DCT have previously been expressed in terms of DDDvet (Kuipers et al., 2016; Stevens et al., 2016b). For this reason, we conducted back-calculations to allow comparison. The lactation sales data were used to estimate antimicrobial usage (DDDvet).

This study has highlighted the urgent need for standardized indicators for quantification of antimicrobial usage in farm animals, to allow country-level comparisons. Collineau et al. (2017) have recently addressed this issue in a comprehensive review, and have suggested technical units, indicators, and data sources to address 4 different study objectives, including monitoring usage trends over time, comparing usage between species or countries, benchmarking between farms, and studying the association between antimicrobial usage and resistance. The recommendations of Collineau et al. (2017) were followed in the current study.

CONCLUSIONS

This study provided detailed insight into on-farm usage of intramammary antimicrobials in Ireland. It demonstrates positive national progress, particularly with respect to lactation antimicrobial usage, but also highlights areas for review and further research. In particular, blanket dry cow treatment in Ireland should be reconsidered. It is not possible to investigate farm-level variation in antimicrobial usage from national sales data. Several studies have investigated farm-level usage using data collected from drug sales (Kuipers et al., 2016) or garbage can audits (Stevens et al., 2016a,b). Farm-level antimicrobial usage data are now routinely collected in several European countries, including Denmark (Wielinga et al., 2014; DANMAP, 2015) and the Netherlands (Speksnijder et al., 2015; Autoriteit Diergeneesmiddelen, 2016), where it is central to efforts to reduce antimicrobial usage in farm animal production. Central collation of data on farm-level antimicrobial use is also needed in Ireland to allow objective measurement and benchmarking of on-farm usage. More generally, standardized indicators to quantify antimicrobial usage in farm animals are urgently needed to allow country-level comparisons.

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A 100-Year Review: Animal welfare in the *Journal of Dairy Science*—The first 100 years[1]

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ABSTRACT

This paper outlines the history and development of research in the area of animal welfare as reflected in the 100 yr that the *Journal of Dairy Science* has been published. The first paper using the term "animal welfare" was published in 1983; since then (to May 2017), 244 papers that reflect growing interest regarding how farm animals are cared for have been published. Much of the scientific work to date has focused on issues related to cow health, such as lameness, and methodologically many papers use behavioral measures. In addition to this science-based research, the journal has taken on the role of publishing work of social scientists that addresses the role of the human factors relating to animal welfare, including research on citizen, consumer, and farmer attitudes toward welfare issues. We call for further research focused on societal perspectives and for new biological research focused on developing issues, such as cow–calf separation and pasture access.
Key words: animal well-being, animal care, animal behavior, animal rights

INTRODUCTION

This review outlines the history and development of scholarly work on the topic of animal welfare as reflected in the 100 yr that the *Journal of Dairy Science* (**JDS**) has been published. Coverage of this topic has expanded dramatically over the past 30 yr, with the greatest expansion occurring very recently. Animal welfare is an area of application rather than a discipline and is amenable to a variety of disciplinary approaches, including physiology, genetics, nutrition, sociology, and so on. Animal behavior has been an especially useful disciplinary approach to welfare questions, but behavioral studies also address more basic issues (e.g., the nature of social relationships) and practical issues (e.g., heat detection) that are not related to animal welfare. In the current paper we focus on animal welfare but highlight how the field of animal behavior has played a role in finding solutions to improve dairy cattle welfare.

In the sections that follow, we define what we mean by animal welfare and the types of concerns that it encompasses, provide a brief history of animal welfare as a social movement, and focus on how animal welfare issues have been addressed within JDS, identifying how far we have come and key papers where possible. We end with our vision for how research in dairy welfare will continue to develop in the years to come.

What is Animal Welfare?

The study of welfare is focused on improving the lives of animals, but exactly what this means has changed over the past century. Traditionally, a good life has been associated with good health and appropriate levels of production, but scholars working on health or production might not think of themselves as working on welfare. Although both health and production are associated with good biological functioning on the part of the animal, sometimes the methods used to address health and production goals may introduce other types of welfare concerns.

A more modern view of welfare concerns how the animal feels (Duncan, 2004); this view would support changing procedures to minimize negative affective states (e.g., pain) and promote positive states (e.g., pleasure). The main challenge with this approach is scientific, and much research has been devoted to developing and validating methods to assess emotional states in animals (Weary et al., 2017). Some authors have also argued that allowing animals to live reasonably natural lives (e.g., providing the ability to express natural behaviors such as drinking milk through a nipple in calves) is also an important dimension to animal welfare, explaining why some standards require access to more natural environments (e.g., pasture) or the ability to perform key behaviors (e.g., the cow nursing her calf).

In 1997, David Fraser and colleagues published a conceptual paper calling for the integration of all 3

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approaches (biological functioning, natural behavior, and affective states), arguing that welfare problems can emerge in any of these 3 areas and that the best practices will address all 3 areas of concern (Fraser et al., 1997). These arguments were specifically applied to dairy cattle in a review by von Keyserlingk et al. (2009), where it was argued, for example, that allowing cows to seek shade on a hot day (natural behavior) will help prevent the cow from feeling uncomfortably hot (affective state) and reduce the health and production risks associated with heat stress (biological functioning). According to this framework, it would be misguided to address one type of welfare concern (e.g., high rates of enteric and respiratory infections in dairy calves—a biological functioning concern) by imposing a solution that introduces new welfare concerns around natural living and affective state (e.g., the use of individual housing that prevents natural interactions and play).

Animal welfare is an ethical concept and is subject to societal input. Progress on welfare relies on science, in part to provide evidence that can aid in the process of consensus building between the various stakeholders, but this scientific work must not occur in a vacuum. The science should instead be grounded in an understanding of societal values that help identify issues and anticipate objections to new practices (Weary et al., 2016).

Animal Welfare as a Social Movement

Criticisms relating to the standard industry practices associated with the care and handling of farm animals first entered the mainstream media in the mid-1960s following publication of the book *Animal Machines* (Harrison, 1964). This book described housing and production practices for laying hens, broiler birds, and veal calves and highlighted the unnaturalness (i.e., lack of sunshine, fresh air, and space) of these systems. The negative reaction by the British public motivated the UK government to commission the report titled "Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems" (Brambell, 1965). This report argued that animals should have the freedom "to stand up, lie down, turn around, groom themselves and stretch their limbs" and that many of the standard systems for rearing farm animals were morally unacceptable.

The findings of the Brambell (1965) report were used to develop the Five Freedoms by the Farm Animal Welfare Council (FAWC, 1992): (1) freedom from thirst and hunger; (2) freedom from discomfort; (3) freedom from pain, injury, and disease; (4) freedom to express normal behavior; and (5) freedom from fear and distress. Similar events have taken place in other countries. Most notably, Sweden passed animal welfare laws in 1988 effectively banning zero-grazing systems for dairy cattle (Ministry for Rural Affairs—Government Offices of Sweden, 2009). The European Union has promoted farm animal welfare, announcing within its first directive in 1991 that, among other things, focus must be placed on care and housing of dairy calves (for additional discussion see von Keyserlingk and Hötzel, 2015).

At the time of publication of *Animal Machines* (Harrison, 1964), only 2 federal laws in the United States regulated the treatment of farm animals. The Twenty-Eight Hour Law (USDA, 1873), passed to protect livestock during transport to slaughter, required that after 28 h of travel in the United States by rail, steam, sail, or "vessels of any description," livestock must be unloaded and provided feed, water, and a resting area for a minimum of 5 consecutive hours before resuming transport. The *Humane Methods of Slaughter Act* (USDA, 1958) required that livestock must be rendered insensible before slaughter (see also Mench, 2008). More recently, a number of farm animal welfare laws have been enacted at the state level within the United States. The first of these was enacted in Florida in 2008, resulting in a ban on gestation stalls for sows. Since then, 9 states have effectively banned a variety of standard industry practices. A particularly well-known example is the 2008 California ballot initiative (Proposition 2) that, effective January 1, 2015, required that "calves raised for veal, egg-laying hens, and pregnant pigs be confined only in ways that allow these animals to lie down, stand up, fully extend their limbs, and turn around freely." The passing of Proposition 2 in California resulted in additional consequences. Senate bill 135, dated October 11, 2009, made an amendment to section 597n of the penal code, relating to animal abuse, that specifically banned tail docking of cattle (California Legislative Information, 2009).

These types of legislative changes have driven industry-led responses, including the development of guidance documents for farmers and verification procedures to provide assurance that farms are meeting these guidelines. In Canada this process has been led by the Dairy Farmers of Canada and Canada's National Farm Animal Care Committee (NFACC, 2014), who have worked together to create a code of practice for the care and handling of dairy cattle (DFC-NFACC, 2009). The US counterpart was led by the National Milk Producers Federation, who published the first version of the Farmers Assuring Responsible Management (FARM) program in 2009; this document has gone through 2 substantive revisions since then (NMPF, 2016). Whether these industry-led approaches will provide the necessary assurances to the public is unknown.

However, as we have previously highlighted (see Weary and von Keyserlingk, 2017), these types of standards are likely to be challenged in at least 2 ways: (1) there will likely be pressure from within the industry to have standards sufficiently lax to enable almost all farms to comply but, (2) there will likely be external pressure to maintain a supply chain that does not include bad actors (see Weary and von Keyserlingk, 2017; Wolf and Tonsor, 2017).

The North American dairy industry seems to be unwilling to leave the issue of animal welfare solely to farmer-run organizations. Both Dean Foods Inc. (2012) and Saputo Inc. (2015) have shown a willingness to play a major role in driving change for improved welfare on dairy farms. For example, in its animal welfare policy, Saputo Inc. (2015) states that it has "zero tolerance for any act of animal cruelty" and requires "compliance with codes/standards for proper animal care and handling." Saputo Inc. also calls on the industry to eliminate or modify routine management practices that are unnecessary or cause pain and makes specific reference to tail docking and the use of pain mitigation when dehorning or disbudding.

ANIMAL WELFARE WITHIN JDS

We conducted a systematic search of the published literature addressing animal welfare in JDS using Web of Science to search for the specific keywords "animal" and "welfare" or "animal" and "well-being" in the title, abstract, or key words of published articles and reviews from 1917 until May 2017: SO = (JOURNAL OF DAIRY SCIENCE) AND TS = ((animal AND (wellbeing OR well-being OR "well being") OR (animal welfare)). This search identified 244 papers; 228 papers were identified using the search term "animal" and "welfare," and an additional 16 papers were identified when "animal well-being" was included. Although the current review is focused on publications appearing in JDS, much research on the welfare of dairy animals also appears in other journals, most notably *Animal Welfare* and *Applied Animal Behaviour Science*. We refer interested readers to these and other journals for additional papers in this subject area.

The timeline provided in Appendix Table A1 highlights some of the key papers in relation to key events that have helped shape the field of animal welfare. The terms "animal" and "welfare" first appeared in JDS in 1983 in an Our Industry Today article authored by the noted researcher Jack Albright (1983). In a forward-thinking approach to this topic, the journal published in the same issue a paper by animal advocate Michael Fox (1983). These 2 papers (plus another written 4 yr later; Albright, 1987) are still recommended reading, in part because they provide a clear list of welfare research topics, many of which are still relevant today.

In the 1990s JDS published very little on the topic of animal welfare, but the relevant number of publications has increased rapidly since the turn of the new millennium (Figure 1). By 2009 a total of 50 papers on some aspect of dairy welfare had been published. By 2012, 100 papers had been published, and as of May 2017, 244 papers had been published.

To illustrate the variety of issues and methods addressed in these 244 papers, we generated a word cloud illustrating the most common words that appeared in these papers (Figure 2). As discussed above, behavioral studies have played a key role in welfare research, so it is not surprising that "behavior" is the largest word in the cloud and that words describing specific behaviors (lying, feed, and locomotion) are also prominent. Also, given that good health has long been considered an important element of good welfare, it not surprising to see that the word "health" is larger than any of the other issue-type words appearing. This also explains why related words (e.g., lameness, disease, mastitis, lesions, prevalence, clinical) are also prominent.

Health as a Welfare Concern

Although the word "animal welfare" is used only in more recent papers, dairy scientists have long been interested in maintaining and improving animal health, even though this has not necessarily been explicitly linked to welfare. Using the same search methodology described earlier, we found that the first paper to use the term "health" appeared in JDS in 1945 (Seath et al., 1945). Since then, this term has appeared in more than 1,800 papers, including in many relevant articles, such as the reviews on transition cow health by Drackley (1999) and LeBlanc et al. (2006). Only more recently have authors specified a link between good health and good welfare. The first time "animal" and "welfare" or "animal" and "well-being" appeared in our search was in Wells et al. (1998). These 2 terms have since appeared together in a total of 118 papers (as of May 2017), suggesting that a more explicit linkage is now important.

Affective States as Welfare Concerns

Welfare often emphasizes the animal's affective state (including negative experiences such as fear, pain, and hunger). Pain appears in our word cloud, as do the painful procedures dehorning and disbudding as well as painful ailments such as lameness, injuries, and dystocia. The first paper using the word "pain" appeared in JDS in 1999 in a paper related to mastitis (Hoeben et

al., 1999); in this case the authors were using clinical signs of pain as an outcome measure for their study, but they clearly link infection to pain. Earlier papers may have discussed pain in the body of the paper even though the word did not appear in the sections of the paper that were covered by our search criteria. For example, the first paper with the word "dehorning" appeared in 1994 (Wohlt et al., 1994). These authors did not use "pain" in the title, abstract, or key words but did discuss pain in relation to studies on humans. Pain is now recognized as an important issue in dairy cattle welfare—for example, as related to disbudding (Faulkner and Weary, 2000), tail docking (Eicher et al., 2000), and dystocia (Barrier and Haskell, 2011). Since 1999 more than 70 papers using the word "pain" have been published in JDS.

Another affective state with relatively unambiguous welfare connotations is fear. The word "fear" fails to appear in the word cloud, showing that little research has been published on this topic. Indeed, our search found only 13 papers using this term in JDS. Of these, the first published was titled "Discrimination of People by Dairy Cows Based on Handling" (Munksgaard et al., 1997). The ideas that animal handling methods may contribute to fearfulness and that fear responses can be used to improve handling procedures are important for JDS to consider.

Other negative affective states are considered in a few papers, although connotations for animal welfare are not always clear. For example, the term "hunger" first appears in a paper in 1971, but this study ("Hormones and Amino Acids as Possible Factors in Control of Hunger and Satiety in Sheep"; Baile and Martin, 1971) did not specifically consider hunger as a negative emotional state or its relevance to animal welfare. More recently, hunger has been treated as an important welfare concern, with a special focus on problems associated with underfeeding of the preweaned calf (reviewed by Khan et al., 2011).

Although much of the welfare-relevant work on affective states has focused on negative states such as those discussed above, more recent work in the field has also considered positive emotional states (for review see Boissy et al., 2007). Although play is a behavior rather than an emotional state, many have argued that the presence of play is indicative of a positive emotional state. Play is more common in young animal than in adults, and papers published to date on play have all

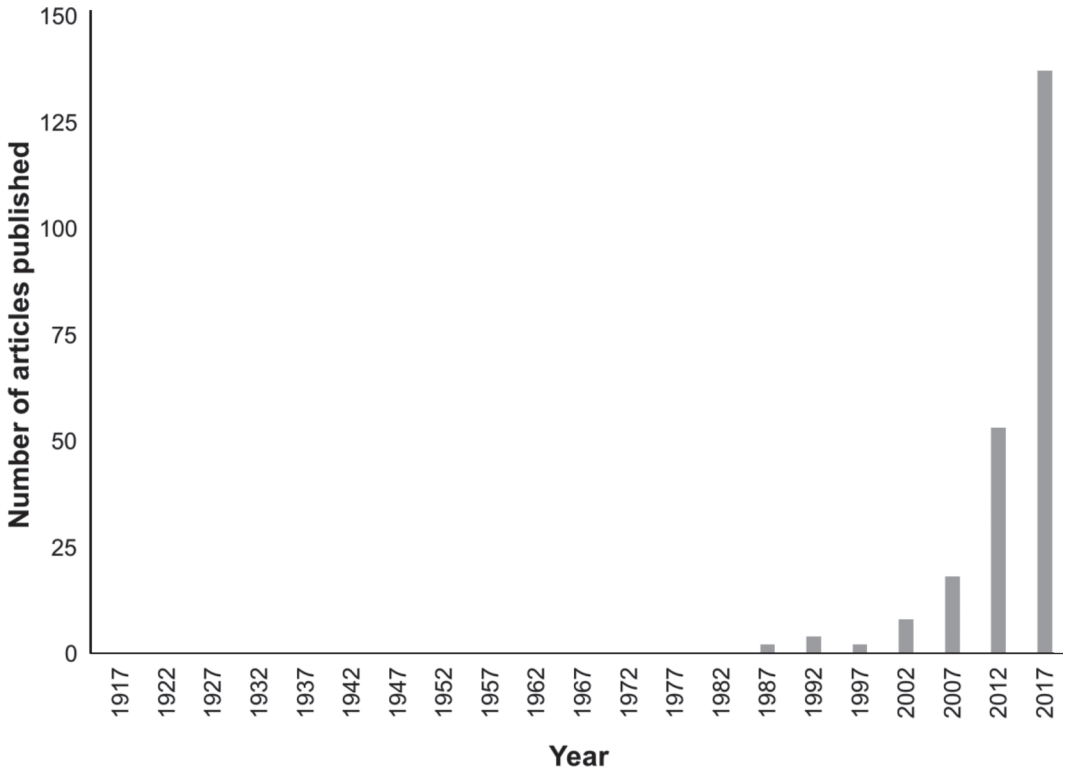

Figure 1. The number of papers published on the topic of animal welfare as reflected in the 100 yr that the *Journal of Dairy Science* (JDS) has been published (1917–May 2017) with the words "animal" and "welfare" or "animal" and "well-being" in the title, abstract, or key words. Results are shown by 5-yr periods (i.e., 1917–1922, 1923–1927, and so on). The first paper meeting these search criteria appeared in 1983; a total of 244 papers have appeared in JDS as of the time this review was written.

Figure 2. A word cloud generated using the titles, abstracts, and key words of the 244 papers resulting from our search of papers published in the *Journal of Dairy Science* between 1983 and the time this review was written (May 2017). The cloud shows the 100 most commonly appearing words related to animal welfare; those appearing in larger type are used more frequently. The most common word was "behavior" (appearing 334 times), and the least common word was "weaning" (appearing just 28 times).

focused on calves. For example, a recent JDS paper from the Czech Republic ("The Effect of Age at Separation from the Dam and Presence of Social Companions on Play Behavior and Weight Gain in Dairy Calves"; Valníčková et al., 2015) showed that play behavior is more common when calves are housed socially versus individually, suggesting that social housing allows for more positive affective states in these animals. We encourage more work on positive states in the years to come.

The term "stress" has some relevance to animal welfare. For example, painful experiences are often accompanied by characteristic physiological responses (e.g., the spike in serum cortisol typically observed in the hours after disbudding; Heinrich et al., 2009). However, physiological stress responses can accompany neutral or even positive experiences, making it difficult to draw clear welfare inferences from these physiological responses. More than 700 papers using the word "stress" have appeared in JDS, but many of these did not explicitly address welfare. We found only 37 papers with both "stress" and "animal welfare"; the first was a review by Ingvartsen and Andersen (2000) titled "Integration of Metabolism and Intake Regulation: A Review Focusing on Periparturient Animals." The first empirical paper using these terms, published in 2002 (Schreiner and Ruegg, 2002), addressed behavioral and physiological responses to tail docking in calves and heifers.

Naturalness as a Welfare Concern

The third sphere of concern, according to the framework of Fraser et al. (1997), is natural living; this includes the animal's ability to access reasonably natural environments and to perform natural behaviors that they are motivated to perform. In many ways this is the most problematic of the 3 spheres. Some authors argue that aspects of natural living are inherent to definitions of a good life in animals (e.g., Fraser et al., 1997), but others argue that natural living is only important in that it improves the animal's affective state (Dawkins, 1988). The natural criterion also may seem awkward for those who see their responsibility as maintaining a managed system rather than a natural one, including the protection of animals from natural risks such as predation, parasite infection, and climatic extremes. That said, concerns about naturalness are deeply rooted in traditional thinking about dairy systems. For example, some of the earliest papers in JDS addressed the issue of providing calves more naturalistic opportunities to ingest milk. In the words of Wise and Anderson (1939), "The unthrifty appearance commonly observed in young dairy calves in modern dairy herds has been attributed, in many cases, to the deviation from 'nature's way' of feeding." We found only 18 papers with both "natural*" and "animal welfare/animal wellbeing"; the first time that these 2 terms were mentioned together was in a review article by Albright (1993) titled "Feeding Behavior of Dairy Cattle." As we argue below, public conceptions of welfare often focus more on natural living aspects, and we hope that future JDS publications will increasingly consider these measures.

To some degree, the presence of abnormal behaviors, including the repetitive and apparently functionless stereotypic behaviors, can be seen as the antithesis of the natural living ideal. Thus, what is unnatural serves to remind us of what is natural, and it is sometimes argued that abnormal behaviors are the result of housing animals in unnatural environments that fail to meet their needs (Mason, 2008). A few papers published in JDS have addressed abnormal (n = 3) and stereotypic (n = 6) behaviors. One example, authored by a group of Dutch researchers, examined risk factors for nonnutritive oral behaviors (e.g., tongue rolling) on commercial veal farms (Leruste et al., 2014). Clearly more work is required in this area.

An Issue-By-Issue Approach

In addition to considering the literature from the perspective of the 3 spheres, we can consider papers on an issue-by-issue basis (e.g., overstocking). The list of potential welfare issues to be considered is long, but relatively few specific issues have been addressed in more than just a handful of papers in JDS. A few of the best-researched examples have been featured in curated "top 10" lists of papers appearing in the "Collections" section of the JDS website (http://www.journalofdairyscience.org/collections). Specifically regarding animal welfare, collections are currently available featuring disbudding and pain management for calves (the first published being Faulkner and Weary, 2000), stocking density (the first published being Huzzey et al., 2006), and tail docking (the first published being Eicher et al., 2000). We encourage future additions to the collections on welfare-relevant topics, including limit feeding of calves, social housing for calves, heat stress for lactating cows, and pasture access for cows, to name a few.

Social Science Contributions

Animal welfare is an important social concern and one of several issues (e.g., environmental, social justice) that need to be addressed to keep industry practices in line with evolving community standards. Unlike some areas of dairy science in which only scientific expertise is required, socially mandated research requires input and interplay between the science and the society that frames the issues. Sometimes this input can happen informally, via discussions with neighbors and others, but a more systematic approach is to turn the social questions into an important research agenda in their own right. Research undertaken in the social sciences can aid us in understanding the attitudes of different stakeholders, including people who work within the dairy industry (e.g., farmers, veterinarians, dairy industry specialists) and those who are not affiliated with the industry (e.g., the customer who buys milk at the grocery store, the neighbor to the dairy). Research in social sciences allows us to identify contentious topics as well as areas of agreement and thus plays a role in efforts to harmonize industry practices with societal expectations (Weary et al., 2016). *Journal of Dairy Science* has shown remarkable openness in publishing work using social science methodologies. Indeed, the word cloud includes the term "attitudes," illustrating the volume of work that has been published describing stakeholder views relevant to animal welfare. This work is still very recent. The first example appeared in 2008; this study described the reactions of citizens in a Vermont community in which a local dairy farm had recently expanded from about 225 cows to 684 cows (Smith et al., 2008). This study was important in that it documented community concerns associated with large farms, including perceived negative effects on the environment (including water quality) and animal welfare. This type of research can help identify needs for future research that addresses these concerns.

One example where JDS has made a particular contribution is in the area of pasture access. Several social science papers have shown that the public considers access to pasture to be important to the welfare of dairy cows both in the United States (i.e., Cardoso et al., 2016) and elsewhere (i.e., Brazil; Hötzel et al., 2017). Interestingly, there is evidence that farmers also consider pasture to be important to cattle (Schuppli et al., 2014). This same work showed that farmers perceive a lack of ability to provide pasture to their cows—for example, because of a lack of land or because of unfavorable climatic conditions. *Journal of Dairy Science* has published a series of studies showing that cows preferentially use pasture at night but stay indoors during the day (e.g., Charlton et al., 2011). One reason why cows prefer to remain indoors during the day is that higher energy total mixed diets are typically provided indoors, and feed intake is highest during daylight hours (DeVries et al., 2003). These studies illustrate the potential of hybrid systems that allow cows to go to pasture but also maintain access to a well-designed barn and mixed ration. This serves as an example of the how research in the biological and social sciences can work in harmony.

Scientific work of this nature may not always adequately address the societal concern, and societal concerns can evolve just as quickly as the science. Thus, the science that leads to developing new approaches needs to happen together with the social science research that seeks to document and understand the underlying concerns. Ideally, this may form a type of feedback loop going from the social science to the science and back again.

HOW FAR HAVE WE COME?

To get a sense of the progress we have seen in animal welfare research published in JDS, we return to research priorities summarized in the pioneering papers of Albright (1983) and Fox (1983). This process shows that in some areas we have made considerable strides, whereas in other cases much remains to be done.

Albright (1983) lists several items in his "Future Research and Solutions" section (p. 66), including confinement housing, (i.e., concrete flooring), different stall types, overstocking and group size, and, more broadly, cow comfort in different housing systems. More specific

concerns included the use of crowd gates in the milking parlor and behavioral problems such as cross-sucking in calves.

The issue of cow comfort has received considerable coverage in JDS. This line of research has addressed topics such as stocking density (as mentioned above), the effects of various flooring options on cow health (Somers et al., 2003) and behavior (Telezhenko et al., 2007, 2008), and the effect of lying surface on preferences for freestalls (Natzke et al., 1982). We note that little work has contrasted cow comfort in different housing systems, but we also caution readers that well-replicated studies assessing system-level differences are difficult to achieve. We also could find no research published in JDS on the welfare effects of crowd gates in the milking parlor, although there has been research on how best to manage cow flow in automatic milking systems (for review see Jacobs and Siegford, 2012). The topic of cross-sucking in calves has received some interest, with the first paper in our search being Kopp et al. (1986). Interestingly, Albright (1983), and to some degree Fox (1983), argued that individual housing is an appropriate solution to this problem due in part to perceived health benefits. We view individual housing as more of a "Band-Aid" solution (i.e., preventing the expression of the behavior but not the underlying motivation). More recent work has shown multiple problems associated with individual housing for calves and no added benefit in terms of health when compared with calves housed in small groups (for review see Costa et al., 2016). Other work published in JDS has also shown that the motivation to suck (including on pen mates, pen fixtures, and so on) is the result of underfeeding and unnatural feeding methods (i.e., buckets versus a teat; Jensen and Budde, 2006).

The article by Fox (1983) provides a summary of welfare issues (pages 2221–2223). This list includes concerns regarding increasing farm size and the resulting lack of individualized care. A recent review argued that there is no clear 1-way relationship between farm size and welfare (Robbins et al., 2016). For example, in a recent paper in JDS (Beggs et al., 2015), it was found that larger Australian herds had better trained workers (presumably favorable for welfare) but were also more likely to overstock their cows (presumably to the detriment of the cows). As summarized by Robbins et al. (2016), the cow-to-worker ratio is typically higher on larger farms, but "farm workers tend to be better paid, better trained, more specialized, and more satisfied" (page 5448) on these farms, perhaps compensating for the reduction in individualized care. Public concerns about cattle welfare are often rooted in the value of individualized care (Ventura et al., 2016a), and more work is required to directly assess the type and quality of care that is provided and how this relates to farm size.

Other issues raised by Fox (1983) included restriction of movement and social deprivation, as illustrated in the following quote regarding the housing of milk-fed heifers and veal calves: "Continued confinement in small crates in which they cannot walk, run around, or interact freely with others is ethically questionable" (page 2222). As we described above, much work has now addressed the welfare issues associated with individual housing for calves.

Unfortunately, relatively little has been published in JDS specifically addressing the welfare issues associated with other types of confinement in dairy cattle. One obvious question is how long periods of housing in tiestalls may affect cow welfare. We suggest that many issues may be inherent to long periods of movement restriction, even if some improvements can be achieved by changes in tiestall design (Zurbrigg et al., 2005). The phasing out of tiestalls has already begun in some regions; for example, Norway banned new construction of this popular housing type in 2004, and a complete ban takes effect in 2023. The welfare impact of other types of restriction, including routine restriction of cows in head lockers for management and health procedures, is largely unknown.

A still contentious issue raised by Fox (1983) is that of cow–calf separation. Unfortunately, we could not find a single empirical paper published in JDS that directly addressed the welfare issues associated with this practice. This is an unfortunate gap given that we now know that that separation of cow and calf is an important area of public concern (Ventura et al., 2013; Hötzel et al., 2017). Another contentious and largely unresolved issue raised by Fox (1983) is the fate of bull calves that are often low-value by-products of dairy production.

The issues raised by Albright (1983) fall in some aspect or another into the biological functioning and health construct of animal welfare. In contrast, Fox (1983) was clearly also concerned about a lack of naturalness and negative affective states. More progress has been made on the biological issues, perhaps because welfare improvements in this domain often are associated with production benefits. For example, reductions in lameness benefit both the cow and the farmer's bottom line. The lack of progress on issues relating to naturalness and affective states may be attributable to a lack of research funding for this type of work. As noted by Albright (1987), "very little organized U.S. research on dairy animal welfare is underway"; the same could be said of the situation today. A significant portion of agricultural research is sponsored by corporations and thus is more focused on production and product testing

than on general benefit to the dairy industry or broader society (see von Keyserlingk et al., 2013).

Our review shows that of the 9,190 publications with US-based corresponding authors since 1983, only 0.7% address the topic of animal welfare. In contrast, of the 7,576 publications with non-US-based corresponding authors over the same time period, 2.3% address the topic of welfare and a greater than 3-fold higher proportion of publications related to animal welfare. Thus, scientific guidance on the topic of animal welfare in the United States is largely dependent on the research and advice provided by scientists working outside of the United States (including the authors of the current review).

From the section above we can conclude that there is some basis for pride in the progress made within the journal to address priority issues in dairy welfare but that there are also areas where little or no progress has been made. We turn to some of these in the next section.

FUTURE DIRECTIONS

Below we speculate on what the future may hold for dairy cattle welfare research. We have organized this section in terms of the time horizon (short, medium, and long term) over which we believe that most progress will occur. Whether acceptable solutions are found over these periods will depend on resources, including funding, the availability of qualified individuals who are able to take a leadership role, and the continued interest and enthusiasm of stakeholders, including the public, producers, and industry professionals.

Short Term

In the short term (over the next decade especially), the dairy industry must work to implement proven science-based welfare solutions. This includes implementing pain control protocols for procedures such as dehorning (Stafford and Mellor, 2011), eliminating tail docking (Sutherland and Tucker, 2011), and providing calves more milk (Khan et al., 2011) and social housing (Costa et al., 2016).

There is also an immediate need to develop a scientific basis for new policies and practices regarding other well-recognized welfare issues. This includes the care, handling, and slaughter of surplus calves (predominantly bull calves) and downer cows. In some cases, we require new research (e.g., to identify practices that result in better recovery by downer cows; see Poulton et al., 2016; Stojkov et al., 2016). In other cases, the development of systems that help prevent the problem are required (e.g., the use of sexed semen for the production of replacement heifers and cross-breeding with beef breeds to create high-value calves that can be reared humanely for beef; see Barkema et al., 2015).

In the short term there is also a need to study not just the dairy animal but also the humans who decide how cattle are to be cared for (farmers), those who advise farmers (e.g., veterinarians and dairy industry specialists), those who buy the dairy products (consumers), and those who ultimately decide what farming systems and associated management practices will be tolerated within their community (citizens; e.g., Smith et al., 2008). Specifically, we urge new work to assess public expectations for dairy farming (e.g., Cardoso et al., 2016) and perceived barriers to changing practices on farms (Weary et al., 2016) as well as understanding the role of dairy professionals in facilitating change on farms (Ventura et al., 2016b). This focus on the social as well as the natural sciences has been helpful for other areas of dairy science; in particular, we note the work on understanding barriers to changing practices in relation to mastitis control (e.g., Swinkels et al., 2015). Specifically, we hope that within 10 yr JDS will have a section devoted to work in the social sciences that embraces all aspects of dairy production.

Medium Term

Over the medium term (likely continuing for at least the next 20 yr), the dairy scientist community will continue to develop research-based solutions to health issues that affect many animals and cause considerable suffering. Key examples include the high rates of disease in transition cows and milk-fed calves. One special concern is lameness in dairy cattle because of the high prevalence and long duration of cases as well as the pain that cows experience. Indeed, we call for more research to better understand the effects of other important dairy cattle diseases, particularly during the transition from pregnancy to lactation, including mastitis and metritis, and the pain and malaise that affected cattle feel (Weary et al., 2009). We also see much room for the social sciences to help identify barriers to implementing proven welfare solutions on farms with regards to culling decisions and timely euthanasia.

Long Term

Over the longer term (likely continuing for at least the next 50 yr), scientists and professionals working within the dairy industry must also show the courage and innovation necessary to develop solutions that address societal expectations. We suggest that there will

be at least 2 urgent priorities for the development and implementation of welfare-friendly practices: pasture access and cow–calf rearing.

As we outlined previously, we have found that both farmers and people outside of the dairy industry see pasture access as an important component of high-welfare dairy systems (Cardoso et al., 2016; Ventura et al., 2016a; Hötzel et al., 2017), but farmers often perceive barriers to implementing pasture access on their farms (Schuppli et al., 2014). Moreover, what is best from the cow's perspective may not always agree with public or even industry perspectives, so having a research basis that underpins recommendations is especially important. For example, although some may feel that pasture is always better for cows, research has shown that cows will often choose to come inside a well-designed and well-managed barn, especially to avoid direct sunlight when temperatures are high (Legrand et al., 2009) and during inclement weather (Charlton et al., 2013). Thus, we see the need for new research on systems based on free-choice access between well-managed indoor and outdoor facilities. These will allow cows to vary their choices depending on conditions and will allow different individuals to express their own idiosyncratic preferences. We also call for new work examining a range of different types of outdoor access. We have found that cows will often choose to consume a well-formulated mixed diet inside the barn rather than graze (Chapinal et al., 2010), meaning that outdoor systems may not need to be grass based or could take advantage of hardy, slow-growing grass varieties designed to provide a comfortable lying and standing surface rather than an important source of nutrients.

There appears to be little public support for the standard management practice of cow–calf separation (United States: Ventura et al., 2013; Brazil: Hötzel et al., 2017; United States and Germany: Busch et al., 2017). Very little research is available to either justify this practice or provide alternatives. The few studies to date suggest that the practice increases the risk of postpartum disease in early lactation (e.g., Krohn et al., 1990). Moreover, the available health data indicate that despite early separation of cow and calf, morbidity and mortality rates are high for both the calf (see review by Costa et al., 2016) and the transition cow (McConnel et al., 2008). On the basis of this evidence, critics could argue that separation may be detrimental to the health and welfare of both the cow and the calf. Some initial guidance on possible solutions may be found in the few studies done on the use of nurse cows (e.g., Loberg and Lidfors, 2001), *Bos indicus* systems that allow the dam some contact with her calf (Das et al., 2000), and recent work by Johnsen et al. (2015a,b) on keeping calves with access to the dam and a supplementary milk supply. Given the complexity of the issue, including the difficulty in developing practical methods that are economically sustainable for farmers, much new research will be required.

CONCLUSIONS

Animal welfare is a relatively new area of application; the first papers directly focused on this topic were published in JDS in 1983. Over the last 30 yr, JDS publications have made important contributions to animal welfare issues such as tail docking, providing calves more milk, and pain mitigation during dehorning, but little has been published on other important issues (e.g., cow–calf separation). The field of animal behavior has played a central role in many of these studies. Animal welfare is a socially mandated science requiring input from all stakeholders, including the public. Research in the social sciences can help document the shared and divergent values of different stakeholders, beliefs regarding the available evidence, and barriers to implementing change, all of which are needed to develop practices that resonate with societal values.

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APPENDIX

Table A1. A timeline of the key contributions to the field of animal welfare

Date	Milestone	Reference
1939	Wise and Anderson in their introduction state, "The unthrifty appearance commonly observed in young dairy calves in modern dairy herds has been attributed, in many cases, to the deviation from 'nature's way' of feeding."	Wise and Anderson, 1939
1945	The word "health" appears in a *Journal of Dairy Science* (JDS) publication for the first time.	Seath et al., 1945
1953	Graf and Petersen publish the first paper in JDS to address "stress," though not linked with animal welfare.	Graf and Petersen, 1953
1958	The *Humane Methods of Slaughter Act* is signed into law in the United States.	USDA, 1958
1964	*Animal Machines* criticizes standard industry practices associated with the care and handling of farm animals; this criticism enters the UK mainstream media.	Harrison, 1964
1965	The UK public reacts negatively to the descriptions in *Animal Machines*, motivating the UK government to commission the Brambell report.	Brambell, 1965
1966	The *Animal Welfare Act* is signed into law in the United States.	*Laboratory Animal Welfare Act* of 1966; https://www.nal.usda.gov/awic/animal-welfare-act
1975	The Five Freedoms (arising from the Brambell report) are formally adopted by the United Kingdom Farm Animal Welfare Council.	FAWC, 1992
1983	The first papers addressing animal welfare are published in JDS by an animal scientist (Albright) and an animal advocate (Fox).	Albright, 1983; Fox, 1983
1988	Sweden passes the Animal Welfare Ordinance into law, effectively banning zero-grazing systems for dairy cattle.	Ministry for Rural Affairs—Government Offices of Sweden, 2009
1997	Fraser et al. publish a seminal paper titled "A Scientific Conception of Animal Welfare that Reflects Ethical Concerns" in *Animal Welfare*.	Fraser et al., 1997
1997	Munksgaard et al. publish the first JDS article addressing fear in dairy cattle.	Munksgaard et al., 1997
1999	Hoeben et al. publish the first paper using the term "pain" in JDS.	Hoeben et al., 1999
2002	Schreiner and Ruegg publish the first JDS paper on tail docking, where they link stress and animal welfare.	Schreiner and Ruegg, 2002

Continued

Table A1 (Continued). A timeline of the key contributions to the field of animal welfare

Date	Milestone	Reference
2004	Norway bans new construction of tiestall housing (with a complete ban by 2024).	Skarstad and Borgen, 2007
2008	Smith et al. publish the first social science paper in JDS.	Smith et al., 2008
2009	State of California bans tail docking of cattle.	California Legislative Information, 2009
2009	Dairy Farmers of Canada and the US-based National Federation of Milk Producers publish industry guidelines for care and handling of dairy cattle.	DFC-NFACC, 2009; NMPF, 2016
2009	von Keyserlingk et al. publish "The Welfare of Dairy Cattle—Key Concepts and the Role of Science" in JDS.	von Keyserlingk et al., 2009
2009	The 50th paper addressing animal welfare is published in JDS.	von Keyserlingk et al., 2009
2012	The 100th paper addressing animal welfare is published in JDS.	Thomsen et al., 2012
Jan. 2016	The 200th article addressing animal welfare is published in JDS.	Westin et al., 2016

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A 100-Year Review: Lactating dairy cattle housing management[1]

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ABSTRACT

Since the mid-1800s, farmers have been housing livestock. What began as a part-time solution for cold winters, stormy days, or injured animals has evolved into the main or only area in which cows spend their adult lives. With this change, farmers, academic researchers, and industry innovators have shaped the farm landscape, literally. Over the last 100 years, changes have been made for productivity, health, milk quality, reproduction, animal well-being, and farm profitability. We review a snapshot of those changes and look ahead to the future of lactating dairy cattle housing. All housing systems are moving toward improved cow comfort. Stalls in tiestall and freestall systems are now designed to accommodate cows based on body size and, in some cases, stage of lactation. Farmers may choose to build a compost bedded or traditional bedded-pack barn to maximize cattle rest or accommodate various breeds or sizes of cows. Looking to the future, external pressure and public perception may push farmers to consider other alternatives to total confinement. Future housing plans may include access to pasture or exercise lots, allowing cows to express their preferences for being outside or inside. Housing that allows natural expression of behavior while maintaining cow cleanliness and health may improve the lives of cows and farmers.
Key words: housing, management, lactating dairy cow, 100-year review

INTRODUCTION

Choosing the environment in which lactating dairy cows will spend most of their time is an important decision for dairy producers. This choice has considerable influence on productivity, health, milk quality, reproduction, animal well-being, and farm profitability. In the decision-making process, dairy producers must consider what system will work best for their respective situations, given local climatic conditions, construction and building costs, labor availability, long-term maintenance and upkeep costs, and return on investment. Although pasture generally allows for excellent cow comfort, inclement weather and muddy conditions may present challenges related to cow cleanliness, milk quality, and heat or cold stress. These challenges have created the need for confinement housing. Traditionally, housing options for lactating dairy cows have consisted of conventional bedded-pack, tiestall, freestall, and compost bedded-pack barns. The early years of many peer-reviewed journals do not include information on dairy cattle housing. Early information on origins of housing types and initial building recommendations was collected from patents, extension publications, and some journal articles. We will describe the research of and advances in housing of dairy cattle in various systems over the last century (Appendix Table A1).

CONVENTIONAL BEDDED-PACK BARNS

Bedded-pack barns (**BP**), or straw yards, were a recognized form of loose housing facility by the mid-1950s (Bickert and Light, 1982; Kammel, 2005). They consist of large resting areas of 5.6 to 9.3 m^2 per animal (Bickert and Light, 1982; Thurgood et al., 2009), or 7.0 and 5.6 m^2 per animal for large and small breeds, respectively (Kammel, 2005). Although BP easily accommodate different breeds and double as manure storage, they require intensive management and large amounts of bedding relative to tiestall or freestall housing to be effective (Bickert and Light, 1982; Thurgood et al., 2009; Benson, 2012).

A layer of gravel or concrete may be required as a base in the barn, depending on regulations, to prevent leaching of water laden with urine, manure, or other nutrients into the environment. Similar to other housing systems, good ventilation is necessary to ensure cow health, aid in pack drying, and reduce barn odor (Kammel, 2005; Benson, 2012). In a study of New York and Vermont dairies, Benson (2012) reported the following bedded-pack barn advantages: producer satisfaction, increased cow comfort, better manure storage, greater soil amendment values, and enhanced conservation practices.

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The reduced capital cost to build a BP compared with a freestall barn can make them an attractive option (Kammel, 2005). However, by the year after building erection, 1,549 kg of bedding per cow was required for adequate bedding of the pack area, increasing annual costs to maintain the barn (Benson, 2012). Similarly, Thurgood et al. (2009) noted an increase from 18 t of bedding for the winter before moving into the bedded pack to 59 t of bedding for the winter in years 1 and 2 of BP operation. This led to an annual amount of 391 kg/animal before moving into the bedded pack, 1,347 kg/animal in the first year of housing in the pack, and 2,910 kg/animal in the second year of housing in the pack. Thurgood et al. (2009) suggested that the increased bedding required and the high cost of bedding led to no overall economic benefit with the BP system. However, increasing capital cost for a greater space allowance (e.g., 9.3 instead of 7.0 m^2 per animal) may reduce variable costs later through reduced bedding requirements. The higher surface area per cow increases the total water evaporated per cow, resulting in lower bedding requirements.

For cow cleanliness and health, dry bedding (<15% moisture) should be used to maintain a clean environment and maintain low bacterial counts. Bedding requirements can range from 4.5 to 15.9 kg/cow per day (Kammel, 2005). Allen (2007) suggested a minimum of 7 m^2 per cow with 18 kg of straw per cow per day to minimize environmental mastitis and maximize cow comfort. Bedding use may be reduced through removal of manure from the pack area or removing accumulated manure from feed alleys and water areas (Kammel, 2005; Thurgood et al., 2009).

Health and Behavior

Providing 10 m^2 per cow instead of 9 m^2 per cow improved lying time (Fregonesi and Leaver, 2002; Kammel, 2005; Fregonesi et al., 2009), increased SCC (Fregonesi and Leaver, 2001), and increased clinical mastitis incidence (Fregonesi and Leaver, 2001). Bedded-pack barns may lead to improved cow health and welfare compared with other housing systems. Less severe hoof disorders and reduced wear have been reported in BP-housed cows compared with cows in freestalls (Phillips and Schofield, 1994; Livesey et al., 1998; Webster, 2001).

Hoof Health

Livesey et al. (1998) noted that first-calf heifers housed on bedded packs versus those housed in freestalls with rubber mats and a straw layer experienced less severe claw horn lesions and less risk of developing white line and sole hemorrhages. Only heel erosion cases were more severe in BP-housed heifers, with heifers having thick, spongy heels that were "pitted" by heel erosion instead of worn down like the freestall-housed heifers. Neither system was the initial cause of claw horn lesions, but freestalls exacerbated claw horn lesion, white line hemorrhage, and sole hemorrhage severity (Livesey et al., 1998; Webster, 2001). Similarly, Phillips and Schofield (1994) reported that BP-housed cows exhibited deeper heels than those housed in freestalls. However, Phillips and Schofield (1994) and Livesey et al. (1998) reported no significant differences observed for lameness, with both housing systems maintaining a low change in locomotion score and no observed clinical lameness through the 6-mo study.

Somers et al. (2003) investigated causes of lameness in pasture herds, housed herds, and straw-bedded packs (BP) where cows were not housed on concrete. Mean percent of herds infected with interdigital dermatitis or heel erosion, digital dermatitis, and sole hemorrhages was lowest in BP compared with housed and pastured herds. Conversely, white line separation was greater in BP compared with housed and pastured herds (Somers et al., 2003).

Lying Time

Increased lying times have been reported in BP compared with freestalls (Kammel, 2005; Fregonesi et al., 2009). When 10 m^2 of space was provided per cow, increased lying and rumination times were observed in BP relative to freestalls by Fregonesi and Leaver (2001). With 9.2 m^2 per cow, no differences were noted in lying time, bed occupancy, and rumination time. High-yielding cows spent less time lying in straw BP, with no difference between high- and low-yielding cows in freestalls (Fregonesi and Leaver, 2002). When offered a choice between freestalls and BP, cows spent more time in BP and increased overall lying time. Phillips and Schofield (1994) noted that cows in all stages of the estrous cycle spent more time lying or feeding (interaction between housing and estrous period) and less time walking or standing in BP than in freestalls.

Reproduction and Behavior

Play behavior (mock fleeing, mock aggression, and environmental exploration) was noted when cows were moved from freestalls into the bedded pack, which may indicate greater animal welfare benefits (Fregonesi and Leaver, 2001). More estrous behaviors were recorded in bedded pack barns compared with freestall barns, including standing to be mounted, mounting without standing, successful mounting, chin rubbing on rump, sniffing or licking of the genital area, and fewer unsuc-

cessful mounting attempts. Consequently, pregnancy rate at first service improved in BP compared with freestalls (Phillips and Schofield, 1994).

Disadvantages

Some disadvantages have been associated with BP. The amount of bedding needed and the potential cost of bedding were greater in BP compared with freestalls (Kammel, 2005; Allen, 2007). However, Buli et al. (2010) suggested using 20 to 25 kg of sand per cow per day in the United States and 5 to 8 kg of sand per cow per day in Europe in freestall barns, similar to the amount of bedding required in bedded packs. Although milk yield did not change, Phillips and Schofield (1994) reported that cows decreased in body condition in straw-bedded pack barns compared with freestall barns. Consumption of bedding straw by cows when in the pack area could have increased rumen fill with a low energy source.

Barn orientation and space per animal are important in maintaining hygiene score and avoiding detrimental effect in straw-bedded packs (Fregonesi and Leaver, 2001, 2002; Kammel, 2005). Cows housed in straw-bedded packs with low space allowance produced increased SCC, clinical mastitis incidence, and hygiene scores with an accompanying milk yield reduction (Fregonesi and Leaver, 2001). This may be because of greater environmental pathogen exposure in bedded-pack barns (Kammel, 2005). Additionally, Kammel (2005) noted that access from the short side of the barn instead of the long side of the barn decreased space efficiency and cleanliness of the entryway.

TIESTALL BARNS

Tiestall or stanchion barns were the traditional type of dairy housing for many years, and stayed the most popular type of housing until the advent of larger herds and other dairy industry changes in the 20th century.

In 1850, the first US patent for a stanchion barn was awarded. Before 1900, the stanchion referred to a stall fitted within a barn designed to house a variety of animals. Stanchions were not intended to house the animals in total confinement but instead were seasonal housing during winter and year-round during milking (Hatch, 1850). Throughout the end of the 19th century, varied designs for stanchions were patented. The debate of optimum housing type dates to this period with tie chains, chainless gated stalls, and headlock-type devices all considered in stanchion stall design. (Huse, 1880; Gibbs, 1887; McCartney, 1887). In 1897, the first US patent for a type of tiestall housing was awarded. This tiestall consisted only of a divider, attached to the floor, in which feed was on one side and the cow on the other. The tie chain was fastened to this floor-level piece of either wood or metal (Witt, 1897). The tiestall is distinct from the previous patents for stanchions, as "tiestall" referred to a stall to go in a barn designed specifically to house only dairy animals. Before 1900, cows were housed with other animals in multipurpose barns, often containing tiestalls to house dairy cattle alongside other various livestock.

After the turn of the century, cows were still rarely housed in total confinement; however, these dairy-cattle-only stalls paved the way for a total confinement option. In 1911, the design for a tiestall was improved upon by M. S. Batchelder through the addition of a trough, dividing wall, and a device to prevent cows from stepping into or lying in the gutter (Batchelder, 1911). By 1930, a design for a tiestall had been patented that was very similar to the basic design that is seen today, with tubular metal stall dividers, concrete floors, curbs, and manure gutters (Hibbs and Miller, 1932).

Stall Design

Some studies attribute the decrease in cow comfort in freestall herds to the size of the stalls. The same consideration applies to tiestalls. In a study by Tucker et al. (2004), cows in wider stalls (126 cm) spent more time lying down (10.8 vs. 9.6 h/d) and less time standing with only the front hooves in stalls (58 vs. 85 min/d) compared with cows in narrower stalls (106 cm). Stall size is vital to the success of a tiestall system because cows do not have a stall size choice. The Ontario Ministry of Agriculture suggests that tiestall barns should accommodate the needs of cows in various life stages, and therefore body sizes, by having 3 different stall types: for first lactation, milking, and dry cows (Anderson, 2014).

Another consideration in tiestall barns is the ability of the cows to lunge forward naturally when rising to the standing position. Cows need 76 to 112 cm of forward lunge space ahead of where the front knees are during resting position. The neck rail height should be at least 15 cm above the stall surface and provide 81 cm of vertical clearance. In 1964, a modification to the tiestall was patented, which included a tie that allowed for forward lunging with the lack of a board in front of the cow (Berg, 1964). This addition has been widely adopted and improved upon to include ties that do not have any forward rails, only rubber-covered chains that prevent injury and allow for more free forward movement.

In a study by Zurbrigg et al. (2005), the height of the tie rail, another indicator of proper lunging space,

was correlated with neck lesions and udder cleanliness. Farms with high tie-rails (116 to 132 cm) had 70% fewer neck lesions compared with mid-range tie-rail height (99 to 114 cm). In addition, for every 2.5-cm increase in tie rail height, the prevalence of clean udders increased 0.2% (Zurbrigg et al., 2005). Tiestall rails have been improved to include options with and without stall dividers, water bowl attachments, feed bunk dividers, various types of neck rails, and different points of chain attachment.

In 2009, stall dividers made of flexible plastic were patented. Several companies patented their own version of the flexible stall parturition between 2009 and 2013. Flexible stall dividers could provide an advantage in cow comfort in tiestall and in freestall facilities through reducing cow injuries from stall dividers (Cow-Welfare, 2017). Gutters for waste removal have also been adapted over time. In 1890, W. H. Brewer patented a stall with a slatted floor at the rear and a gutter behind that to facilitate easier waste removal. Prior to the advent of the gutter system, "it was customary to clean the stable floor thoroughly before milking" (Brewer, 1890).

Barn Design

Equally as important as the stall is the barn in which the stalls are housed. Because tiestall facilities are often total confinement systems, factors such as ventilation and manure management become vital. Ventilation has improved significantly in recent years for all dairy housing systems. Ventilation for tiestalls can be both natural and mechanical. Natural ventilation relies upon thermal buoyancy, or the temperature difference inside and outside of the barn, and air exchange driven by wind. Natural ventilation relies on inlets at the eaves or inlets in the sidewalls of the barn for airflow in and ventilation stacks along the roof for airflow out. Pennsylvania State recommendations for types of natural ventilation systems in existing facilities specify local airspeed, orientation and placement of the barn, roof slope, and the size and placement of the openings (Graves, 2017). Mechanical ventilation in tiestall barns can include tunnel ventilation and other fan-based systems. Tunnel ventilation is a special system designed only for summer warm weather conditions. Tunnel ventilation is optimized for a 2-row tiestall barn with more than 40 cows. It relies upon large fans in the end of a barn and large outlet spaces with exhaust fans in the other end. Tunnel ventilation is preferred in weather that could cause heat stress conditions due to the high-speed airflow provided (Tyson et al., 2014). Other types of mechanical ventilation installations include the use of fans and inlets to promote airflow. Selection of fans and inlets is based upon the consideration of static pressure, airflow, and efficiency. Pennsylvania State recommends a combination of these types based on specific farm considerations (Graves, 2015).

Technology

Technology available to complement tiestalls has also been invented. One notable technology is the addition of individual automatic waterers available to each cow or every other cow. In 1932, a patent for a tiestall design included a place for such a water bowl, stating that they were common in "modern" dairy barns at the time (Hibbs and Miller, 1932).

Another technology created specifically for tiestalls is trainers. Trainers teach cows to step back when arching their back to defecate or urinate to encourage manure in the gutter and improve stall cleanliness (Anderson, 2008b). The first patent for a tiestall trainer was in 1930 (Jost, 1930) and it functioned as a spring-loaded whip. The first electric version was patented in 1966 (Treangen, 1966) and improved upon to the type in use today (Rousseau, Desjardins, and Rousseau, 1999). Research has shown that stalls are no dirtier with the use of the trainer 2 d per week than 7 d per week, and that 90% of shocks received from the trainer do not happen during defecation (Anderson, 2008b).

Tiestall Milking

In many tiestall barns, the milk pipeline runs throughout the barn and the milk machine itself is mobile and travels to each stall. This is a stark contrast to the freestall system, which requires moving the cows for each milking and the building of a holding pen facility. Tiestall milking brings many advantages, including the individual attention given to each cow, and the producer does not need to build additional milking facilities. The design of this system has greatly evolved over the past 100 years as milking technology in general has changed. The first milking machine was built in 1875, and was simply a catheter inserted into the teat to promote milk letdown. The first modern milking machine, the pulsating milker, was patented in 1893 by Dr. Alexander Sheils (Sheils, 1894). The entire milking machine, including all of the lines and the milking can, had to be moved to each cow in a tiestall system. In the modern pipeline system, there is a vacuum and milk pipeline outlet at each tiestall, and only the milking apparatus is moved from stall to stall. The cow is milked and all milk is transported via the pipeline system in the barn to the bulk tank.

Cow Health

Housing in tiestall barns reduces the stress from constantly shifting social orders and has been shown, in some studies, to reduce mortality rates compared with freestall facilities. Dechow et al. (2011) reported that cows in tiestall barns had lower mortality and early-lactation cull rate than cows housed in freestall barns, but both tiestall and freestall herds had higher mortality than bedded-pack facilities.

However, tiestalls present issues in cow comfort and adequate exercise. Cows housed in tiestalls are more prone to lameness and sole disorders than cattle housed in loose housing (Bielfeldt et al., 2005). Konggaard (1977) stated that dairy cattle housed in tiestall barns had reduced reproductive performance and higher cull rate (23% for tiestall herd, 18% for freestall, and 15% for loose housing) because of reproductive failures compared with cows in freestall and loose housing systems over 4 yr. In total, 69 cows were confirmed pregnant over the 4-yr period in tiestall housing, compared with 81 in freestall cubicles and 86 in deep-bedded loose housing. A mean of 2.33 inseminations was required to impregnate cows in a tiestall barn, compared with 2.32 in a freestall and 1.96 in loose housing facilities.

FREESTALL BARNS

Freestall housing facilities have been in use since the 1960s. Adolph Oien developed the first barn identified as freestall in 1959, in Washington State, in which individual stalls were installed in an existing loose housing shed. The primary driver identified was a lower bedding requirement per cow while maintaining clean animals. Later advantages included easy separation of lactating cow groups (by days in milk, parity, and health status) and feeding of a TMR (Bickert and Light, 1982).

Barn Design

Two early freestall types were developed: a U-shaped and an L-shaped system. The U-shaped system consisted of resting and feeding areas that formed legs of the U, with the milking parlor forming the base. This resulted in a large amount of paved area that needed to be scraped. The development of L-shaped barns reduced the amount of paved area. These barns were generally oriented with a south-facing resting area, an east-facing feeding area, and the milking parlor at the system apex. The importance of paved area space per cow was downgraded after 1962. In 1962, Paul Varney (in Turner, Maine) used an inventive freestall design on his farm. This design, built by Rodney Martin, integrated feeding and resting areas, albeit still separated, under a single roof. This barn adhered to the prior recommendation of 9.3 m^2 of paved area per cow with 13 to 15 m^2 of total area provided per cow. After adoption of this style, stall design—not paved area per cow—was reported to be a predictor of cow cleanliness. Consequently, the recommended paved space per cow was reduced to 7.4 m^2 (Bickert and Light, 1982).

In 1975, modified environment housing styles were introduced. Cold housing systems were not fully enclosed and had internal temperatures similar to ambient temperatures, whereas completely enclosed housing kept the internal temperature higher than ambient. The modified freestalls attempted to keep animals warmer in winter than in cold housing but not as warm as in completely enclosed barns. These barns relied upon natural ventilation for temperature and moisture control. Covered barns with a modified environment were particularly advantageous for reducing frozen manure in alleyways and controlling snow and rain runoff. An additional freestall design was the corral style; in this barn design, only the freestall area was covered, with or without a cover over the concrete feed alley and bunk, generally with an adjoining dirt lot (Bickert and Light, 1982).

More recently, the design of individual stalls has been standardized by animal weight range. The areas of design concern are body space, head space, and lunge space (Bickert et al., 2000). Body space defines the area from the front of the cow's knees to her rear, head space is the area in front of the cow's body occupied by her head, and lunge space is the additional space required for the cow to thrust her head when lunging forward to rise. The 2 main types of freestall designs are forward lunge and side lunge. A forward lunge provides longer stalls to allow animals to move forward to propel themselves up, whereas side lunge allows animals to move their heads into adjacent stalls and lunge sideways to stand. Side lunge freestalls allow the animal to place their heads above or below the lower partition (Bickert et al., 2000). Lunge space is the forward space required when lying down or rising up. The bob zone is the area within the lunge space where the chin nearly touches the ground when rising. When head lunge space is downward and upward, lunge space extends forward. Standing requires space vertically and forward, whereas hindquarters require space laterally (Buli et al., 2010).

Stalls consist of side partitions defining each cow's individual area, a brisket board to define their total lying area, a neck rail to prevent the animal from standing too far forward in stalls, and a base for the animal to rest on. The brisket board and neck rail limit the cow's lying and standing area, respectively, to prevent manure and urine from filling the rear end of the stall. Brisket boards and neck rails may be placed too far

forward, allowing manure and urine to enter the stall's rear. Shorter stalls, or brisket boards placed too far to the rear, limit the resting area for cows and may inhibit comfort when the cow is resting (Bickert et al., 2000). Brisket boards, particularly wooden boards, may reduce stall usage. In a Canadian study, lying time and length of lying bout were shorter when brisket boards were present in stalls than when they were absent (Tucker et al., 2006). Tucker et al. (2006) suggested that contact with stall features was uncomfortable for resting animals, decreasing the length of lying bouts. This change may be less severe with softer, more rounded, or lower brisket boards than the wooden ones used in the Canadian study.

Neck rails define the stall standing space and alter the time spent standing in a stall. When the neck rail is absent, more time is spent standing; and when the neck rail height is lower, standing time decreases. Changes in neck rail height did not alter time spent lying or the amount of defecation or urination in the stall (Tucker et al., 2005). Stalls are generally raised above the concrete alleyway, resulting in a curb (15–20 cm). A curb permits cows to avoid resting in manure or slurry and prevents manure or slurry from entering the stall during alley scraping or flushing (Bickert et al., 2000).

If a mattress or pad is used as the bedding surface, additional bedding material may be added to improve stall comfort, reduce injury potential, add resilience, absorb moisture, and collect manure tracked into the stall. Mattresses consist of a filler in a heavyweight polypropylene or other heavy fabric material (Bickert et al., 2000). Shredded rubber, water, and gel have been used as filler for mattresses (Bickert et al., 2000; Fulwider et al., 2007; Main, 2013). Pads can be rubber mats, plastic mats, carpeting materials, or other compressed products. Compressed earth or a concrete base may be the sole resting surface. However, compressed earth allows deep pockets to form at the front and rear of the stalls. These pockets trap manure and urine and increase the cow's difficulty in standing and lying. Pads, compressed earth, and concrete resting areas are not satisfactory for cushioning and require the addition of larger amounts of bedding to keep cows comfortable.

In a deep-bedding system, at least 15 cm of bedding depth should be provided (Bickert et al., 2000). Bedding choices include sawdust, straw, wood chips, shredded newspaper, composted or dried separated manure solids, corn stalks, bark, sunflower hulls, rice hulls, peanut hulls, sand, and ground limestone (Hogan et al., 1989, Bickert et al., 2000; NYSCHAP, 2002). Bickert et al. (2000) suggested twice-daily maintenance to remove wet bedding and manure, and the addition of bedding once or twice a week. Alleyways in all housing systems need to be scraped or flushed clean a minimum of twice a day (Bickert et al., 2000).

Hygiene

Hygiene depends on the bedding material used, management style, barn design, and stall dimensions. Different areas of the barn affect hygiene on different parts of the cow. Generally, bedding surface and bedding type have more influence on udder hygiene. Scraping frequency, ease of movement, and manure management system have more influence on leg hygiene (Schreiner and Ruegg, 2003).

Cows housed in sand freestalls had a greater proportion of higher hygiene scores than cows housed on mattresses or waterbeds (Fulwider et al., 2007). However, cows housed in sand freestalls were cleaner than cows housed in straw-bedded freestalls (Norring et al., 2008). Three main areas showed significant differences between straw and sand: mid-legs, upper legs, and belly. Conversely, van Gastelen et al. (2011) reported no differences in cow cleanliness for the udder, leg, flank, or total rear body score when animals were housed in freestalls using box compost, horse manure, sand, or foam mattresses, respectively, as a stall base.

Inorganic Bedding

Inorganic materials, namely sand, have been considered the gold standard for deep-bedded freestalls (Bickert, 1999; Allen, 2007). Sand can be sourced from a variety of locations: mason sand, dredged sand, beach sand, or quarried sand (Stowell and Inglis, 2000; Buli et al., 2010). Sand has 5 main qualities that make it an ideal bedding source: (1) it provides a comfortable resting surface, improving cow comfort; (2) it limits bacterial growth; (3) it has a low initial moisture content, reducing moisture build-up; (4) it remains cool and reduces heat stress through lower lying temperature than other bedding materials; and (5) it reduces slipping through improved traction (Stowell and Inglis, 2000; Allen, 2007; Buli et al., 2010). The nature of loose sand allows for movement with the animal, reducing friction on the hocks and increasing cushion for the animal (Bickert, 1999). A top layer of sand dries quickly due to water binding to single grains of sand, limiting both growth and survivability of bacteria (Stowell and Inglis, 2000; Allen, 2007; Buli et al., 2010). Sand has a lower environmental impact than other bedding types because of its natural and reusable nature (Buli et al., 2010). Increased traction allows cows to express natural behaviors (mounting) with less risk of slipping (Anderson, 2008a). A film of sand in alleyways increased

hoof growth and wear due to a more abrasive surface, particularly when concrete flooring was present (Vokey et al., 2001). Vokey et al. (2001) suggested that a rubber alley in conjunction with concrete or sand stalls balanced hind claw growth and wear, whereas concrete alleys with mattress stalls caused imbalances in wear and growth in both lateral and medial hind claws.

Large quantities of sand are required per cow per day (Buli et al., 2010). A stall base of sand requires 15 to 20 cm, although a minimum of 25 cm has been suggested, with complete sand bedding replacement necessary every 12 to 14 d (Bickert, 1999; Buli et al., 2010; Cook, 2010). Additional bedding may be needed during the week to cover the curb, and the stall should be cleaned twice daily (Allen, 2007; Buli et al., 2010). The amount of bedding used will change with the sand level relative to the curb. If sand is above the curb, 20 to 25 kg/d may be kicked out, instead of 10 to 15 kg/d when the level is below the curb. Failure to replace sand can decrease sand usage, but it will also decrease effective resting space and lead to decreased stall usage (Buli et al., 2010). Fulwider et al. (2007) noted that short sand lying areas and stalls that were not maintained at or above the curb often resulted in greater incidence of hock lesions. This could be due to abrasions from the exposed concrete curb. Recycled sand caused more knee injuries than fresh sand (Fulwider et al., 2007).

Bickert (1999) claimed that sand's only detriment was its handling difficulty in manure management. Similarly, Rodenburg (2000) referred to sand as the most laborious bedding option due to its difficult handling. These difficulties are expressed through increased wear on equipment and difficulty in slurry management (Buli et al., 2010).

Organic Bedding

Organic materials have been used extensively as bedding material, both as a top dressing for mattresses or pads and in deep-bedded stalls. Allen (2007) advised a minimum of 3 kg per cow per day of sawdust and 5 kg per cow per day of straw as top dressing for mattresses, waterbeds, or gel mattresses. Kiln-dried bedding wood sources undergo a drying process that kills most bacteria. Because of this, kiln-dried bedding is often recommended for freestall and compost bedded-pack barns (Janni et al., 2006; Allen, 2007). Green bedding materials are from wood sources that have not undergone a drying process (Briggs, 1994). The greatest physical difference between green and dried wood sources is their moisture content, with kiln-dried bedding being able to absorb more moisture than green bedding (Briggs, 1994; LeBlanc and Anderson, 2013).

Although Newman and Kowalski (1973) inferred that green bedding materials sustained higher bacterial counts, specifically *Klebsiella pneumoniae*, green sawdust was only compared against sand bedding. Fairchild et al. (1982) compared several bedding materials and teat swabs for total counts of coliforms and *Klebsiella* spp. One trial compared bedding and teat-end bacterial counts in stalls bedded with either green softwood sawdust or the same sawdust mixed with agricultural lime. No differences were found for total coliform or *Klebsiella* counts between the 2 bedding types for the trial duration. There appeared to be little benefit of mixing lime into sawdust in the rear of freestall, as the increase in *Klebsiella* spp. was numerically small and the pH of both beddings fell within a range of 6 to 8, compatible with coliform growth. When green sawdust was compared strictly with bedding with agricultural lime, lower growth of total coliforms and *Klebsiella* spp. was reported for lime bedding and teat ends.

Fairchild et al. (1982) compared total coliforms and *Klebsiella* spp. counts for green softwood sawdust, washed concrete-grade sand, agricultural lime, and cellulose fiber from old newspaper. Total coliform counts were higher in sawdust and newspaper than in sand and lime. Similarly, *Klebsiella* spp. counts were higher in sawdust and newspaper than in sand and lime. In both studies, lime and sand exhibited a pH outside the acceptable growth range for coliforms and offered a lack of nutrients and moisture (Fairchild et al., 1982). Total coliform and *Klebsiella* spp. in "fresh" sawdust bedding counts were generally higher than those in stockpiled bedding (Fairchild et al., 1982). Fairchild et al. (1982) suggested that high bacterial populations did not definitively lead to udder infection under good management conditions, as no cases of clinical mastitis were reported.

COMPOST BEDDED-PACK BARNS

Compost bedded-pack barns (**CBP**) have become a housing system of interest globally. In the United States, the first CBP were developed by Virginia dairy producers in the 1980s to increase cow comfort and longevity (Wagner, 2002). Minnesota (Janni et al., 2006; Barberg et al., 2007a; Shane et al., 2010), Kentucky (Damasceno, 2012; Black et al., 2014; Eckelkamp et al., 2014), Ohio (Douridas, 2012; Zhao et al., 2012), and New York (Petzen et al., 2009) researchers have examined CBP and herds housed within them. Israel, Germany, Austria, the Netherlands, South Korea, Italy, and Canada have begun using CBP as housing systems (Ferrari and Moscatelli, 2009; Galama, 2011; LeBlanc and Anderson, 2013). South Korea, Brazil, Argentina,

and Columbia have also recently adopted the system (J. L. Taraba, University of Kentucky, Lexington, personal communication; Albino et al., 2017).

Barn Design

Compost bedded-pack barns may be a more sustainable system than traditional dairy housing facilities. As a loose housing system, a CBP does not include the stalls and partitions found in freestall housing. Without stalls, the cows' resting and exercise areas are combined (Barberg et al., 2007a; Galama, 2011; Black et al., 2013). This combination of resting and exercise space for animals concurrently reduces greenhouse gas emissions and cost compared with freestall barns, and maintains cow health and well-being (Galama, 2011). The large, open resting areas (7 to 30 m^2 per cow) are generally separated from a concrete feed alley by a 1.2-m-high retaining wall (Janni et al., 2006; Klaas et al., 2010; Black et al., 2013). If space per cow decreased below 9.3 m^3, compaction and moisture could increase, inhibiting compost efficacy (Janni et al., 2006).

To allow easy access to the feed alley and water, walkways onto the pack should be provided every 35 to 40 m (Janni et al., 2006). Feed bunk space per cow should be 46 to 76 cm (Janni et al., 2006; Bewley et al., 2013) with at least 2 water troughs in the feed alley. Water troughs should be separated from the pack by a retaining wall to decrease moisture on the pack (0.91 m of water space per 15 to 20 cows; Janni et al., 2006; Bewley et al., 2013).

To maintain adequate natural ventilation and offset the height of the retaining wall, high sidewalls (4 to 5 m) are required in the barn (Janni et al., 2006; Bewley et al., 2013). Further requirements to maintain adequate natural ventilation include a roof pitch of at least 4:12, ridge vent opening of at least 7.6 cm for every 3.0 m of roof width with a minimum opening width of 30.5 cm, and an east–west orientation (Bewley et al., 2013). To prevent excess moisture from entering the pack, roof overhangs should be no less than 1 m and preferably a length one-third the height of the sidewall opening (Janni et al., 2006; Bewley et al., 2013). A base of clay, gravel, or concrete may be required to prevent leaching of urine, slurry, or other nutrients into the environment, depending on current government restrictions (Bewley et al., 2013). Additional lighting or mechanical ventilation may be used in the barns, depending on dairy management style (Janni et al., 2006; Damasceno, 2012). Fan speeds of 9.6 km/h may increase the length of time bedding will last in a barn, decreasing bedding costs by $11,800 per year (Eckelkamp, 2014). Any features in the barn need to be high enough to allow tractors entry onto the pack regardless of bedding depth for normal tilling and maintenance (Janni et al., 2006).

Composting the pack area allows feces and urine to be handled as solids (Janni et al., 2006). Barns can store manure for 6 to 12 mo before cleaning is required. The solid portion is commonly used for direct land application in the fall, although one-half to two-thirds could be removed in spring to allow adequate summer storage. This allows reestablishment of the barn before cold weather starts (Janni et al., 2006). The concrete feed alley collects 25 to 30% of manure and urine produced. This slurry needs to be handled and stored in a manure handling facility such as a lagoon, mini-manure pit, or stack slabs, or with daily manure hauling (Janni et al., 2006; Barberg et al., 2007a). Manure from the feed alley should not be spread back onto the pack, as the microbial activity of drying should equal the amount of manure voided on the pack. If the manure is spread onto the pack, it may increase the amount of bedding needed and create wet spots or overly manure-laden areas if not evenly spread. In addition, the extra traffic (from tractor or spreader) may increase compaction and reduce compost activity through reduced aeration (Janni et al., 2006).

Pack Management

Compost bedded-pack barns require periodic bedding addition and twice-daily tilling (Janni et al., 2006; Barberg et al., 2007a; Black et al., 2013). Aeration (tilling) incorporates manure and air (oxygen) into the pack (Shane et al., 2010). This process promotes aerobic microbiological activity, heating and drying the pack. Tilling of the pack also exposes greater pack surface area for drying (Janni et al., 2006). Heating and drying the pack provides a fresh, dry surface for cattle to lie on (Shane et al., 2010). Ideally, the internal temperature for CPB at a depth of 15 to 31 cm ranges from 43.3 to 65.0°C (Janni et al., 2006; Bewley et al., 2013). Areas of the barn that are chunky and compacted after stirring indicate pockets of anaerobic activity and lower temperature (Janni et al., 2006). The depth of tilling varies by individual producer and the specific tillage tool used (18 to 30 cm; Janni et al., 2006; Barberg et al., 2007a). Unlike US CBP, Israeli and Denmark compost barns are only tilled once per day (Klaas et al., 2010; Bjerg and Klaas, 2014). However, some US producers chose to till once or 3 times per day, depending on management style (Black et al., 2014).

To start a CBP, a bedding layer from 25 to 50 cm deep is added (Janni et al., 2006; Barberg et al., 2007a; Black et al., 2013). In Kentucky, producers began these barns with 25 cm of shavings. Bedding material is

added to the pack at a mean layer of 8.8 cm over the entire barn area (m^2), ranging from a dusting of bedding to a full load of bedding (Black et al., 2013). The influx of moisture in CBP comes from feces, urine, and moisture from microbial activity (Janni et al., 2006). In Minnesota, a full semi-load (14 to 16 t) is added when moisture content enables bedding to stick to cattle (every 1 to 5 wk; Janni et al., 2006; Barberg et al., 2007b; Endres and Janni, 2007). Israeli researchers did not add additional bedding in their CBP, instead relying solely on the composting process to maintain dry bedding (Klaas et al., 2010). Bewley et al. (2013) recommended a moisture range of 40 to 60% before adding new bedding. Shane et al. (2010) recommended keeping moisture content in the top 15 cm of the pack below 65%. More recently, 55% moisture was suggested as the benchmark for adding new bedding (Bewley et al., 2012; Eckelkamp et al., 2016b).

Bedding Material

Several different types of bedding have been used in CBP. Janni et al. (2006) and Barberg et al. (2007a) recommended sawdust and dry, fine wood shavings, although ground soybean straw was also effective. Kentucky dairy producers used green and kiln-dried shavings or sawdust, a mixture of shavings and sawdust, a mixture of soy hulls and shavings, or a mixture of soy hulls and sawdust (Black et al., 2013). Shavings have the potential to improve handling, mixing, aeration, and biological activity due to their large surface area (Janni et al., 2006). This increases the ability of microbial populations to grow and break down manure and urine added but prevents excessive compaction of the bedding between tillings (Janni et al., 2006).

Farmers in Israel and the Netherlands have used dried manure or compost from other sources (food or paper industry waste) in compost barns (Klaas et al., 2010; Galama, 2011). Bjerg and Klaas (2014) reported that in Denmark, CBP were bedded with wood chips with leaves, roots, and garden residuals, a combination of sawdust and wood shavings, or heathland vegetation. Israeli CBP established composting using layers of an inorganic residual product from oil extraction (similar to cat litter), paper industry waste, or a layer of dried manure obtained before establishment of the compost system. However, only the barn that used dried manure achieved a heat greater than ambient temperature (Klaas et al., 2010).

Some bedding materials are unsuitable for CBP. Coarse hay and cereal grain straw matted and clumped, decreasing effectiveness as bedding material. Corn stover does not maintain a coarse particle size, decreasing air incorporation into the pack (Petzen et al., 2009). Wallboard (92% calcium sulfate, 7% paper, and <1% impurities or additives) did not promote heat production (LeBlanc and Anderson, 2013). Janni et al. (2006) did not recommend straw, corn stalks, and wet or green sawdust. Straw and corn stalks proved difficult for proper tilling using the various tillage tools. Wet or green sawdust decreased the amount of water that could be absorbed from the pack (Janni et al., 2006). The increased possibility of *Klebsiella* spp. counts in bedding also increased concerns regarding green sawdust (Newman and Kowalski, 1973; Janni et al., 2006; Bewley et al., 2013). Cedar, black walnut, and cherry were not recommended for CBP bedding because of antimicrobial properties or the potential to cause diseases such as laminitis (Janni et al., 2006; Bewley et al., 2013).

Bedding Bacteria and Cow Health

Early CBP research suggested that maintaining a temperature of 54 to 65°C for 3 to 4 d had the potential to inactivate pathogens and viruses, destroy weed seeds and fly larvae, and decrease odor emanating from the pack. Inactivating mastitis pathogens was of particular interest (Janni et al., 2006). However, research by Black et al. (2014), Barberg et al. (2007a), Eckelkamp et al. (2016b), and Petzen et al. (2009) indicated that managing for good composting allowed proliferation of coliforms, staphylococci, streptococci, and bacilli species in the pack. Although bacterial levels were high, the expected effects on SCC, bulk tank SCC, and clinical mastitis have not been seen (Barberg et al., 2007b; Black et al., 2014; Eckelkamp et al., 2016a).

After transitioning to CBP, Minnesota dairy producers reported SCC to be 325,000 ± 172,000 cells/mL, below the state average of 357,000 cells/mL (Barberg et al., 2007b). The average prevalence of mastitis infection decreased after moving into the CBP from previous housing facilities (Barberg et al., 2007b). When comparing well-managed CBP against well-managed sand freestall barns, no differences were found between SCC, clinical mastitis incidence, or bulk tank SCC (Eckelkamp et al., 2016a).

Hygiene scores for cows housed on compost barns were similar to those housed on waterbeds in freestalls and lower than those for cows housed in sand freestalls or rubber-filled mattresses (Fulwider et al., 2007). Eckelkamp et al. (2016a) also reported no difference between CBP and sand-bedded freestall barns. Conversely, Lobeck et al. (2011) found that animals housed in CBP exhibited greater overall hygiene scores than animals in sand-bedded cross-ventilated and naturally ventilated freestall barns.

Hock scores were lower or nonexistent in CBP compared with freestall barns (Barberg et al., 2007b; Fulwider et al., 2007; Lobeck et al., 2011). Barberg et al. (2007b), Black et al. (2013), Lobeck et al. (2011), and Petzen et al. (2009) witnessed decreased lameness in cows housed in CBP compared with freestall systems. Eckelkamp et al. (2016a) did not report a decrease in lameness or hock lesions, but no differences were detected between CBP and sand freestall barns.

ROBOTIC MILKING

The newest addition to housing systems are automatic milking systems (**AMS**; Hulsen and Rodenburg, 2008). The first AMS was installed in the Netherlands in 1992, increasing to over 8,000 worldwide in 2009 (Svennersten-Sjaunja and Pettersson, 2008; de Koning, 2010). Although AMS reduce the manual labor attached to traditional milk parlors, AMS require cows to voluntarily come to be milked (Hulsen and Rodenburg, 2008; Jacobs and Siegford, 2012). Consequently, barns must be designed to encourage cows to visit the AMS voluntarily or by guided flow (Jacobs and Siegford, 2012). In the mid-2000s, barns began to be designed around AMS systems instead of AMS being added to an existing freestall barn. To encourage visits by cows to an AMS, a barn must be designed for excellent cow traffic. Cow traffic is governed by a series of gates (or pathways in the absence of gates) that guide cows throughout the barn (Jacobs and Siegford, 2012).

Free-flow systems allow cows to move freely between the AMS, feed area, and resting area (Melin et al., 2006; Tremblay et al., 2016). Forced, one-way, or guided flow leads cows to be milked at the AMS before eating at the feed alley (Jacobs and Siegford, 2012). This system can be adjusted with selection gates to only guide cows over the time allotted between milking to the AMS (Melin et al., 2006). Building this system may reduce the number of cows that need to be fetched, but may also reduce eating time and resting time. However, conflicting reports exist on the effect of guided flow on eating time and lying time (Jacobs and Siegford, 2012). A recent paper associated greater milk yield with free-flow systems compared with forced flow (Tremblay et al., 2016). Ipema (1997) suggested that the choice of free or forced flow should be made based on productivity, lying behavior, and individual cow reactions to the system.

Designing facilities to improve cow comfort (e.g., resting area, walkways, cleanliness) will improve AMS use. Lame cows will require more "fetches" than their nonlame counterparts (Ipema et al., 1987; Ipema, 1997). When facilities are designed to promote cow flow with positive enforcement of concentrates fed in the AMS, as few as 0.8% of milkings/cow will require fetching (Ipema, 1997). Placing selection units (gates guiding cows to the AMS or the feed alley) in the passage from the resting area to the feed alley can decrease unsuccessful visits to an AMS system. An unsuccessful visit would be one when a cow has been milked too recently to be milked again and is entering the robot for another reason. Although the time lag from this may seem small, on a costly investment such as an AMS, having only viable cows enter increases productivity. Placing the exit of the AMS near the feed alley will capitalize on cows' drive to eat following a milking event (Metz-Stefanowska et al., 1993; Halachmi et al., 2000).

Providing enough space for cows to comfortably enter and exit the AMS is important. Free-flow lanes should range between 4 and 7 m wide. Within a holding pen in guided flow, allowing animals 1.3 m^2 per cow (the number the pen is intended to hold) and 33 m^2 of free space around the selection unit is preferable (Ipema, 1997). Adding automatic alley scrapers in freestall barns can also improve cow hygiene (Buitink, 1991; Ipema, 1997).

In the early 2000s, Halachmi (2000) suggested changes in AMS design based on a simulation model. Using process orientation, Halachmi (2000) determined that a 40-cow herd used space more efficiently in a robotic milking barn than what is typically seen in a freestall barn. To maximize efficiency, cows were provided with 35 stalls and 12 feed spaces (equivalent to recommended space per cow). With concentrate supplied at the AMS, and in the case of Halachmi (2000), 2 additional concentrate feeders, less area was required to devote to a partially mixed ration. Based on time management and allowing cows to decide their own schedule, barn design may be more successful through techniques described in Halachmi et al. (2000) and Halachmi (2000).

FUTURE DIRECTIONS

Freestall and cubicle housing systems are the primary housing system for lactating dairy cattle in many parts of the world. Early freestall barns lacked features that maximized cow comfort. Today, modern freestall barns are generally cow-centered with a large focus on cow comfort. Among freestall barns, the sand-bedded freestall barn is often promoted as the ideal system. However, after decades of housing cows in freestalls, limitations remain. Lameness levels remain high in many freestall barns, and manure handling is challenging. Further, as the science of animal behavior grows and consumers place more pressure on dairy farms, new questions arise around natural behavior within freestalls. Should freestall barns be the housing of the future? Would other housing options promote more natural behaviors and remove some of the limitations of

freestall barns? What alternative systems hold promise? The term "freewalk housing" has been used to describe housing systems that provide animals the opportunity to walk more freely within the barn, often in combination with grazing access. Compost bedded-pack barns and cow gardens are 2 examples of freewalk housing. A compost bedded-pack barn consists of a large, open resting area, usually bedded with sawdust or dry, fine wood shavings. Bedding material is composted in place, along with manure, when mechanically stirred on a regular basis. Producers report reduced incidence of lameness and improved hoof health resulting from greater lying times and a softer, drier surface for standing. The cow garden system has a multi-layer, semi-permeable floor. Manure is removed by robotic scrapers, and trees and plants are planted within the barn. Questions remain about the suitability of these facilities.

Advancements in monitoring technologies and materials science may provide new refinements to dairy cattle housing. Real-time location systems may enable targeted cooling based on presence or absence of cows in a particular part of the barn. Physiological and behavioral technologies may be incorporated into feedback loops to control whole or micro-environments within dairy facilities. Innovations in materials science may lead to better solutions for cow resting surfaces or beddings. As the industry has grasped the importance of cow comfort in optimizing production, health, and well-being, more solutions will be sought to continue to improve lactating cow housing environments.

In evaluation of alternative systems, a systems approach must be applied that addresses animal behavior, economics, and environmental sustainability. The dairy farm is a complex system, and lactating cow housing plays an important role within that system. No one factor can or should dictate what type of housing is chosen. Rather, the choice is a balance of many internal and external factors. Dairy cattle housing of the future will likely reflect continued emphasis on consumer demands, animal behavior, and environmental impact. It is difficult to predict what future housing systems will be developed and most widely adopted. However, when one considers how dramatically housing systems have changed in the last 100 years, we should expect similar, if not more rapid, progress in the next 100 years.

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APPENDIX

Table A1. Timeline of developments in lactating dairy cattle housing

Date	Milestone	Reference
1850	First dairy tiestall or stanchion is patented.	Hatch, 1850
1894	First pulsation milker is introduced.	Sheils, 1894
1897	First US patent is assigned for a tiestall.	Witt, 1897
1929	Individual waterers on tiestalls became "common."	Hibbs and Miller, 1932
1930	Basic version of modern tiestall is patented.	Hibbs and Miller, 1932
1950	Bedded-pack barns are recognized as a loose-housing facility.	Bickert and Light, 1982
1961	The first freestall barn is built in Washington State.	Bickert and Light, 1982
1962	Feeding and resting areas are integrated into a single freestall barn – Rodney Martin; stall design becomes indicator of cow cleanliness.	Bickert and Light, 1982
1964	First tiestall with open front (no forward board) is introduced to allow for less restricted movement and lunge space.	Berg, 1964
1966	Electric trainer is patented.	Treangen, 1966
1975	Modified environmental housing styles (freestalls) are introduced to keep animals warmer in winter and cooler in summer.	Bickert and Light, 1982
1980	First compost bedded-pack barn is developed in Virginia.	Wagner, 2002
1992	First automatic milking system (AMS) is installed in the Netherlands.	Svennersten-Sjaunja and Pettersson, 2008
2000	Animal weight becomes the standard for stall dimensions. First robotic dairy is built in the United States.	Bickert et al., 2000
2001	Play behavior is observed in bedded pack barns after animals are moved from freestalls.	Fregonesi and Leaver, 2001
2005	Tie rail height is shown to be related to neck lesions and udder cleanliness.	Zurbrigg et al., 2005
2006	Resting quality is shown to be affected by stall design.	Tucker et al., 2006
2007	Flexible plastic tiestall dividers are invented.	Cow-Welfare, 2017

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A 100-Year Review: Metabolic health indicators and management of dairy cattle[1]

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ABSTRACT

Our aim in this *Journal of Dairy Science* centennial review is to describe the evolution of focus on metabolic indicators, from discovery and description to evaluation at the individual cow and subsequently herd levels, over the past 100 yr. Furthermore, we discuss current and future technologies that will be used in the dairy industry to utilize these indicators widely going forward. Knowledge of chemical changes in various fluids (e.g., blood, urine, and milk) accompanying numerous metabolic disease states in the dairy cow has existed since almost the beginning of the *Journal of Dairy Science* 100 yr ago. However, only during the last 25 yr have these metabolic indicators been developed into useful tools for cow- and herd-level monitoring for disease and management. From the 1920s through the 1940s, our understanding of the changes in blood chemistry accompanying milk fever and ketosis increased, as did our understanding of the underlying biology. In the 1950s and 1960s, workers studying ketosis and energy metabolism began to evaluate changes in lipid metabolism reflected by concentrations of circulating nonesterified fatty acids; furthermore, initial development occurred for on-farm tests of milk ketones. During the 1970s, blood metabolic profiling was applied to dairy farms but found to be of varied and limited usefulness. The turning point occurred when large epidemiologic studies of periparturient cow disease were pioneered in the United States, Canada, and Europe in the 1980s; these studies further solidified our understanding of risk factors and epidemiological interrelationships among disease, production, and reproduction. In the early 1990s, scientists first incorporated indicators of metabolic health into large observational studies and determined important epidemiological relationships between these indicators and outcomes of interest. This field of study blossomed during the 2000s as several research groups conducted multiple investigations into metabolic indicators related to energy metabolism and began to develop cow-level thresholds and herd-level alarms for use in monitoring and management. This work was accompanied by additional studies to validate point-of-care instruments that could be used to implement these strategies at the cow and herd levels. Work in the 2000s continued to identify and evaluate other physiological indicators of inflammation and oxidative stress; however, these have yet to be incorporated into large-scale cohort studies. Finally, use of technology (e.g., activity monitoring, cow-monitoring collars and tags, milk-based analysis using Fourier transform infrared spectroscopy) continues to receive significant attention going forward to eventually allow for real-time and automatic monitoring of metabolic indicators and improved health and herd management on dairy farms.
Key words: transition cow, technology, metabolic disease

INTRODUCTION

Recognition that clinical diseases and disorders are accompanied by chemical changes in blood, urine, or milk of dairy cattle has existed for almost 100 yr. Remarkably, only during the past 25 yr or so have we begun to realize the value of analytes measured in these fluids for both cow- and herd-level disease detection and monitoring. This realization, combined with advances in technology that enable practical measurement at the cow and herd levels through on-farm tests, has created a rapidly growing appetite among dairy farm managers, veterinary practitioners, and other herd consultants for this type of information. Continued gains of knowledge related to the chemistry of fluids that are readily measurable on farm and their relationships with disease and other economically important outcomes related to milk yield and reproductive performance, along with the rapid advancement of technologies that will enable real-time, automated measurement, will continue to revolutionize how we approach the monitoring of metabolic function and health in dairy cattle.

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In this *Journal of Dairy Science* (**JDS**) centennial review, we describe the evolution of knowledge of metabolic health indicators from the early days of discovery, description, and eventual use in individual cow medicine to the determination—beginning in the 1990s and rapidly expanding through the 2000s—that these indicators are associated more broadly with cow- and herd-level disease, milk production, and reproduction. Furthermore, we review the progression of focus from clinical to subclinical disease detection and monitoring at the herd level and how use of these metabolic health indicators has been an integral part of both the research and eventual on-farm application of programs targeting subclinical disease. We present what we think are the key milestones in Appendix Table A1. Concurrent with research on the biology and epidemiology of subclinical disease and metabolic dysfunction has been progress on the development and validation of technologies that can be applied on farm; we describe the state of our knowledge in this rapidly evolving area. Finally, we provide our perspectives on the development and use of metabolic health indicators in the management of dairy cattle going forward. We focus primarily on contributions made within JDS; however, given the nature of this topic, we also refer to selected works in other journals as appropriate.

THE EARLY DAYS: THE FIRST 75 YEARS

Evidence in the scientific literature that clinical health disorders such as milk fever and ketosis were related to changes in blood and urine chemistry began appearing in the 1920s and 1930s. Various scientists and veterinary practitioners (e.g., Hayden and Scholl, 1923; Dryerre and Greig, 1925; Little and Wright, 1925) determined that cows with milk fever had decreased blood Ca concentrations and hypothesized that dysfunction in the regulation of Ca metabolism in the cow caused milk fever. Others (e.g., Stinson, 1928; Sampson et al., 1933; Lormore, 1934; Boddie, 1935) reported that concentrations of acetone in blood and urine were elevated dramatically in cows diagnosed with ketosis, although causative and predisposing factors were not well recognized at the time. This early work provided the basis for what was to come with more focused work on these chemical changes in blood and urine.

Metabolic Indicators and Hypocalcemia

The first reports in JDS on blood Ca and P and relationships with milk fever appeared during the 1930s. Initially, these papers largely characterized "normal" concentration ranges (later commonly referred to as reference intervals) of these minerals (Anderson et al., 1930; Palmer et al., 1930; Palmer and Eckles, 1930) and provided some initial characterization of how nutrition and management factors may affect them. Wilson and Hart (1932) assessed the dynamics of Ca and P during the immediate periparturient period in both healthy cows and cows diagnosed with milk fever and suggested that blood samples should be collected at multiple time points relative to calving to adequately characterize the decrease at parturition and recovery of blood Ca concentrations postcalving that occurred even in normal cows; furthermore, patterns of blood P concentrations were much more variable than blood Ca concentrations during the same period.

With the exception of additional reports (Kennedy et al., 1939; Vanlandingham et al., 1942) related to concentrations of Ca and P in blood and various factors affecting them related to age and stage of development, further studies of the relationships of blood chemistry with milk fever were not reported in JDS until the 1950s. Blosser and Smith (1950a) measured serum Ca and citric acid concentrations in both healthy cows and cows with milk fever and determined that citric acid and Ca concentrations generally followed the same pattern in normally calving cows and cows with milk fever; however, concentrations of citric acid were elevated during the immediate prepartum period in cows that subsequently developed milk fever. They determined that cows that developed milk fever had increased urinary excretion of citric acid during the immediate prepartum period and few other changes in urine chemistry other than increased excretion of Mg from d 3 to 16 prepartum. These cows also had increased serum Mg accompanying milk fever during the postpartum period (Blosser and Smith, 1950b). Van Soest and Blosser (1954) noted that cows with milk fever had elevated concentrations of blood glucose and strongly negative correlations between blood glucose and P concentrations.

By this time, milk fever was a well-recognized disorder and the linkage between milk fever and blood Ca concentrations was well established; therefore, scientific focus turned to the mechanisms underpinning Ca metabolism in the cow (e.g., Mayer et al., 1969; Ramberg et al., 1970; Ramberg et al., 1976; Horst et al., 1977) and management strategies to prevent milk fever (reviewed by Horst et al., 1997). Few additional reports relating to the specific use of blood Ca and related analytes as indicators of Ca metabolism appeared in JDS until Ballantine and Herbein (1991) evaluated the relationships of ionized (more metabolically active) and total Ca and determined that the relationships between the two varied by time relative to parturition; the proportion of total Ca in the ionized form was highest during the early postpartum period. Furthermore, Szenci

et al. (1994) determined that ionized Ca concentrations were more stable than total Ca concentrations during the periparturient period. We provide more discussion on testing technologies for Ca status and perspectives going forward in a subsequent section of this review.

Metabolic Indicators and Hyperketonemia

The first reports of the relationships of ketosis with blood and urine chemistry in JDS emerged from a series of studies conducted by workers at the University of Connecticut and published in the early 1940s. Knodt et al. (1942a) measured concentrations of ketone bodies (acetone, acetoacetate, and BHB) in blood and urine of cows and reported variation in ketone body concentrations both within day related to feeding time and across various parts of the year as cows moved from pasture to hay to grass silage feeding. In other work, they determined relationships of blood and urine ketone bodies with time relative to parturition and compared concentrations of ketone bodies in blood and urine, noting a correlation (but with r values ranging from 0.33 to 0.51) for concentrations of BHB, acetone, and acetoacetic acid and total acetone bodies in blood and urine (Knodt et al., 1942b). This group conducted numerous other studies (e.g., Knodt et al., 1942c; Shaw et al., 1942; Shaw, 1943) during this period to examine the dynamics of ketone body concentrations in growing animals and the quantitative utilization of ketone bodies related to milk fat synthesis and oxidative metabolism in the cow as well as the onset of ketosis. Furthermore, they noted that cows with complicated ketosis, particularly during the later stages of ketosis, also had elevated concentrations of free fatty acids in blood (Saarinen and Shaw, 1950).

During the late 1950s and 1960s, research groups at the University of Wisconsin and Michigan State University renewed this focus on ketosis and lipid metabolism in the periparturient dairy cow, with additional emphasis on strategies that could be used at the farm level to detect and potentially treat ketotic cows. Schultz and Myers (1959) evaluated a milk test for ketosis that could be used at the farm level, largely over concern that the urine tests available at the time resulted in false positives. Their evaluation suggested high correlations between blood and milk levels related to acetoacetic acid and acetone and lower correlations between blood and milk BHB but high specificity and sensitivity between blood and milk when assessed using total ketone body concentrations. Subsequently, Emery et al. (1964) used a milk ketone test on 2 dairy farms to detect ketosis followed by assignment of cows detected with ketosis to control or propylene glycol treatment groups; they reported that treatment decreased milk ketones in both herds and increased milk production in one of the herds.

Radloff et al. (1966) conducted a series of studies to extend the information previously determined on blood ketones to also include and evaluate relationships with blood glucose and nonesterified fatty acids (**NEFA**). They measured sharply decreased circulating NEFA concentrations following feeding, particularly in higher producing cows, and large increases in circulating NEFA following fasting in goats, particularly in lactating compared with nonlactating animals. Finally, they characterized a pronounced increase in plasma NEFA concentrations near the time of parturition followed by decreasing concentrations for the first 5 to 6 wk of lactation. Correlations of blood ketones were much stronger with blood glucose than with plasma NEFA; in their study, there was little relationship of plasma NEFA with milk yield and a low correlation (~0.10) of blood ketones and plasma NEFA. In further work focused on ketotic cows (Radloff and Schultz, 1967), they determined much higher positive correlations (~0.85) between blood ketones and plasma NEFA following the onset of ketosis.

These groups continued to evaluate testing approaches for ketones and their application. Emery et al. (1968) determined that use of a milk test that would detect acetone plus acetoacetic acid identified 68% of the ketotic cows before the onset of clinical symptoms and claimed that the test used by Schultz and Myers (1959) would have detected only 41% of these cases; furthermore, 75% of the cows detected as ketotic developed another disease within 14 d after the positive test. Menahan et al. (1967) sought to further refine understanding of the relationships between various components of ketone bodies within blood and urine of goats experimentally induced to create varying degrees of hyperketonemia and hypoglycemia. They determined that BHB was the predominant ketone body at low concentrations but that the ratio of BHB to acetoacetate plus acetone decreased as total ketone bodies increased, suggesting greater relative concentrations of the latter at higher total ketone body concentrations. Despite this potential variation in the relationship between BHB and other ketone bodies of interest, BHB remains the sole blood ketone typically evaluated.

Blood Proteins as Metabolic Indicators

The first reports of changes in blood proteins in periparturient cows in JDS appeared in the 1950s following several papers published during the 1940s in *Journal of Biological Chemistry* focused on the relationships between blood proteins in the cow and transfer to the calf through colostrum. Larson and Kendall (1957) mea-

sured changes in various blood proteins during the dry period and continuing into lactation and determined that concentrations of total serum proteins decreased beginning about 4 wk before calving and were lowest at calving before increasing during lactation. Changes in several of the globulin fractions (i.e., β2 and γ1) were largely responsible for the decrease, as serum albumin concentrations and concentrations of other globulin fractions (i.e., β1 and γ2) were similar across the time period. Reports of changes in blood proteins and other indicators related to protein metabolism were absent from JDS until Blum et al. (1985) described changes in plasma 3-methylhistidine as a marker of protein breakdown and its relationships with other blood indicators during the periparturient period and lactation. They determined that plasma 3-methylhistidine concentrations increased sharply after calving, peaking during wk 1 postcalving and then decreasing until wk 5 postcalving. The spike in 3-methyhistidine coincided with lower concentrations of insulin, thyroid hormones, glucose, protein, and urea and increased concentrations of NEFA. Although we generally have an understanding of the dynamics of protein mobilization in the periparturient dairy cow, we still lack full understanding of the regulation thereof as well as dietary and management factors that affect it.

Metabolic Profile and Its Application

Following several papers in veterinary journals (e.g., Payne et al., 1970; Blowey, 1975; Parker and Blowey, 1976) in which results from blood metabolic profiles in dairy herds were reported and related to herd veterinary investigation or herd status, papers describing blood metabolic profiles, including the Compton metabolic profile and others, began appearing in JDS. Lee et al. (1978) reported means and variances for several blood analytes, including macrominerals, various indicators related to protein metabolism, and glucose in 5 normal and 25 "problem" herds in Illinois. Blood was collected from a representative sample of cows in high-producing, low-producing, and dry cow groups; although not reported, we suspect that most or all of the lactating cows sampled were in established lactation and likely not in the immediate postpartum period. The researchers determined that there were strong relationships of herd, production, stage of lactation, and season with several of the analytes and that several of the analytes were related to intake of certain nutrients; however, there were no clear patterns between normal and problem herds. Interestingly, they concluded that "concentrations of metabolites are of almost no practical use for individual cows because of the extreme variations in diet required to generate abnormal concentrations of blood metabolites."

Adams et al. (1978) published an excellent review based on results from blood chemistries from 750 problem herds submitted to the Penn State Large Animal Diagnostic Laboratory beginning in 1968 as well as an in-depth study of 15 high-producing herds with good health and reproduction. They noted statistically significant but low correlations of some blood indicators with nutrient intake along with very inconsistent patterns for herds varying in disease and reproductive status. They provided an excellent overall discussion of the limitations of interpretation of metabolic profiles, including the determination of reference ranges and the appropriate determination of deviations of indicators from normal, and concluded that metabolic profiles could be a useful adjunct in investigations of problem herds but that there was also great "potential for misuse."

Several additional studies published in JDS in the 1980s focused on the application of blood metabolic profiles. Jones et al. (1982) studied profiles from 30 herds in Virginia varying in production level and concluded that metabolic profile testing was of limited value in assessing problems or nutritional deficiencies in herds. Kronfeld et al. (1982) analyzed blood profiles from 395 cows in 21 herds in Pennsylvania and suggested that distributions of values for many of the blood indicators did not fit normal distributions and that this nonnormality should be considered when developing ranges for interpretation. Furthermore, multiple regression analyses involving multiple ration characteristics improved correlations with blood analytes. These authors were more optimistic than previous authors about the potential value of metabolic profiling given these considerations.

Metabolic Indicators Related to Oxidative Metabolism

During the 1970s, papers began appearing in JDS on the determination of status of vitamins and trace minerals related to oxidative metabolism and immune function in dairy cattle. Schingoethe et al. (1978) assessed vitamin E concentrations of milk from cows fed only stored feed year round and from cows pastured during the summer. They determined that concentrations of vitamin E in milk were increased when cows were fed pasture but did not affect blood cell composition, hemoglobin and related indicators, serum glutamic oxalaocetic transaminase, services per conception, calving interval, or retained placenta. However, the number of cows in the study was limited (20 cows per treatment

at the beginning and 12 at the end of the 4-yr study). Maus et al. (1980) reported that increasing dietary Se intake from basal sources and supplemental sodium selenite in the range from 2 to 6 mg/d increased both plasma and milk Se concentrations; however, further increases in Se intake did not further increase plasma or milk Se. Although vitamin E and Se are commonly thought to interrelate, Van Saun et al. (1989) measured vitamin E (α-tocopherol) and Se concentrations in serum from paired dams and fetuses during pregnancy and determined minimal interactions between these nutrients in both the dams and the fetuses.

Chew et al. (1982) determined that concentrations of plasma vitamin A, β-carotene, and total vitamin A equivalent were inversely related to California mastitis test scores indicative of mastitis. In a subsequent study, the same group determined that plasma concentrations of vitamin A and β-carotene decreased sharply during the prepartum period and were lowest during the first week postpartum; vitamin A concentrations were lower postpartum and, curiously, β-carotene concentrations were higher prepartum in cows that developed mastitis during the postpartum period (Johnston and Chew, 1984).

THE TURNING POINT

In the mid-1980s, researchers at Cornell University reported for the first time in JDS the results of large-scale observational studies involving commercial dairy farms and evaluating risk factors for and interrelationships among various periparturient diseases and disorders (Curtis et al., 1984, 1985). At about the same time, Canadian researchers (e.g., Dohoo et al., 1983, 1984; Dohoo and Martin, 1984) published an extensive series of papers from their own large-scale observational study of commercial dairy farms focused on disease, production, and culling in dairy cows. In addition, researchers in Sweden (Andersson and Emanuelson, 1985) published the results of a large observational study focused on hyperketonemia using concentrations of milk acetone to measure hyperketonemia, and Markusfeld (1986) published a large observational study focused on the epidemiological risk factors for displaced abomasum and relationships with other disorders in Israeli dairy herds. Until this time, much of our knowledge regarding risk factors for periparturient disease and relationships between diseases or metabolic indicators and production and reproduction had generally come from much smaller studies (e.g., Schingoethe et al., 1978; Larson et al., 1980) or observations stemming from randomized controlled trials evaluating various dietary treatments (e.g., dry period energy levels investigated by Coppock et al., 1972). Results from these and other (e.g., Rowlands and Lucey, 1986; Gröhn et al., 1989; Detilleux et al., 1994) large-scale epidemiologic studies of periparturient health, production, and reproduction truly provided the foundation on which our modern understanding of the relationships among these in the periparturient dairy cow is built and facilitated the generation of innumerable testable hypotheses that we continue to evaluate today.

In the early 1990s, work continued to further refine our understanding of the relationships of various metabolic indicators with production, DMI, and energy balance of dairy cattle. In a comparatively small study involving 14 multiparous cows, Lean et al. (1992) investigated concurrent and temporal relationships of serum BHB, NEFA, glucose, cholesterol, milk yield, DMI, and predicted energy balance during the postpartum period using repeated measurements and sampling for all variables. They determined positive associations between BHB and milk yield; a similar relationship between NEFA and BHB; and negative correlations between DMI and BHB, energy balance and BHB, and glucose and BHB.

In our opinion, the study that truly integrated metabolic health indicators within the context of large-scale rigorous analytical studies of periparturient health, production, and reproduction and laid the groundwork for the approach that has been used so successfully by many other research groups, including ours, was conducted by researchers at Michigan State University in the early to mid 1990s and published in part in JDS (Cameron et al., 1998) and as a master's thesis (Dyk, 1995). They enrolled a total of 104 high-producing commercial dairy farms in Michigan, visited each farm 4 times within a 6-wk period, assigned BCS to cows at each visit, collected blood samples from each cow for subsequent analysis of plasma NEFA beginning 5 wk before the expected calving date until calving, and collected detailed health, nutrition, and management information from each farm. They conducted their statistical analyses at both the cow and herd levels. Among other findings, they determined that elevated prepartum plasma NEFA, high BCS, poor feed bunk management, and higher energy content of the prepartum rations were important risk factors for displaced abomasum (Cameron et al., 1998). Furthermore, they determined that elevated prepartum plasma NEFA concentrations were associated with many (dystocia, retained placenta, ketosis, displaced abomasum, and mastitis) but not all (milk fever) periparturient disorders.

In addition to the work above expanding the use of metabolic health indicators related to energy metabolism into large-scale observational studies, researchers interested in oxidative metabolism and immune func-

tion began to integrate these indicators into larger scale studies. Weiss et al. (1990) enrolled 9 herds in a year-long study relating selenium, vitamin E, and SCC. They analyzed blood samples collected from a subset of cows from each herd between 60 d prepartum and 60 d postpartum for plasma Se, α-tocopherol, and glutathione peroxidase and determined that bulk tank SCC and herd mean plasma Se concentrations were correlated negatively. Jukola et al. (1996) evaluated relationships of clinical mastitis, bacterial infections, fertility treatments, and fertility with serum concentrations of vitamin E, vitamin A, and β-carotene and whole-blood Se and glutathione peroxidase in 511 cows across 30 dairy herds in Finland. They determined that cows with higher concentrations of whole-blood Se had decreased mammary infections, but with a low coefficient of determination between serum vitamin A and SCC. However, the DIM for sampling was not well described in their paper, which makes further interpretation difficult.

EXPANDING USE OF METABOLIC HEALTH INDICATORS THROUGH EPIDEMIOLOGY OF PERIPARTURIENT COW- AND HERD-LEVEL OUTCOMES

Energy- and Macromineral-Related Indicators

The first rigorous epidemiologic study beginning to define cow-level cut-points of metabolite concentrations and their association with postpartum health events was conducted by scientists at the University of Guelph and published in 2005 (LeBlanc et al., 2005). In 20 predominantly tie stall herds, the researchers explored several metabolites (e.g., NEFA, cholesterol, BHB, glucose, urea, Ca, and P) and their association with left displaced abomasum (**LDA**) in 1,044 cows in which the incidence risk of LDA was 5.1%. This work indicated that cows with an NEFA concentration >0.5 mEq/L 4 to 10 d prepartum had 3.6 greater odds of developing an LDA and that cows with a postpartum BHB concentration >1,200 μmol/L had 8 times greater odds of developing an LDA compared with cows with lower concentrations, whereas Ca concentration was not associated with LDA. These results were similar to those of a future Guelph study from a different set of predominately tie stall-housed, component-fed herds (n = 25) in Ontario in which the investigators (Duffield et al., 2009) also noted a strong association [odds ratio (**OR**) = 2.6] with LDA in cows with BHB concentrations >1,200 μmol/L in wk 1 postpartum and negative effects on DHIA test milk yields.

In a series of prospective cohort studies in the northeastern United States by Ospina et al. (2010a,b), Cornell investigators examined (1) the NEFA and BHB concentration cut-points that are associated with a higher risk of postpartum diseases, higher risk of poorer reproductive performance, and lower milk production; (2) the magnitudes of these risks; and (3) the frequency of elevated NEFA and BHB concentrations in predominately free stall-housed, TMR-fed herds. In 100 herds (average size = 840 cows) and 2,758 cows, they found strong associations between elevations in these metabolites and unwanted subsequent events in otherwise apparently healthy cows. Those cows with NEFA concentrations >0.3 mEq/L between 2 and 14 d prepartum had 2 times higher risk of any postpartum metabolic disease (LDA, metritis, or clinical ketosis), were >15% less likely to be pregnant by 70 d after the voluntary waiting period, and made approximately 680 kg less 305-d milk than those with lower concentrations. Those cows with NEFA concentrations >0.6 mEq/L between 3 and 14 d postpartum had >4 times higher risk of postpartum diseases (LDA, metritis, or clinical ketosis) and were >15% less likely to be pregnant by 70 d after the voluntary waiting period; multiparous but not primiparous cows made approximately 500 kg less 305-d milk than those with lower concentrations. Those cows with BHB concentrations >1.0 mmol/L between 3 and 14 d postpartum had >4 times higher risk of postpartum diseases (e.g., LDA, metritis, clinical ketosis) and were >15% less likely to be pregnant by 70 d after the voluntary waiting period; the cows but not the heifers made approximately 390 kg less 305-d milk than those with lower concentrations. The prevalence of elevated NEFA concentrations—about 25% of the cows and almost 50% of the heifers—was high during the prepartum period. More than 25% of the cows and heifers sampled had elevated NEFA or BHB concentrations during the postpartum period.

Ospina et al. (2010c) then set out to define herd alarm levels to identify when a herd is at risk of poor health, performance, or reproduction as a result of the transition period so that management opportunities can be better identified. Herd alarm levels can be defined as the proportion of statistically sampled animals above cow-level thresholds that are associated with herd-level effects on health, reproduction, and milk production. Ospina et al. (2010c) showed that when >15% of the sampled animals are above the cow-level NEFA and BHB cut-points there is an association with increased metabolic disease incidence, decreased reproductive performance, and lower milk yield in the herd. Indeed, there is a dose–response effect of increasing metabolic disease incidence when the proportion of animals with elevated NEFA or BHB increases (Ospina et al., 2013).

In a similar series of prospective cohort studies asking related questions, researchers led by the group at the

University of Guelph (Chapinal et al., 2011, 2012a,b) corroborated the findings of Ospina et al. (2013) in a large geographical range including a total of 55 herds from Ontario, New York, Minnesota, Wisconsin, California, and the southeastern United States. In general, with similar thresholds, elevated concentrations of NEFA and BHB were associated with a higher incidence of retained placenta, LDA, and metritis. Further, they showed an association with decreased milk yield but did not observe the same effect on reproduction. At the herd level they also showed an association of the proportion of transition cows above cow-level cut-points with metabolic disease, reproduction, and milk production, albeit at a slightly higher herd alarm level (usually >25%, depending on the outcome).

A comprehensive cohort study by Wolfgang Heuwieser's productive group from Germany (Suthar et al., 2013) showed similar risk for disease on 528 dairy farms from 10 European countries when studying hyperketonemia. In addition, they found an association between lameness and hyperketonemia. A notable recommendation from this study, based on the results from this study and others, was that a universal single threshold of 1.2 mmol of BHB/L for hyperketonemia was practical for farm management and monitoring.

McArt et al. (2012b) intensively examined the ecology of hyperketonemia in 4 high-producing large herds by testing BHB concentrations 6 times between 3 and 16 DIM. Doing so allowed for measurement of the incidence of this condition, which was on average 43%, for the first time in commercial herds; the majority of the new cases of hyperketonemia occurred within the first 7 DIM. Furthermore, the negative effect of hyperketonemia on LDA, culling, conception to first AI, and milk production was found to be much more profound when it first occurred in the first week after parturition compared with the second week. This intensive prospective cohort study established that the prevalence of hyperketonemia was generally half of the incidence. Again, the magnitude of the effect of hyperketonemia on metabolic disease and milk production was similar to that in the aforementioned studies. A randomized controlled trial also showed the value of oral propylene glycol therapy for cows with subclinical ketosis (McArt et al., 2011, 2012a) and described the cost and benefit of routine screening and treatment for hyperketonemia (McArt et al., 2014).

With the intent of preventing hyperketonemia, risk factors and nutritional management have been studied in the United States (McArt et al., 2013; Mann et al., 2015) and the Netherlands (Vanholder et al., 2015), among other places. These studies found that feeding a controlled-energy diet in the dry period greatly decreased the risk for hyperketonemia in the early postpartum period without negatively affecting milk production compared with feeding cows well above ME requirements (Mann et al., 2015). Consistent and strong risk factors for postpartum hyperketonemia across studies are increasing parity, elevated concentrations of prepartum NEFA, and higher BCS. Hyperketonemia costs approximately US$290 per case (McArt et al., 2015) or about Can$200 per case (Gohary et al., 2016) and has a worldwide average prevalence of >20% (i.e., incidence of >40%); therefore, it is likely cost effective to allocate some resources to prevent hyperketonemia.

Ostergaard and Larsen (2000) investigated the relationship between early postpartum blood Ca and milk yield in 153 cows from 27 Danish dairy herds and did not observe an association with fat- and protein-corrected milk yield at any period in the lactation. This mirrors the lack of association noted previously by LeBlanc et al. (2005) between Ca and LDA. However, the series of studies by Chapinal et al. (2011, 2012a,b) previously described also examined the effect of Ca. They observed a 3.1 greater odds of subsequent LDA when serum Ca concentration in the first week postpartum was <2.2 mmol/L as well as a negative effect on first test DHIA milk production and pregnancy at first AI. Further investigating the association of immediate postpartum Ca with subsequent health and production events in the concurrent lactation, Chamberlin et al. (2013) defined hypocalcemia as <1.0 mmol/L of whole-blood ionized Ca. Although they observed an interesting association between hypocalcemia and higher NEFA concentrations as well as greater hepatic fat percentage, they did not find a relationship between milk production or common postpartum diseases like Ostergaard and Larsen (2000) and LeBlanc et al. (2005). However, this study was likely underpowered to detect differences in disease risk. An interesting and often overlooked finding in this study is that the dynamics of whole-blood ionized Ca and plasma total Ca were very similar, suggesting that measuring either is suitable. In an attempt to determine a cow-level cut-point for Ca concentration, Martinez et al. (2012) used receiver operator characteristic curves to find the threshold that had an association with subsequent metritis. In that study, cows with serum Ca <2.15 mmol/L from 0 to 3 d postpartum had an increased incidence of metritis and puerperal metritis compared with normocalcemic cows in one herd with a high incidence of metritis. This cut-point was similar to the 2.2 mmol/L noted by Chapinal et al. (2011) and mentioned previously.

The Guelph group was again the first to examine the relationship between BHB and subsequent reproductive performance (Walsh et al., 2007). In this study, they noted that cows with serum BHB concentrations >1,000 μmol/L in the first week postpartum and those

with concentrations >1,400 μmol/L in the second week postpartum were 20% less likely to be pregnant at first insemination than those cows with lower concentrations. These cut-points are the same as or similar to those found by Ospina et al. (2010b) and Chapinal et al. (2012b), who also studied other outcomes. Looking for some of the reasons for poorer reproductive performance in cows with hyperketonemia, Dubuc et al. (2010) studied some of the risk factors for uterine diseases such as metritis and cytological (subclinical) endometritis. They noted that hyperketonemic cows had greater odds (OR = 1.4) of cytological endometritis, as did cows with higher concentrations of haptoglobin (OR = 1.59). In a prospective cohort study of 779 cows from 38 herds, Cheong et al. (2011) found that multiparous cows with ketosis had a 5.6 greater odds of subclinical endometritis and suggested that it is likely the result of impaired uterine immune function due to negative energy balance. This concept was echoed by the University of Missouri group (Garverick et al., 2013), who noted that nutritional status during the early postpartum period, as indicated by blood NEFA and glucose concentrations, might affect subsequent fertility by a mechanism that is independent from interval to first ovulation. In support of this, Galvão et al. (2010) determined that, in addition to having higher circulating NEFA and BHB, diagnosis of metritis or cytological endometritis was preceded by or concurrent with decreased concentrations of glycogen in neutrophils harvested from the same animals.

Work from other groups has further investigated these relationships between indicators of metabolic health and reproductive diseases. Giuliodori et al. (2013) suggested that high prepartum NEFA and high postpartum BHB concentrations increased risk for clinical endometritis in Argentina. However, Burke et al. (2010) determined that concentrations of NEFA and BHBA in grazing cattle were not different between cows that did or did not develop cytological endometritis, although plasma albumin concentrations were lower and plasma glutamate dehydrogenase and aspartate aminotransferase were higher in cows that had cytological endometritis.

Energy-related analytes also are related to other infectious disease in the periparturient cow. Using a data set of 634 lactations from 317 cows in Denmark, Moyes et al. (2009) determined that cows with clinical mastitis during early lactation had higher NEFA and BHB concentrations compared with healthy cows and that cows with clinical mastitis during wk 1 postcalving had higher circulating glucose concentrations during the week before parturition. From wk 2 to 13 of lactation, aspartate aminotransferase activities were higher in cows developing clinical mastitis than in healthy cows. In a series of elegant experiments in which they induced hyperketonemia, Bruckmaier's group in Switzerland suggested that the immune suppression observed during spontaneous hyperketonemia (and its association with increased susceptibility to mastitis) is likely directly caused by elevated concentration of BHB (Zarrin et al., 2014). However, Ster et al. (2012) reported that in vitro addition of NEFA, but not BHB, decreased proliferation and oxidative burst of immune cells. Further mechanistic work is needed to determine the effects of NEFA and BHB on immunity in transition cows.

In addition to relationships with health, reproduction, and milk yield, concentrations of energy-related analytes and Ca are related to risk of herd removal. Seifi et al. (2011) compiled a data set consisting of 849 cows across 16 herds and determined that elevated concentrations of BHB during the postpartum period were associated with increased odds of displaced abomasum, clinical ketosis, and culling by 60 d postpartum. Elevated postpartum NEFA was associated with increased clinical ketosis and culling, and lower serum Ca during wk 1 and 2 was associated with increased culling by 60 d postpartum. Roberts et al. (2012) pooled data from several of the aforementioned studies and found that high concentrations of NEFA and BHB and low concentrations of Ca during the periparturient period were associated with increased risk of culling during the first 60 d postpartum.

Ingvartsen and colleagues (Ingvartsen et al., 2003; Ingvartsen, 2006) postulated that markers or indicators that reflect physiological imbalance would be better than individual biomarkers for assessing periparturient cow status. Moyes et al. (2013) used a large data set (634 lactations from 317 cows, as described above) to evaluate a physiological index comprising plasma concentrations of NEFA, BHB, and glucose weighted with coefficients within week and compare it with calculated energy balance and the individual biomarkers. They reported that cows with a higher physiological imbalance prepartum had greater risk of many postpartum diseases; furthermore, the calculated index and NEFA during the prepartum period were better indicators of postpartum disease than were calculated energy balance, BHB, or glucose. Finally, the authors clearly articulated the need for automated, real-time information based on milk measurements that would accurately reflect physiological imbalance in the periparturient cow.

Blood Proteins

Limited data are available that characterize changes in major circulating proteins during the periparturient period, and relatively little is known about their sensitivity and specificity as potential diagnostic tools. Piccione et al. (2011) measured serum protein fractions

in cows during the late prepartum and early postpartum periods and determined that serum total protein concentrations decreased from the prepartum period to wk 1 postpartum and that decreased concentrations of globulins largely were responsible for the decline in total protein concentrations. Concentrations of serum albumin were relatively stable during the periparturient period but increased slightly in the sample collected at or around the time of calving. Cows with elevated inflammatory response during the postcalving period had decreased serum albumin concentrations and changes in a variety of other serum and plasma components (Bossaert et al., 2012; Trevisi et al., 2012). Consistent with this, Burke et al. (2010) reported that pasture-fed cows diagnosed with endometritis during early lactation had decreased concentrations of plasma albumin and a lower albumin:globulin ratio than cows without endometritis. Furthermore, Rezamand et al. (2007) reported that cows with new IMI postcalving had lower concentrations of plasma albumin.

Although it appears that serum protein or protein fractions may be associated with aspects of health at the cow level, the sensitivity and specificity of using them as markers of herd-level opportunities is not known. Furthermore, responses of these to dietary factors or management have not been well characterized. Cozzi et al. (2011) measured concentrations of a variety of blood-based markers in 740 Holstein cows in 33 dairy herds. They reported significant herd variance components for albumin as well as parity and season of production effects on total protein and globulin; however, detailed study of diets or management practices was not conducted in their survey.

Overall, it appears that changes in plasma protein fractions, particularly albumin, may be associated with health and reproduction in cows. Furthermore, herd-level variance suggests the potential for evaluation of these as metabolic indicators; however, large data sets will be required to assess the robustness (e.g., sensitivity and specificity) of these markers for cow- or herd-level evaluation.

Vitamin-Related Indicators and Disease

Several research groups continued to explore relationships between vitamin status and disease during this time period. LeBlanc et al. (2004) measured serum concentrations of α-tocopherol, β-carotene, and retinol during wk −1 and +1 relative to calving in 1,057 cows on 20 farms. They determined that increased α-tocopherol concentrations during the last week prepartum were associated with decreased risk of retained placenta and that increased retinol concentrations during the last week prepartum were associated with decreased risk of clinical mastitis during early lactation. More recently, using a much smaller number of cows but more frequent blood sampling during the periparturient period, Qu et al. (2014) determined that cows that went on to have retained placenta and other diseases had lower serum α-tocopherol concentrations during the prepartum period along with higher NEFA and BHB. The same group also reported that lower serum α-tocopherol concentrations preceded displaced abomasum and that cows with displaced abomasum had lower serum cholesterol and higher NEFA, BHB, haptoglobin, and serum amyloid A concentrations during the postpartum period (Qu et al., 2013).

Putative Indicators of Oxidative Stress and Inflammation

During the early 2000s, Italian researchers moved past the prior work focused on measuring concentrations of vitamins or trace elements in blood or tissues as indicators of antioxidant status and started to evaluate indicators of oxidative stress more directly. Bernabucci et al. (2002) reported that periparturient cows subjected to moderate heat stress had increased activity of glutathione peroxidase and superoxide dismutase and concentrations of intracellular thiols in erythrocytes compared with spring-calving cows; however, differences in these indicators were not significant when measured in plasma. In subsequent work, they demonstrated that cows with higher BCS prepartum and greater BCS loss postcalving had higher concentrations of reactive oxygen metabolites, thiobarbituric acid reactive substances, and thiol groups and lower plasma superoxide dismutase activity and erythrocyte thiol groups postpartum, collectively suggestive of increased oxidative stress in cows of higher BCS and with greater BCS loss postcalving concurrent with higher NEFA and BHB concentrations postpartum in the same group (Bernabucci et al., 2005). More recently, Politis et al. (2012) reported that concentrations of α-tocopherol in cows receiving high dietary levels of vitamin E (3,000 IU/d) during the dry period were inversely related with concentrations of serum reactive oxygen metabolites and thiol groups at dry-off and calving; however, these markers of oxidative stress were not related to subsequent development of mastitis.

At about the same time, another research group in Italy begin exploring relationships of indicators related to inflammation with metabolism. Bionaz et al. (2007) evaluated relationships of plasma paraoxonase as a marker of liver function. They determined that cows with lower plasma paraoxonase concentrations postpartum also had the lowest concentrations of negative acute phase proteins and the highest concentrations of

positive acute phase proteins such as haptoglobin and oxidative stress indicators such as reactive oxygen metabolites. Further, cows with low plasma paraoxonase produced about 10 kg/d less milk during the first 30 d postpartum compared with cows with the highest concentrations of paraoxonase in plasma. Interestingly, there were no clear relationships with plasma NEFA or BHB, suggesting that inflammatory status can be altered without direct associations with indicators of energy metabolism. Bertoni et al. (2008) proposed a liver activity index based on an aggregate of plasma concentrations of albumin, total cholesterol as a proxy for lipoproteins, and retinol-binding protein and determined that cows with lower liver activity index had higher plasma concentrations of haptoglobin and globulin during the first week postpartum and lower milk yield in early lactation. Cows with lower liver activity index also had more health disorders postpartum and generally poorer reproductive performance, although the final data set was quite small (n = 77 cows) for evaluating reproductive and health outcomes.

Circulating acute phase proteins and relationships with health, milk yield, and reproduction continued to receive focus during the late 2000s. Huzzey et al. (2009) reported that cows subsequently diagnosed as healthy, mildly metritic, or severely metritic based on clinical signs had increasing serum haptoglobin concentrations before the diagnosis. Stengärde et al. (2010) found that cows with displaced abomasum had elevated concentrations of serum haptoglobin in addition to elevated plasma concentrations of NEFA and BHB. Huzzey et al. (2011) determined that prepartum plasma haptoglobin had only modest relationships with postpartum health events; however, cows with increased plasma haptoglobin concentrations during wk 1 postpartum had subsequently decreased milk yield and lower risk of pregnancy by 150 DIM (Huzzey et al., 2015). Although circulating haptoglobin concentrations were related to clinical metritis as described above and were reported to be related to increased risk for purulent vaginal discharge and cytological endometritis (Dubuc et al., 2010), other work suggested that there was no difference in plasma haptoglobin concentrations during the postpartum period for cows that subsequently developed cytological endometritis (Yasui et al., 2014). Recently, Pohl et al. (2015) reported that serum haptoglobin concentrations during the early postpartum period were higher in primiparous cows than in multiparous cows and that serum concentrations of haptoglobin were higher in multiparous but not primiparous cows following assisted calving or retained placenta and were positively associated with metritis across all parities. Further, serum haptoglobin was positively associated with BHB but was not related to serum NEFA concentrations.

All of the studies above that have focused on indicators of oxidative stress and inflammation, with the exception of Huzzey et al. (2011, 2015), Politis et al. (2012), and Pohl et al. (2015), were conducted using small (generally 100 cows or less) data sets, and studies that had larger numbers of cows were conducted in 1 or 2 dairy herds. To date, we lack the type of information for these indicators that has been gleaned from large-scale observational studies described above that related NEFA and BHB to outcomes across many herds with many cows. These types of studies would potentially allow these indicators to advance to become useful in cow- and herd-level assessment and decision making.

CURRENT AND FUTURE TECHNOLOGIES FOR MEASUREMENT OF METABOLIC HEALTH INDICATORS

Blood

Hypocalcemia. Calcium status can be measured directly using either total Ca or ionized Ca concentrations in blood. Although ionized Ca is considered to be the more biologically active fraction and recent work (Leno et al., 2017) suggests that ionized Ca concentrations are more consistently related to neutrophil function in vitro than total Ca, research also suggests that the relationship between ionized Ca and total Ca varies in the days after calving (Ballantine and Herbein, 1991; Leno et al., 2017) and thus cannot be used interchangeably as indicators of Ca status. Notably, the large observational studies referenced above relating Ca status with postpartum outcomes related to disease, milk production, and reproduction all used total Ca as their indicator, and ionized Ca has received limited evaluation as a predictor of these outcomes.

Technologies for measurement of total Ca have not progressed far in the past few decades, and benchtop analyzers remain the gold standard. On-farm tests for ionized Ca, although available, are expensive and have not been validated for use with bovine blood with the exception of the i-STAT analyzer (Abbott Laboratories, Abbott Park, IL) which was evaluated using only 24 samples (Peiró et al., 2010). Due to the difficulty of handling samples to ensure accurate results and cost, ionized Ca is not commonly measured in research settings and is used even less for individual animal treatment or farm management decisions. However, a recent abstract suggested that technologies for cost-effective on-farm measurement of ionized Ca are in development (Neves et al., 2015).

The historical clinical impression of cold ears as an indicator of hypocalcemia was recently evaluated by Venjakob et al. (2016) using an infrared thermometer to determine ear skin temperature. Although cows with clinical hypocalcemia had lower ear temperatures than cows with subclinical hypocalcemia, ambient temperature was a major confounder. Thus, ear temperature alone could not be recommended for accurate diagnosis of subclinical hypocalcemia.

Hyperketonemia. The use of milk powders and milk and urine dip strips for the detection of ketosis gave way to more precise technologies in the late 2000s, when JDS published the validation of a handheld point-of-care blood BHB meter for use as a cow-side test (Iwersen et al., 2009). Laboratory validation of the Precision Xtra meter (Abbott Laboratories) provided 100% sensitivity and 100% specificity against serum BHB determined photometrically at a cut-point of 1.4 mmol/L, and subsequent work with 35 veterinary practices showed that the meter was an accurate and valuable tool for on-farm use to determine blood BHB concentration using cut-points of 1.2 or 1.4 mmol/L. The availability of such an accurate and economic tool allowed for huge advancements in our knowledge of hyperketonemia as mentioned above. Recently, several other handheld BHB meters have been validated for on-farm use in whole blood (Iwersen et al., 2013; Bach et al., 2016). These meters have become the norm for routine monitoring of herd-level hyperketonemia prevalence and individual animal testing for hyperketonemia treatment decisions; some have been validated for use in identifying elevated prepartum BHB concentrations as well (Tatone et al., 2015).

Alongside publication of handheld BHB meter performance, numerous reports have provided information regarding the preferred type, timing, and location of sample collection. Iwersen et al. (2013) and Pineda and Cardoso (2015) showed that plasma and serum samples can be accurately used with handheld BHB meters (some requiring adjusted thresholds) for classification of hyperketonemia. Use of these meters for on-farm whole-blood or stored plasma or serum BHB determination is thus an attractive and accurate method of BHB measurement for many researchers.

Although storage temperature of the meters and testing strips has not been found to confound results with tested devices, the temperature of blood samples has an important effect on the results: the difference between meter BHB and laboratory BHB concentrations decreases as blood temperature increases (Iwersen et al., 2013). Thus, samples should be as close to body temperature as possible to ensure accurate results. Work from Mahrt et al. (2014) showed that blood samples can be collected at any time of day for precise measurement of BHB concentration in continuously fed dairy cows; however, the jugular vein or coccygeal vessels should be used for blood collection because blood BHB concentrations in the mammary vein are quite a bit lower. Other European colleagues (Kanz et al., 2015; Süss et al., 2016) have found that capillary measurement of blood BHB concentrations using a lancet device in the skin of the exterior vulva or ear is an acceptable and minimally invasive method for classifying cows with hyperketonemia.

Point-of-care meters have also been examined for cow-side determination of blood glucose concentration. Wittrock et al. (2013) found a strong correlation between blood glucose measured on the Precision Extra meter and serum samples run on a chemical analyzer; however, the prevalence of hypoglycemic samples was low and the standard deviation of difference in glucose concentrations was high, which may limit the meter's utility for classification of hypoglycemia. Additional work suggests using adjusted cattle-specific algorithms for both blood and plasma with a recommendation for plasma as the measured analyte (Megahed et al., 2015). Further investigation is thus warranted to optimize the use of point-of-care meters for cow-side measurement of blood glucose as well as to determine the health and production implications of blood glucose concentrations in early-lactation dairy cattle.

NEFA and Haptoglobin. Although there are numerous reports in JDS regarding the association of elevated pre- and postpartum serum NEFA concentrations with subsequent negative disease, production, and reproduction effects at both the individual animal and herd levels (Ospina et al., 2010a,b,c; Chapinal et al., 2011, 2012a,b), there remains no rapid cow-side test for this important energy metabolite. Thus, measurement of NEFA concentrations is currently evaluated either to monitor herd-level prevalence of excessive negative energy balance or for research projects, with samples being sent through a veterinary diagnostic laboratory or run in research laboratories. Both options are still expensive on a per-sample basis, and future development of a rapid, inexpensive cow-side test for blood NEFA concentrations will allow extension of our knowledge to routine use on commercial dairies.

Similar to NEFA, an increase in peripartum haptoglobin has been implicated as an indicator of early-lactation disease such as metritis and has been associated with reduced milk yield and risk of pregnancy (Huzzey et al., 2009, 2011, 2015; Pohl et al., 2015); however, no rapid cow-side test is currently available that limits the utility of this measurement on an individual animal- or herd-level basis. Serum concentrations of haptoglobin are correlated with their milk concentrations (Hiss et al., 2009); however, more comprehensive analysis is

needed before milk concentrations could be considered as a proxy for blood. Future development of a simple and economic testing method to determine blood haptoglobin concentration will allow further investigation into the use of this acute phase protein as a predictor of early-lactation disease, milk production, and reproduction.

Application of Metabolomics. The emerging area of metabolomics offers additional promise to enhance or move beyond the biomarkers of choice at the present time. Hailemariam et al. (2014) used a targeted quantitative metabolomics approach using plasma collected from 12 periparturient cows and assessed about 120 metabolites in plasma from each of 6 healthy and 6 diseased cows. They further subjected these data to principal component analysis and identified several compounds related to fatty acid metabolism (carnitine, propionyl carnitine, and lysophosphatidylcholine acyl C14:0 along with 2 other forms of plasma phosphatidylcholine) that were related to disease in this small data set. Rico et al. (2015) assessed changes in plasma sphingolipids such as ceramides as potential biomarkers of insulin resistance in periparturient dairy cows and determined that specific ceramides were positively correlated with NEFA and inversely correlated with insulin sensitivity. Furthermore, the authors noted marked changes in the plasma sphingolipidome during the periparturient period. We are confident that studies such as these focused on various aspects of metabolomics will provide additional biomarkers for further development into potential indicators for use in practical monitoring and management in the future.

Milk

Current measurement and monitoring of metabolic health using milk parameters rests on the assessment of prediction of the degree of or adaptation to negative energy balance. In the late 1990s and early 2000s, JDS published numerous reports regarding cow-side measurement of ketones in milk using dip strips and powders in relation to prediction of diseases such as LDA as well as their association with blood BHB (Geishauser et al., 1997, 1998, 2000; Enjalbert et al., 2001; Carrier et al., 2004). Their generally poor sensitivity, even with good specificity, has increased widespread adoption of cow-side blood BHB testing methods for disease diagnosis, as discussed above.

Investigation of in-line milk measurement for ketosis monitoring and diagnosis began in the late 1990s concurrent with cow-side milk tests. Duffield et al. (1997) published results in *Canadian Veterinary Journal* on a large study using 1,333 cows from 93 Canadian dairies to assess the association of test-day milk fat and protein percentages with elevated serum BHB ($\geq$1.2 mmol/L). They found that although both test-day fat and protein percentage were associated with elevated serum BHB, neither of these measurements alone or combined provided an accurate screening method. Even at an optimal protein:fat ratio cut-point of 0.75, the sensitivity and specificity of detection of elevated serum BHB were only 58 and 69%, respectively.

Furthering the evaluation of in-line techniques for analysis of metabolic health, the use of infrared spectroscopy as a screening tool for ketosis—an improvement over previously used wet chemistry methods—was first described in JDS by Hansen (1999). Although direct measurement of milk BHB using fluorometric methods was found to be straightforward even with the opacity issues with milk (Larsen and Nielsen, 2005), the lack of this type of measurement system in laboratories handling large quantities of milk samples most likely prevented its widespread adoption. Fourier transform infrared spectroscopy (**FTIR**) was already in place for measurement of milk fat, protein, lactose, and urea and thus provided a fast and inexpensive method of estimating milk acetone. First reports of the accuracy of mid-infrared FTIR for predicting elevated milk acetone concentrations compared with gold standard milk detection methods showed that it had potential as a screening method, with results varying based on the prevalence of elevated milk acetone in the tested population (Hansen, 1999; Heuer et al., 2001). This line of research has continued with further investigation as a ketosis screening method using FTIR with additional analysis of milk BHB as well as other milk biomarkers such as citrate (de Roos et al., 2007; Grelet et al., 2016). These analytes, and specifically the combination of milk BHB and acetone, increased the accuracy of FTIR predictions for detection of ketosis compared with older milk measurement methods. Similarly, biological models have been reported that use additional animal-specific data (e.g., parity, BCS, DIM, milk yield) along with milk BHB concentrations to predict the risk of ketosis and the suggested time to retesting of milk (Nielsen et al., 2005); however, none of these studies using milk BHB examined the correlation of these measurements with on-farm diagnosis of hyperketonemia.

In 2010, following advancements in cow-side blood BHB measurement accuracy, JDS published a report comparing the milk ketone concentrations determined through FTIR of 69 high-producing dairy cows with blood BHB determination (van Knegsel et al., 2010). This study determined that FTIR measurement of milk acetone or milk BHB provided a higher sensitivity for detecting hyperketonemia (blood BHB $\geq$1.2 mmol/L) compared with a milk fat:protein ratio at 80, 80, and 66%, respectively. However, all 3 methods of classifica-

tion had specificities of approximately 70% compared with blood BHB, which allowed for a high number of false-positive diagnoses for hyperketonemia using any of these methods.

Recent studies in JDS have aimed at exploring the association of milk components taken at monthly DHIA tests with blood BHB concentrations as well as optimizing milk BHB thresholds for prediction of hyperketonemia. Using blood BHB data from 163 cows in 37 herds in Canada with flow-injection analysis for milk BHB and acetone and FTIR analysis for milk fat and protein, Denis-Robichaud et al. (2014) found good correlation of blood BHB with milk BHB and acetone ($r = 0.89$ and 0.73, respectively) but poor correlation of blood BHB with fat percentage, protein percentage, and fat:protein ratio ($r = 0.21$, 0.04, and 0.71, respectively). They found that the optimal threshold for prediction of hyperketonemia was milk BHB $\geq$0.20 mmol/L and milk acetone $\geq$0.08 mmol/L, which provided sensitivities of 84 and 87%, respectively, and specificities of 96 and 95%, respectively. The first study reporting the association of elevated milk BHB with production was published by Santschi et al. (2016) using 498,310 milk samples from Holstein cows in 4,242 herds in Canada between 3 and 35 DIM on monthly DHI test. Cows with elevated milk BHB ($\geq$0.20 mmol/L) produced 2.3 kg/d less milk and had lower protein yield and concentration, higher fat yield and concentration, and higher SCC than cows with milk BHB <0.20 mmol/L. A limitation of the current literature evaluating production outcomes based on milk BHB or acetone is that studies have focused on testing during routine DHI tests. Although commercial systems such a Herd Navigator (DeLaval, Kansas City, MO) and AfiLab (Afimilk Ltd., Kibbnutz Afikim, Israel) make in-line milk measurements available for use in commercial dairies, individual animal health and production outcomes based on these technologies are not yet known. It is likely that systems that monitor milk BHB or other components on a continuous basis have better prediction accuracy; however, this idea needs to be investigated further.

Fourier transform infrared spectroscopy has good potential for measuring ketone bodies and other milk components such a fatty acid profiles (Ferrand-Calmels et al., 2014). However, a review by De Marchi et al. (2014) emphasized the variability of reporting results depending on the reference method used and robustness of equation model development. These factors can vary greatly between studies due to the variation in biology under different study conditions (e.g., breed, herd location, diet, and stage of lactation), and the authors highlighted the importance of data exchange between commercial and research groups to maximize future advancement in this area. Use of FTIR to assess energy balance in early lactation has also been explored (Friggens et al., 2007; Løvendahl et al., 2010; McParland and Berry, 2016), with initial promise for use with additional information regarding individual animal physical traits and adjustment for type of production system. Additional methods of milk metabolomic analysis, including nuclear magnetic resonance spectroscopy, have recently been investigated (Klein et al., 2010), and we expect further exploration of methods for rapid analysis of milk constituents to continue with vast improvement in the near future.

In an attempt to improve prediction of individual animal early-lactation metabolic disease, multiple studies in the last decade of JDS have looked to milk to identify cows with elevated NEFA or hyperketonemia. The first of these was a case-control study from Van Haelst et al. (2008) investigating the milk fatty acid profiles of 8 healthy and 8 hyperketonemic (plasma BHB $\geq$1.2 mmol/L) cows; they found that hyperketonemic cows had elevated levels of C18:1 *cis*-9 in milk fat 2 wk before diagnosis. A larger data set using 92 early-lactation Holstein-Friesian cows from a research herd in the Netherlands found a 50% increased risk of plasma NEFA $\geq$0.6 mmol/L in cows with at least 240 g/kg of C18:1 *cis*-9 in milk fat; however, sensitivity for this method was just under 50% (Jorjong et al., 2014). This group also reported that a milk fat ratio of C18:1 *cis*-9 to C15:0 >40 was present in 70% of hyperketonemic animals (Jorjong et al., 2015). Mann et al. (2016) investigated the diagnostic value of milk fatty acids and fatty acid ratios for correct classification of 84 multiparous cows in a research herd in New York State as having high NEFA or BHB concentrations. They found that although several fatty acid concentrations were associated with elevated NEFA and BHB, correct classification was only moderate; thus, they could not recommend this measurement method over direct blood detection methods. Both Jorjong et al. (2014, 2015) and Mann et al. (2016) stated that practical use of this information most likely requires routine analysis of milk fatty acids and repeated sampling of an individual animal; however, current technology does not support a method of on-farm milk fatty acid determination that is rapid and cost effective.

Ongoing work in this area attempts to predict blood BHB and NEFA concentrations using various milk components (e.g., BHB, acetone, fat, protein) in conjunction with cow-specific characteristics such a parity, DIM, and breed (Chandler et al., 2015). A potential complication with this comparison is that even when samples are taken repeatedly, the nature of milk sample collection (foremilk vs. composite), timing of sample collection relative to feeding or time of day, and milking frequency can affect milk components. As blood

concentrations of energy metabolites are taken at a single point in time, the association of this timing with milk components of interest, which are some combination of concentration over 8 to 12 h, needs further investigation. In addition, the correlation between milk components of interest specific to certain disease processes (e.g., hyperketonemia and milk BHB) is likely nonlinear and need to be explored further.

Rumination Collars and Tags

Rumination collars were first validated in JDS as an accurate method of determining rumination (Schirmann et al., 2009). The reported electronic rumination monitoring system, HR-Tag (SCR Engineers Ltd., Netanya, Israel), was highly correlated (r = 0.93) with direct observation by a human over fifty-one 2-h periods in 27 Holstein cows. Schirmann et al. (2013) continued to describe rumination times across the periparturient period in 11 Holstein cows. They found that rumination time decreased in the 24-h period before calving by an average of approximately 1 h (15%) compared with the baseline rumination time of 426 min/d in the 3 to 4 d preceding calving. Total rumination time in the 24 h after calving decreased by an average of a little more than 2 h (31%) compared with baseline but returned to baseline rumination times between 24 and 48 h after calving. When 2-h time periods were considered, rumination time declined in the 4 h before parturition, showing potential for this measurement system to identify cows nearing calving.

In the past 3 yr, multiple studies have investigated the use of rumination time as a tool for predicting early-lactation disease, with the number of enrolled cows in each trial increasing with time. Calamari et al. (2014) retrospectively assigned 23 Italian Friesian cows to a high or low rumination group using data from 3 through 6 DIM. They found that 90% of cows with low total rumination times during this period were diagnosed with clinical disease in early lactation compared with only 42% of cows with high total rumination times. Milk yield was also greater by an average of almost 8 kg/d for those cows with high compared with low total rumination times in the first month of lactation. In 296 Holstein cows on 1 dairy farm in Wisconsin, delivery of a stillborn was associated with a reduced rumination time of approximately 60 min/d for both the pre- and postpartum periods, and delivery of twins similarly reduced rumination time by approximately 60 min/d compared with delivery of a singleton (Liboreiro et al., 2015). This group also found that the rumination time of cows diagnosed with metritis was 30 min/d less than that of healthy herdmates. In 64 Holstein dairy cows on 1 farm in Canada, Schirmann et al. (2016) examined rumination time from 10 d before until 21 d after calving and found that cows with postpartum hyperketonemia (BHB $\geq$1.2 mmol/L) spent 14% less time ruminating during the prepartum period than healthy cows.

In 2016, JDS published multiple reports regarding the association of ruination time and disease on a large-scale basis in commercial dairies, focusing on differences in rumination time between healthy and sick cows as well as the predictive capabilities of the system. In a study using 339 Holstein cows from 4 commercial herds in Canada, Kaufman et al. (2016) evaluated the association of rumination time with postpartum hyperketonemia. They found no difference in rumination time from 2 wk before until 4 wk after calving between primiparous healthy cows, hyperketonemic cows, and hyperketonemic cows with additional early-lactation diseases. However, multiparous healthy cows, on average, ruminated 25 and 44 min/d more than hyperketonemic cows and hyperketonemic cows with additional early-lactation diseases, respectively. The largest difference in rumination time in multiparous cows in these groups was seen in the weeks before and 2 wk after parturition. This study showed increased odds of a cow being hyperketonemic or hyperketonemic with additional early-lactation disease if it had reduced rumination time in the week immediately before calving or the week immediately following calving, respectively, which suggests that rumination monitoring across the periparturient period might contribute to identification of early-lactation disease in individual animals.

In a large observational field trial in 1,121 Holstein cows on 1 commercial dairy in New York State, Stangaferro et al. (2016a,b,c) evaluated the performance of an automated health-monitoring collar system, which used a combination of rumination time and physical activity, in detecting cows with displaced abomasum, hyperketonemia, indigestion, mastitis, or metritis and described the time from system alert to diagnosis of disease by farm employees as well as the patterns of rumination and activity surrounding disease diagnosis. The sensitivity of the system for disease alert, assuming a positive system identification from −5 to 2 d after diagnosis of disease by farm personnel, was 93, 91, 89, 58, and 55% for displaced abomasum, hyperketonemia, indigestion, mastitis, and metritis, respectively, with an increase in effectiveness for cows with severe cases of mastitis or metritis. When all diseases of interest were combined, the overall sensitivity of the alert system was 59%; specificity was 98%; positive and negative predictive values were 58 and 98%, respectively; and accuracy was 96%. Of note is that this study was conducted on a single dairy. As the use of rumination and activity collars becomes more widespread in the dairy industry,

our knowledge of within- and between-herd variation in these parameters and their association with disease prediction will improve.

WHERE DO WE GO FROM HERE?

Multiple articles in this review discuss the key issues surrounding maintenance of metabolic health throughout the periparturient period, notably transition and periparturient nutrition and housing management. Other articles in this review discuss selection and effect of genetic traits in reducing disease and improving milk production. These areas should be our focus for preventing early-lactation metabolic disorders. However, the effect of genetic, nutritional, and management strategies on periparturient cow well-being cannot be assessed without appropriate tools for measuring metabolic health at both the individual and herd levels.

Future emphasis in this area will likely continue to be on measurement of constituents in blood or milk that have strong associations with economically important outcomes related to metabolic health in early lactation, such as disease occurrence, milk yield, and reproductive performance. To this end, accurate and precise cow-side or inline tests for these constituents are necessary to further explore the epidemiology of metabolic health during the periparturient period in large field-based studies.

Further advances in technology will allow for real-time measurement and automatic monitoring of metabolic health, providing us with immediate feedback and information on effectiveness of herd-level feeding and management strategies. The ability to directly measure or estimate biologically important items in milk or to develop prediction models for blood concentrations using milk analyses and cow-level information will improve our detection of disease with minimal disruption to farm and cow routines. Integration of these forthcoming discoveries with epidemiological evidence for herd-level strategies to optimize cow health will greatly enhance our knowledge and ability to improve the metabolic health of dairy cattle throughout the periparturient period and lead not only to better detection of opportunities but also to better and more specific and actionable cow- and herd-level recommendations.

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APPENDIX

Table A1. Timeline of advances in the understanding, development, and use of metabolic indicators in management of dairy cattle (the reader is referred to the text for citation of specific studies and dates)

Date	Milestone
Mid 1920s	Milk fever is characterized by decreased concentrations of blood Ca.
Late 1920s	Concentrations of acetone are determined to be elevated in urine and blood of cows with ketosis.
1930s	Dynamics of blood Ca and P in healthy cows and those with milk fever are characterized.
1940s	Rapid advances are made in understanding changes in blood and urine chemistry with ketosis and underlying physiology.
1950s	First on-farm milk tests for ketosis are developed and evaluated.
1950s	Changes in blood proteins and relationships to colostrogenesis are determined.
1960s	Ketosis research is extended to lipid metabolism and circulating nonesterified fatty acid concentrations; on-farm milk ketone tests are further refined.
1970s	Blood metabolic profiling is applied to herds and herd investigations with varying results.
1980s	Epidemiological approaches are first applied to determine risk factors for and interrelationships of periparturient disorders.
Mid 1990s	Nonesterified fatty acids are the first metabolic indicator to be incorporated into large-scale observational studies of periparturient health, production, and reproduction.
Late 1990s	Infrared testing of milk ketones is evaluated with a focus on milk acetone.
Mid 2000s	First epidemiological studies to rigorously define cow-level thresholds of metabolite concentrations and postpartum health are conducted.
Mid to late 2000s	Metabolic indicators related to inflammation and oxidative stress are characterized.
Late 2000s	Cow-level thresholds and herd alarm levels for nonesterified fatty acids and BHB are determined.
Late 2000s	Point of care meter for blood BHB is validated, enabling low cost and convenient on-farm measurement.
Early 2010s	Prevalence and implications of subclinical hypocalcemia are revisited.
Early 2010s	Ecology and economics of hyperketonemia are determined.
Early 2010s	Milk ketone testing is expanded to milk BHB using Fourier transform infrared spectroscopy.
Early to mid 2010s	Further evaluation is performed on rumination collars and other electronic monitoring of cow health.
Mid 2010s	Interest in using milk-based constituents to assess and predict metabolic indicators increases.

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A 100-Year Review: Mastitis detection, management, and prevention[1]

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ABSTRACT

Mastitis is the most frequent disease of dairy cows and has well-recognized detrimental effects on animal wellbeing and dairy farm profitability. Since the beginning of modern dairy farming, producers have sought effective methods to minimize the occurrence of mastitis in their herds. The objective of this paper is to review and highlight important advances in detection, management, and prevention of mastitis that have occurred since the first volume of the *Journal of Dairy Science* was published in 1917. Initial research efforts were directed at understanding the nature of pathogenic bacteria that were responsible for most intramammary infections. For decades, researchers worked to identify effective strategies to control mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus*. To develop successful control programs, mastitis workers first had to identify mechanisms of infection, define the clinical and subclinical states of the disease, discover appropriate screening tests, determine likely points of exposure, identify pathogen-specific characteristics, and develop effective procedures for machine milking. Pioneering researchers eventually recognized that mastitis control was based on preventing new infections from occurring in healthy cows and reducing the duration that cows remained infected. Development of a control program that incorporated post-milking teat dipping, hygienic milking procedures, and strategic use of antibiotic therapy at dry-off resulted in widespread control of contagious pathogens. As herd management changed, researchers were tasked with defining control of mastitis caused by opportunistic pathogens originating from environmental sources. As mastitis pathogens have evolved, researchers have sought to define antimicrobial usage that will maintain animal wellbeing while minimizing unnecessary usage. During the last century, tremendous significant advances in mastitis control have been made but changing herd structure and more rigorous processor standards ensure that mastitis will remain an important subject focus of future research.
Key words: mastitis, prevention, management, 100-year review, *Journal of Dairy Science*

INTRODUCTION

Historical evidence suggests that cows have been milked since at least 3100 BC (Nemet-Nejat, 1998) and it is likely that bovine mastitis has existed since that time. For millennia, the close contact required by hand milking allowed for easy detection of abnormalities of milk and the mammary gland, but little was known of the causes or management of mastitis. A more complete understanding of mastitis was not possible until the development of microscopes that allowed detection of microorganisms that are the primary etiological agents. The earliest mention of bovine mastitis in the *Journal of Dairy Science* (**JDS**) occurred in the third issue of 1917 and was focused on public health risks associated with high bacterial counts of raw milk. In that study, Breed and Brew (1917) described a method of grading dairy farms that included enumeration of bacteria in milk and noted that "long chain streptococci" were frequently found in large numbers, even when signs of inflammation were so slight that "farmers cannot be blamed for having saved the milk." The authors reported bacteriological results from several surveys of raw milk cans and noted in one survey (n = 9,387 cans), that >20% of "high count milk" could be attributable to "udder problems." During that period, streptococci were the primary known cause of mastitis and the concept of subclinical infections was just becoming known. Since then, pathogens, cows, and herd management have changed dramatically but mastitis remains an important disease of dairy cows. Hundreds of research and review articles with the topic of bovine mastitis have been published in JDS and the emphasis has broadened (Appendix Table A1). Effects of mastitis on public health, processing characteristics of milk, milk quality, animal wellbeing, and farm profitability have become well known. Quality standards for acceptable milk have progressed and concern about mastitis has expanded to include the effect of mastitis management programs on farm sustainability and consumer perceptions. The

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number of research articles in JDS that include content about mastitis has steadily increased from about 3 in 1917 to >100 in 2016. The purpose of this review is to highlight advances in detection, management, and prevention of mastitis with an emphasis on research published in JDS that has encapsulated our changing understanding of the disease.

DETECTION AND DIAGNOSIS

Pathogens Past and Present

In a comprehensive review, Plastridge (1958) noted that bacterial causes for mastitis were first advanced in the late 1800s. An early mastitis researcher (Murphy, 1947) defined a 3-phase process for development of mastitis based on (1) invasion of an organism (with or without establishment of infection), (2) infection (the bacteria became established in the gland), and (3) inflammation. This process continues to serve as the basis of our understanding of mastitis. Although numerous bacteria are recognized as able to cause IMI, initial emphasis of mastitis control was directed at pathogens that were known to spread among cows in a contagious manner when teats were exposed to bacteria in milk that originated from an infected mammary gland. For decades, *Streptococcus agalactiae* and *Staphylococcus aureus* were considered the most important contagious pathogens.

Streptococcus agalactiae and *Staphylococcus aureus*

Initial concern about bovine mastitis was based on public health and was directed at reducing bacterial counts of raw milk. Breed and Brew (1917) stated, "we have come to know that mastitis is a cause of high bacterial counts. The mastitis causing high bacterial counts has without exception been due to streptococci." As the dairy industry progressed, a broader understanding of mastitis pathogens emerged. In a manuscript titled "A study of flaky milk," Jones and Little (1927) reported observations of 20 instances where foremilk revealed "flocculent particles." Although streptococci were the most prevalent bacteria identified, hemolytic staphylococci (most likely *Staph. aureus*) accounted for 20% of bacterial pathogens, and only 1 case failed to yield significant bacterial growth. That paper contributed to our understanding of mastitis as they correctly defined the abnormalities observed in milk as clumping of leucocytes as a result of inflammation caused by IMI. Although occurrence of large numbers of bacteria in milk was an obvious public health issue, researchers noted that not all of the bacteria originated from IMI and that many aspects of mastitis remained obscure. By 1927, *Strep. agalactiae* was considered responsible for about 90% of IMI (Williams, 1927) and the subclinical condition was an important reason that milk was de-graded (from grade A to B). During this period, mastitis workers were struggling to find an efficient way to detect infected cows in order to maintain grade A status in infected herds (Williams, 1927). This issue remained important as the prevalence of IMI in the 1950s was estimated to approach 50% of cows and 25% of quarters (Plastridge, 1958). The emphasis on *Strep. agalactiae* as the most important cause of mastitis continued for several decades, although mastitis attributed to *Micrococcus pyogenes* (later defined as *Staph. aureus*) began to be recognized during the 1950s (Plastridge, 1958).

In 1956, at the annual meeting of the American Dairy Science Association, the committee on animal diseases reported that mastitis was "the most costly dairy cattle disease not under satisfactory control," (Murphy, 1956). In a seminal paper titled "Mastitis—The struggle for understanding," Murphy (1956) described years of experience with ineffective mastitis control programs in New York and Connecticut, and concluded that "the problem is larger than any single effort put forth toward its understanding." He then presented 8 points to help define the disease (Table 1). These points serve as the basis of our modern understanding of the disease and succinctly define the challenges inherent in mastitis control. He noted that while >20 types of infections can cause mastitis, "at least 99% are caused by…*Str. agalactiae*, other streptococci, staphylococci and bacillary mastitis (including coliform, pseudomonas etc.)." He identified clinical, nonclinical, and severe states and noted that even though discrimination among pathogens could only be performed by laboratory testing, the clinical and nonclinical states did not occur at the same frequency for all pathogens. Murphy (1956) further stated that shedding (and the chance of negative cultures) varied among pathogens over time and emphasized the need for pathogen-specific control programs so that appropriate treatment could be applied to cows affected with *Strep. agalactiae* while calling for research to identify environmental sources of exposure for other pathogens.

Environmental Pathogens

Until the late 1970s, little emphasis was placed on gram-negative organisms as a cause of mastitis. Eberhart (1977) directed initial attention to the emergence of coliforms as mastitis pathogens and in 1979 a paper titled "Coliform mastitis—A review" was published in JDS by the Coliform Subcommittee of the Research

Committee of the National Mastitis Council (1979). This comprehensive review included a description of growth requirements of various coliform bacteria, mechanisms of IMI (with emphasis on exposure and movement through the teat canal), an explanation of pathogenesis (including recognition that magnitude of inflammation is dependent on host factors), an excellent portrayal of epidemiology and risk factors, and recommendations for a model control program. Publication of this review signaled awareness about the emerging importance of mastitis caused by opportunistic environmental organisms. In 1985, the importance of environmental mastitis was highlighted by a comprehensive symposium paper titled "Environmental mastitis: Cause, prevalence, prevention," (Smith et al., 1985). In that paper, progress in controlling contagious pathogens was contrasted with emergence of mastitis caused by environmental pathogens. They described results of a longitudinal study of a university herd that characterized microbiological characteristics, epidemiology, control, and treatment of both gram-positive and gram-negative pathogens that originate primarily from environmental exposure (Smith et al., 1985). They recognized the importance of reducing teat-end exposure, highlighted differences in susceptibility among cows, and contrasted differences among gram-negative and gram-positive (primarily *Streptococcus* spp.) opportunistic pathogens. Differences among pathogens, the importance of IMI during the dry period, the high rate of spontaneous clearance of gram-negative IMI, and the increased rate of clinical cases (vs. subclinical IMI) associated with environmental pathogens were all thoroughly described. They correctly predicted the challenges of reducing environmental mastitis in herds that have effectively controlled contagious organisms and summarized recommendations for mastitis control that remain relevant for modern intensively managed dairy farms.

The same group (Hogan et al., 1989) later reported that herds with low SCC (indicating successful control

Table 1. Outline for the understanding of mastitis (reproduced from Murphy, 1956)

	Clinical stages based on barn observations		
Disease forms based on laboratory cultures	Non-clinical negative to barn tests*	Mild-clinical positive to barn tests only*	Severe-clinical; also swelling or general illness
Point 1. Each of the four forms of the disease can appear in each of the clinical stages. **Point 2.** Without laboratory cultures, the clinical stages of each form cannot be distinguished from one another.			
Streptococcal, *Strep. agalactiae*	Yes	Yes	Yes
Streptococcal, other	Yes	Yes	Yes
Staphylococcal	Yes	Yes	Yes
Bacillary	Yes	Yes	Yes
Point 3. The clinical stages do not occur with the same frequency in each form of the disease. **Point 4.** All forms of the disease may fluctuate between the clinical stages, except that severe-clinical mastitis due to *Strep. agalactiae* rarely occurs.			
Streptococcal, *Strep. agalactiae*	+++ ↔	++ ↔	Rare
Streptococcal, other	+++ ↔	+ ↔	+
Staphylococcal	++ ↔	++ ↔	+
Bacillary	+ ↔	++ ↔	++
Point 5. The four forms of the disease have different shedding characteristics.			
Streptococcal, *Strep. agalactiae* Long duration, positive most days	+++ ↔	++ ↔	Rare
Streptococcal, other Variable duration, positive most days	+++ ↔	+ ↔	+
Staphylococcal Variable duration, not positive every day	++ ↔	++ ↔	+
Bacillary Short duration, often negative when cultured	+ ↔	++ ↔	++

Point 6. The *Strep. agalactiae* form of the disease is the only one that can be eliminated from herds. This is economically worthwhile.

Point 7. The habitat of these bacteria is the environment. It will be a monumental research task to discover their mode of operation. Until then they cannot be eliminated from herds.

*Barn tests such as strip-cup, bromthymol-blue test, White-side test and the California Mastitis test (CMT).

Point 8. By means of treatment and management, the clinical stages may be cured or forced temporarily into the nonclinical stages. At present, it is not known precisely which management practices are of true value.

of contagious mastitis pathogens) could experience serious udder health problems that are characterized by high rates of clinical cases. In the ensuing decades, this situation has become common. Between 1994 and 2001, isolation of *Strep. agalactiae* and *Staph. aureus* from milk samples submitted to the Wisconsin Veterinary Diagnostic Laboratory declined dramatically (Makovec and Ruegg, 2003) and gram-negative pathogens (or culture-negative results) have become the predominant results of milk samples obtained from cows experiencing clinical cases (Oliveira et al., 2013). National data collected for US herds has demonstrated considerable improvements in bulk tank SCC, reaching a milk-weighted average of 194,000 cells/mL in 2015 (USDA, 2015). In contrast, from 1996 to 2014, the reported incidence of clinical mastitis on US dairy farms increased from 13% (USDA, 1996a) to 25% (USDA, 2016). Although mastitis caused by *Staph. aureus* remains a challenge for some herds that have not effectively implemented well-known control strategies, a variety of opportunistic pathogens (i.e., *Enterobacteriaceae*, *Streptococcus* spp., CNS, *Lactococcus* spp., *Prototheca* spp., and others) are frequently identified as mastitis pathogens in modern dairy herds (Bradley and Green, 2001; Oliveira et al., 2013). Additional challenges with pathogens such as *Mycoplasma* spp. (Jasper, 1967; Fox, 2012) have been recognized as important for expanding herds, especially if animals are commingled from multiple locations. Identifying mechanisms to reduce exposure and enhance resistance to IMI caused by opportunistic and emerging organisms while also defining appropriate interventions for affected cows will continue to be a challenge for future farmers, veterinarians, and researchers.

Diagnosis and Impact of Mastitis

Leukocyte Counting. Development of reliable tests for detection of mastitis was a priority for early researchers who wanted to ensure public safety, produce high-quality dairy products, and have a practical means of managing affected cows (Halversen et al., 1934; Shaw et al., 1937). Detection methods that were evaluated included direct microscopic examination of milk for bacteria, enumeration of milk leukocytes, microbial culture, and detection of various abnormal milk constituents (such as chloride content; Halversen et al., 1934). Leukocyte counting rapidly emerged as a practical and repeatable test but general ignorance about the nature of inflammatory responses to IMI made it difficult for early researchers to agree upon an apparently healthy threshold. Although thresholds used for defining mastitis were highly variable (reaching 3,000,000 cells/mL), an early comparative study noted that most milk samples from apparently healthy glands contained <100,000 cells/mL and identified approximately 200,000 to 250,000 cells/mL as a reasonable threshold for discriminating healthy and abnormal milk samples (Prouty, 1934). However, this threshold was not adopted uniformly for many years, probably because the overall prevalence of cows with subclinical infections was quite high and researchers could not arrive at a consensus for defining normal milk. For many years, the threshold of 500,000 cells/mL combined with isolation of >200 cfu/mL of pathogenic bacteria was commonly used to define subclinical mastitis (Plastridge, 1958).

By 1953, the incidence of subclinical mastitis was found to explain almost 80% of the leukocyte count of milk that was delivered to processors, and this study set the stage for use of leukocyte counting as a herd management tool (MacLeod et al., 1953). The ensuing development of the California Mastitis Test (CMT; Schalm and Noorlander, 1957) and the Wisconsin Mastitis Test (Postle, 1964) provided inexpensive and rapid methods to detect and manage subclinical infections but these tests required producers to collect milk and subjectively evaluate results, thus limiting their applicability. The development of faster and more automated methods to enumerate somatic cells in milk was an area of intense research during the 1960s (Paape et al., 1965). As methods to measure SCC were developed, regulatory authorities began to set limits for bulk tank SCC. In the United States, a maximum bulk tank SCC (1,500,000 cells/mL) was first imposed in 1967. The limit was decreased several times and was stabilized at 750,000 cells/mL in 1993. Limits in northern European counties were much lower; in 1992, the European Union adopted a limit of 400,000 cells/mL, which has become the global standard for milk that is used for products destined for international markets.

Emphasis on reducing bulk tank SCC required identification of infected cows and led to the important step of incorporating SCC tests in monthly DHI programs (Funk et al., 1967). The modern era of managing udder health using monthly SCC testing of individual cows was initiated and, eventually, SCC values came into routine use as a mastitis management tool (Reneau, 1986). The use of monthly SCC values was a departure from previous programs that defined mastitis based almost exclusively on culture of milk samples. Learning how to correctly interpret SCC required knowledge of immunology and physiology, and a comprehensive review of milk SCC published in 1994 remains a relevant reference for understanding factors that influence these values (Harmon, 1994). Today, use of SCC of individual cows is a well-accepted tool that mastitis workers continue to fine tune as pathogens and market needs evolve.

Impact of Mastitis. The negative effects of clinical mastitis were obvious, but the full impact of the disease only gradually became known. Although early researchers recognized that mastitis impeded curd formation (Hansen et al., 1934) and resulted in reduced milk yield (Shaw and Beam, 1935; White et al., 1937), the effect of subclinical mastitis on product quality, milk yield, and overall productivity was not easy to quantify until methods of accurately detecting subclinical infections were developed. The development of the somatic cell score (Ali and Shook, 1980; Wiggans and Shook, 1987) allowed researchers to quantify the linear relationship between subclinical mastitis and reduced milk production. Determining that each 1-unit increase in SCS (or doubling of SCC above 50,000 cells/mL) resulted in a constant production loss (−91 and −181 kg per lactation for parity 1 and >1, respectively) allowed producers to understand the tremendous effect of subclinical mastitis on herd productivity. These values continue to form the basis for estimating the economic impact of mastitis on dairy farms.

Inflammation was known to be detrimental to the mammary gland, but the effect of mastitis beyond the udder did not become apparent until researchers began focusing on environmental pathogens. As researchers studied mastitis caused by gram-negative pathogens, experimental studies indicated that endotoxin could reduce fertility (Gilbert et al., 1990), and several observational studies were subsequently performed to explore this relationship. Initially, researchers recognized that the occurrence of clinical mastitis caused by both gram-negative and gram-positive pathogens resulted in reduced conception rates and increased days to conception (Barker et al., 1998). This research was followed by a study that identified similar detrimental effects for cows affected with subclinical mastitis during the early breeding period (Schrick et al., 2001). Since that time, numerous researchers have confirmed that even relatively modest levels of inflammation can affect fertility, and the effect of inflammation caused by mastitis beyond the mammary gland continues to be an important area of research (Lavon et al., 2011, 2016; Hudson et al., 2012; Fuenzalida et al., 2015).

MANAGEMENT

Definition of Modern Mastitis Control

In 1956, Murphy defined the problem of mastitis (Murphy, 1956; Table 1) but presciently noted that treatment would not be the solution and called for research to define the value of various unproven management practices. Mastitis workers recognized that mastitis was a multifactorial disease but they lacked research that allowed them to prioritize the effect of various preventive practices. In the next decade, UK researchers from the National Institute for Research in Dairying evaluated a management program that focused on understanding the dynamics of IMI (Figure 1; Dodd et al., 1964). They arrived at the simple equation that the percent of infected quarters within a herd was a function of the rate of new infections and the duration of those infections (Figure 2; Dodd et al., 1964). They noted that treatment was effective for reducing duration (and controlling *Strep. agalactiae*) but was of little value for eliminating staphylococcal infections, thus emphasis was directed at reducing the rate of new IMI. While Neave and Dodd were experimenting with the impact of various management practices (Neave et al., 1966), they correctly noted that, "it means that the control is going to depend on being able to persuade thousands of people of different abilities to conform to particular work patterns." Four decades later, researchers continue to study methods to persuade farmers to improve mastitis management (Valeeva et al., 2007).

In 1969, JDS published a series of symposium papers that described progress in mastitis control (Dodd et al., 1969; Neave et al., 1969; Norcross and Stark, 1969; Philpot, 1969; Read, 1969). The series was introduced by Frank Dodd, who is recognized as an important pioneer in the field of mastitis control. He summarized data from a longitudinal study of 721 cows in 14 herds (Dodd et al., 1969). At the beginning of the study, 57% of the cows were affected with subclinical mastitis, 80% of which was attributed to either streptococci or staphylococci. Throughout the yearlong study, they characterized the dynamic nature of new infections, occurrence of clinical mastitis (in cows with IMI), and the effect of various treatment strategies on reducing overall prevalence. They also experimented with various management practices that were referred to as a "hygiene system." They commented that the ideal mastitis control program "must cost much less than the losses caused by the disease, it must be relatively easy to carry out, there should be good experimental evidence that the control works under a range of conditions, and it must be obvious to the farmers who adopt the method that clinical mastitis is much reduced." In an accompanying paper, Neave et al. (1969) described results of field experiments that evaluated the effect of applying a "full hygiene system." They described results of a series of experiments and field trials that systematically evaluated use of premilking teat disinfection with individual towels, use of milking gloves, sanitation of teat cups, and efficacy of post-milking teat dip. They reported that a program of "partial hygiene" (the preceding steps without the practice of sanitizing the teat cups between cow milking) resulted

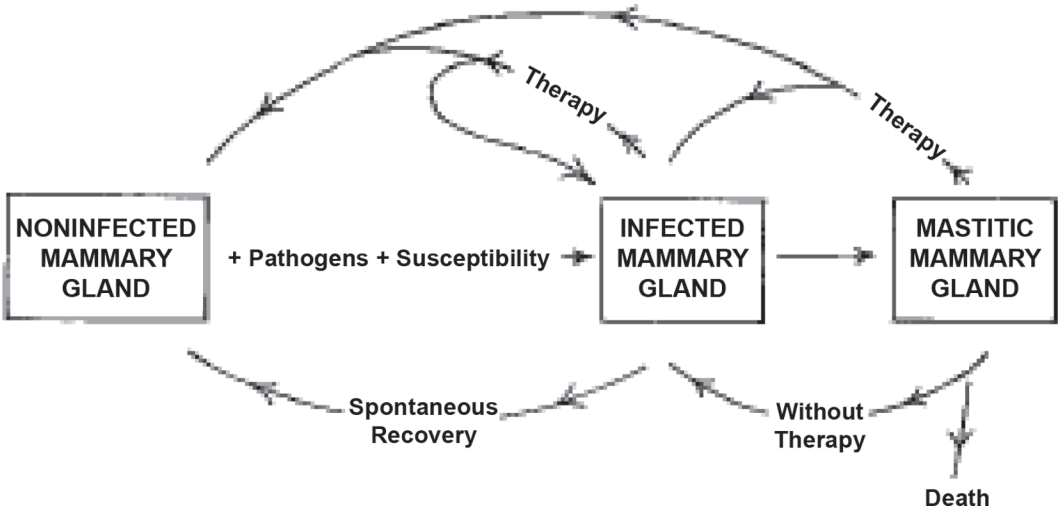

Figure 1. Possible sequence of events in development of infection and mastitis. Reprinted from Dodd et al. (1964) with permission.

in a 44% reduction in new infection rates (Table 2) and advocated use of antibiotic therapy at dry-off to further reduce infections. In the coming years, this plan was widely adopted as the basis of modern mastitis control and the work of Dodd and Neave greatly contributed to improving udder health and milk quality throughout the world. Their work soon led the recently formed National Mastitis Council to develop a mastitis control program known as the "5-Point Plan" that is the basis for controlling contagious mastitis and includes (1) effective post-milking teat dipping, (2) use of antibiotic dry cow therapy in every quarter at the end of each lactation, (3) appropriate treatment of clinical cases, (4) culling of chronically affected cows, and (5) maintenance of milk equipment to ensure stable teat end vacuum.

Antimicrobial Therapy

During the pre-antibiotic era, little could be done with cows that developed IMI and little was known on how to limit transmission. Early researchers determined that periodic examination of milk, followed by segregation and selective culling of affected cows, could be used to establish herds free of *Strep. agalactiae* (Plastridge et al., 1936). However, this control strategy was difficult to implement and when antimicrobials became available researchers rapidly began experiments to determine how to use them. Despite administration of massive doses (that resulted in toxicity in several cows), initial studies with oral sulfanilamide failed to achieve effective concentrations in blood or milk and the researcher noted that "treatment with sulfanilamide was successful in restoring normal flow and normal appearance of milk…but it did not eliminate the streptococci from the udder, nor prevent later acute attacks (Gildow et al., 1938)." This comment is the first indication that clinical impressions can be misleading in determining efficacy of antimicrobial compounds and illustrate the difficulty of separating the occurrence of inflammation from active IMI. Experiments with intramammary penicillin began in the 1940s and the in vitro efficacy of penicillin against gram-positive mastitis organisms was established by 1945 (Seeley et al., 1945). Even in the early years, researchers were aware that treatment using penicillin was much more efficacious against *Strep. agalactiae* than staphylococcal infections (Seeley et al., 1945). The ineffectiveness of controlling mastitis based on treatment of clinical cases was noted very early in the paper by Murphy (1956) and summarized with the memorable statement that "the utter futility of thinking that mastitis can be controlled by the treatment of clinical mastitis only should be obvious. This is merely cutting the tops off the weeds and leaving the roots." However, despite variable success and limited understanding of effective means to reduce new infections, use of antibiotics to treat mastitis was rapidly adopted for both lactating and dry cows. Mastitis remains the most common bacterial disease on most dairy farms, and consequently, mastitis treatment and prevention account for the majority of antimicrobials administered to adult dairy cows (Pol and Ruegg, 2007b; Saini et al., 2012; González Pereyra et al., 2015; Kuipers et al., 2016; Stevens et al., 2016). Such use is of increasing concern to consumers and public health authorities, and additional research is required to define appropriate antimicrobial usage that balances animal wellbeing with societal concerns about the role that farm use of antimicrobials plays in development of antimicrobial resistance.

Table 2. Results of field trials that compared normal herd management (control) to a full or partial hygiene program, showing the proportionate reduction (%) in new infection rate with the 3 hygiene systems (reproduced from data in Table 4 in Neave et al., 1969); significant results are shown in bold

Trial[1]	Hygiene comparison[2]	Decrease in new infections	Decrease in new *Staphylococcus aureus* infections	Decrease in new streptococcal infections
MFE_1	Full vs. control	**45** (**45**)[3]	33 (41)[3]	**60** (**62**)[3]
MFE_2	Full vs. control	**58**	**62**	**70**
MFE_2	Partial vs. control	**44**	**55**	**63**
MFE_2	Full vs. partial	25	17	19

[1]MFE_1 = first field experiment, using 14 herds for 12 mo; MFE_2 = second field experiment, using 15 herds for 18 mo.

[2]Full hygiene = teat cups pasteurized, udders disinfected with separate udder cloths or towels, and teat dip. Partial hygiene = teat cups not disinfected, disinfectant with separate udder cloths or towels, and teat dip. Control = teat cups not disinfected, udders washed with water and common cloth, and no teat dip.

[3]After adjustment for the mean number of infected quarters at the start.

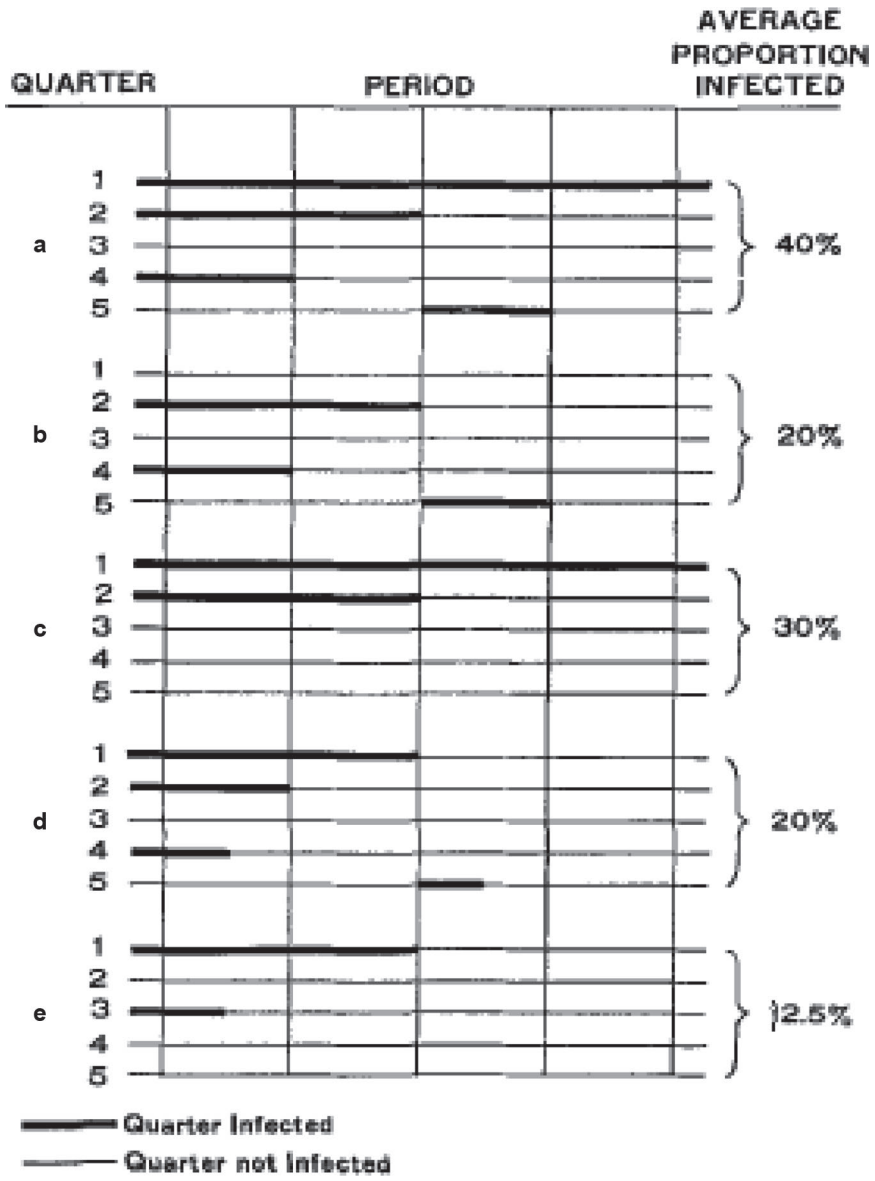

Figure 2. Factors influencing the average level of infection in a herd: prevention of new infection and reduction of duration of infections. Reprinted from Dodd et al. (1964) with permission.

Antibiotic Treatment During Lactation. By 1969, use of antibiotic therapy was well established, and a review article titled "Role of therapy in mastitis control" was published as part of the ADSA mastitis symposium (Philpot, 1969). As appropriate for this period, the emphasis was on treatment of IMI caused by *Strep. agalactiae* and *Staph. aureus*. Recommendations about treatment of subclinical mastitis included a preference for intramammary administration of broad-spectrum drugs suspended in relatively small volumes of aqueous vehicles. Although use of antibiotics to treat mastitis was common, the limitations of therapy were well known by this time. Philpot (1969) emphasized that the excellent prognosis for treatment of *Strep. agalactiae* was partially because of the location of the infection in the milk duct system. In contrast, when referring to *Staph. aureus*, he reported that the "prognosis regarding therapy is disappointingly low" because the organisms "penetrate the duct walls of the udder and become established in numerous foci." He further stated, "tissue barriers within the udder are of infinitely greater importance in therapeutic failures than the matter of drug resistance." Although he documented that a single treatment of penicillin would result in elimination of about 90% of IMI caused by *Strep. agalactiae*, he cited 5 studies indicating an expected efficacy of 50% for treatment of staphylococcal IMI. Importantly, this is the first publication that includes recommendations to review individual animal factors (age, stage of lactation, level of milk production, pedigree, and the severity of infection) before deciding to use antibiotics to treat cows affected with *Staph. aureus*. Three decades later, these recommendations were validated in research evaluating factors associated with bacteriological cure of mastitis caused by *Staph. aureus* (Sol et al., 1997, 2000; Barkema et al., 2006). Similar to Philpot (1969), these studies confirmed low bacteriological cure rates (30–50%) and indicated that age of the cow, SCC, infection in the front quarters, and stage of lactation were the most important determinants of successful outcome. More recently, a highly cited review about cow, pathogen, and treatment factors that contribute to therapeutic success of cows infected with *Staph. aureus* again emphasized that only selected animals will respond to antibiotic therapy (Barkema et al., 2006). Philpot (1969) concluded his paper with the following statement that is as relevant today as it was when originally published (capitalized as in original citation): "Therapy can be a valuable adjunct to an effective program of mastitis control. It should be employed, however, with a full awareness that IT IS LESS THAN DESIRABLY EFFECTIVE IN ELIMINATING MANY EXISTING INFECTIONS AND THAT IT DOES NOT PRECLUDE THE DEVELOPMENT OF MOST NEW INFECTIONS."

As coliform mastitis was recognized as an emerging problem, researchers began to evaluate the unique challenges in treating these infections. Although it was recognized that many cases were not severe, defining effective treatment of peracute and acute cases was a high priority and almost no controlled studies were available to guide treatment decisions (Coliform Subcommittee of the Research Committee of the National Mastitis Council, 1979). Initially, recommendations for treatment were empirical and included frequent milk out, systemic and intramammary administration of antibiotics, supportive fluid, and anti-inflammatory therapy. The authors noted that approved antibiotics with gram-negative spectrum were not available. Thus, choices of antibiotics included drugs that were soon to be banned for use in dairy cows (such as chloramphenicol) and other drugs that did not have Food and Drug Administration–approved withholding periods (Coliform Subcommittee of the Research Committee of the National Mastitis Council, 1979). Until the 1990s, few trials were performed to validate recommendations for treatment of coliform mastitis but initial experiments indicated that antimicrobial therapy did not improve outcomes of mastitis caused by *Escherichia coli* (Pyörälä et al., 1994) and challenged prevailing concepts of how mastitis should be treated. The important role of the host immune response in clearance of coliform infections (rather than antibiotic therapy) has been highlighted by an important body of research (Burvenich et al., 2003, 2007). Although some broader spectrum drugs later became available, the increased proportion of culture-negative clinical cases and increased diversity of etiological agents have encouraged development of selective treatment protocols (Lago et al., 2011a,b). Current recommendations for treatment of clinical mastitis are based on targeted antibiotic usage for most gram-positive cases while allowing time for spontaneous cure of most other cases (Ruegg, 2017). With increasing pressure to reduce antibiotic usage on dairy farms, additional research is needed to develop evidence-based treatment protocols that use antibiotics appropriately and can be practically applied on a variety of dairy farms.

Dry-Cow Antibiotic Therapy. Early mastitis workers recognized that about 50% of cows had IMI so use of antibiotic therapy to reduce duration of IMI was recommended as part of a comprehensive mastitis control program (Neave et al., 1969). The cost of discarded milk and the risk of milk residues (Albright et al., 1961) were recognized as limitations to using antibiotics to treat the large proportion of infected lactating cows so

Table 3. Numbers of new IMI detected during 2 trials to evaluate use of comprehensive dry cow antibiotic therapy and milking hygiene (reproduced from data in Tables 2 and 5 in Eberhart and Buckalew, 1972)

			Quarters infected						
Trial[1]	Group[2]	Period	*Streptococcus agalactiae*	*Staphylococcus aureus*	Other streptococci	Coliform	Other	Total	No. of new IMI/cow-year
1	Control	Lactating	53	51	25	13	4	146	
		Dry	4	12	12	12	0	40	
		Total	57	63	37	25	4	186	1.50
	Treatment	Lactating	14	50	40	27	2	103	
		Dry	2	0	7	10	2	21	
		Total	16	20	47	37	4	124	1.03
2	Control	Lactating	17	1	9	9	1	37	
		Dry	2	1	3	1	1	8	
		Total	19	2	12	10	2	45	0.98
	Treatment	Lactating	0	4	9	3	0	16	
		Dry	0	1	3	4	0	8	
		Total	0	5	12	7	0	24	0.48

[1]Trial 1 used 3 herds with about 60 cows per group; trial lasted 2 yr and prevalence of IMI at start of trial was characterized as high. Trial 2 used 2 herds with about 40 cows per group; trial lasted 60 wk and prevalence of IMI at start of trial was characterized as low.

[2]Control = no post-milking teat dipping and no dry period antibiotic therapy; teats were disinfected before milking and forestripped, and cases of clinical mastitis were treated with antibiotic. Treatment = teats received post-milking disinfection using iodine-based teat dip; all quarters were treated with antibiotic during wk 1 and 2 after dry off; teats were disinfected before milking and forestripped, and cases of clinical mastitis were treated with antibiotic.

use of antibiotic dry-cow therapy (**DCT**) was explored. Researchers had already established that cows were at risk of acquiring IMI during the dry period, and additional benefits of reducing new IMI during this period were hypothesized (Neave et al., 1950). Similar to current concerns about giving antibiotics to animals that may not be infected, early researchers disagreed about which cows should be treated. Some authorities were recommending treatment of all quarters of all cows whereas others believed that only infected cows should be treated (Philpot, 1969). Shortly after DCT was initiated, Natzke (1971) reviewed potential methods of selecting cows for dry treatment. After comparing bacterial culture, use of screening tests (such as CMT), and review of clinical mastitis history, he stated that the limited sensitivity of each of those methods led to the conclusion "that the treatment of all quarters of all cows at the time of drying off is the preferred system. . ." The effectiveness of DCT (combined with teat dipping) was subsequently demonstrated conclusively by several field studies. Use of teat-dipping and comprehensive DCT was shown to reduce new IMI by about 50% in herds with both high and lower prevalence of existing IMI (Table 3), but the authors noted that "other streptococci" were not effectively controlled and one herd that started the trial with high prevalence of IMI experienced increased infections caused by coliform bacteria (Eberhart and Buckalew, 1972). Results of a later study comparing comprehensive DCT to selective DCT (cows selected based on history of clinical mastitis) demonstrated considerably reduced clearance of infections, increased new IMI, and increased cases of clinical mastitis in cows that were in the selective treatment group (Ward and Schultz, 1974), and use of comprehensive DCT became established as an important component of mastitis control in dairy herds in North America and the United Kingdom. While researchers continued to debate the use of antibiotics in apparently uninfected glands (Rindsig et al., 1978; Poutrel and Rainard, 1981; Schultze, 1983), US dairy farmers rapidly adopted the practice of comprehensive DCT; by 1996, about 77% of farmers used antibiotic DCT in all quarters of all cows at dry-off (USDA, 1996b). In contrast, during the same period, dairy herds in Scandinavia had lower rates of IMI and preferred use of selective dry-cow programs (Schultze, 1983).

As researchers learned more about the high risk of mastitis during the nonlactating period, it became evident that antibiotic DCT was not able to prevent new IMI entirely during the periparturient period (Oliver and Sordillo, 1988). The combination of concern about widespread use of antibiotics and the desire to better reduce IMI during the dry period resulted in development and commercial introduction of a nonantibiotic internal teat sealant (Woolford et al., 1998; Hillerton and Kliem, 2002; Huxley et al., 2002), which was rapidly adopted as an adjunct to antibiotic DCT. The continued decline of IMI caused by *Strep. agalactiae* and *Staph. aureus* and availability of a nonantibiotic alternative to prevent new IMI have again ignited debate and research about selective DCT (Halasa et al., 2010; Cameron et al., 2014; Scherpenzeel et al., 2014).

Economic models have demonstrated that the decision to use either selective or comprehensive antibiotic DCT is highly farm specific (Huijps and Hogeveen, 2007) but it is likely that governmental regulations encouraging reduced antibiotic usage will result in less use of comprehensive antibiotics at dry-off in the future.

PREVENTION

Effect of Milking Machines and Milking Management

Machine Milking. During the century covered by this review, methods of milking and milking management underwent revolutionary changes that go far beyond the scope of this paper. The tremendous progress during this period is illustrated by comments of Witzel (1956), who reviewed advances in dairy farm engineering in JDS for the 50th anniversary of the founding of the American Dairy Science Association. He noted that in 1950, the average herd size was 6 cows and, although 93% of farms had electrical power, only 51% of cows were machine milked. As milking machines rapidly replaced hand milking, researchers became concerned that the machines could cause irritation and serve as fomites for spreading mastitis among cows (Cone, 1942). Research was needed to determine how milking machines functioned relative to the physiology of milk secretion and how adoption of machine milking would influence the risk of mastitis.

Early innovative studies about machine milking were performed by Espe and Cannon (1942), who injected barium into the teat sinus and took a series of radiographs that illustrated functioning of the teat sphincter. These experiments contributed greatly to our understanding of the mechanics of the teat and illuminated the mechanism of bacterial penetration through the streak canal. As research progressed, effects of vacuum level, vacuum stability, and milking duration on risk of mastitis were identified (Mochrie et al., 1953a,b; Eberhart et al., 1968). Eventually, investigators determined that both vacuum fluctuations and milking duration should be minimized to reduce the risk of new IMI associated with liner slips (Baxter et al., 1992).

A decade-by-decade review of progress in machine milking research was published in JDS by Thompson (1981) for the 75th anniversary of the founding of the American Dairy Science Association. Thompson (1981) reviewed advances in development of milking machines and highlighted research about the important association between milking vacuum and IMI. He concluded his review by emphasizing the increasing role for automation in the milking process. He noted that the automatic detacher had been the most important development in milking automation, predicted that sensors would be developed that would result in "further automation not only of milking tasks but also of management data recording and analysis." In the decades since his review, automatic milking systems have become commonplace in many regions but effective use of data from the systems is still not optimized (Jacobs and Siegford, 2012). Detection of mastitis and maintaining udder health in automated milking systems remains challenging, and the role of the "competent" herdsperson in managing udder health remains as important today as in past decades (Hovinen and Pyörälä, 2011).

A later reviewer (Spencer, 1998) defined the role of the milking machine in maintaining udder health. By this time, many herds had controlled *Strep. agalactiae* and *Staph. aureus*, and the prevalence of IMI had declined. Advances in milking machines had greatly improved vacuum stability, and installation standards for milking systems had been developed. While Spencer (1998) noted that the milking machine could influence new IMI by serving as a fomite, allowing cross-infections within cows, damaging teat sphincters or creating teat impacts, he was one of the first to point out that the milking machine is rarely a direct cause of new IMI. He cited research that demonstrated that only 6.6% of new IMI were accounted for by milking machine factors and concluded that there was no convincing evidence linking the milking machine to the overall prevalence of herd infection (Spencer, 1998).

Milking Management. As milking machines became popular, defining appropriate milking procedures was an important priority. Early mastitis workers had studied physiological mechanisms of milk secretion and ejection, and "pituitrin" (oxytocin) was identified as a substance that could positively stimulate milk flow (McCandlish, 1918). As milking machines were adopted, factors that could influence milk ejection were studied. In one remarkable experiment, the effect of fright on milk ejection was evaluated by placing a cat on the back of a cow and exploding paper bags every 10 s for 2 min (the authors noted that "later the cat was dispensed with as unnecessary"; Ely and Petersen, 1941). This work clearly demonstrated that fear had a significant effect on reducing milk ejection. This was a potentially important finding because incomplete milking of cows chronically infected with *Strep. agalactiae* was soon shown to result in increased occurrence of clinical mastitis (Schalm and Mead, 1943). This study had a lasting effect influence on milking management. Although the authors did not report milk yield of cows enrolled in their experiment, the volume of milk left in the udder (about 1 kg) was probably close to 15 to 20% of the normal daily milk yield of cows of that period. The authors did not report negative effects in cows free of IMI, but this fear—that leaving milk in the

udder led to mastitis—persisted and likely encouraged widespread use of excessively long attachment times for decades to follow.

The association between bacterial colonization of teat skin and development of IMI has been well established and use of management practices that reduce bacterial contamination of teat ends is a fundamental aspect of mastitis control. Dodd et al. (1964) and Neave et al. (1969) established the importance of postmilking teat disinfection for control of contagious pathogens. In a comprehensive review of postmilking teat disinfection, Pankey et al. (1984) stated, "postmilking teat antisepsis is regarded as the single most effective practice for prevention of IMI of lactating dairy cows" but cautioned that it was not equally effective against coliforms and many streptococci. Smith et al. (1985) concurred and noted that postmilking teat disinfection did not effectively control environmental pathogens. As environmental mastitis emerged, researchers began to investigate other preventive strategies. Historically, premilking sanitation had usually been performed by washing udders and teats with water or disinfectants, but Galton et al. (1984, 1988) demonstrated that premilking disinfection of teats (not udders) followed by effective drying dramatically reduced development of IMI caused by *Streptococcus uberis*. In a field trial, Pankey et al. (1987) demonstrated a 51% reduction in new IMI caused by streptococci and coliforms when pre-dipping was combined with "good udder preparation." "Good udder preparation" included teat sanitation, drying using a single-service towel, forestripping, and application of a pre-dip sanitizer for a minimum of 30 s. Pankey (1989) later recommended standardization of premilking procedures and use of proper udder hygiene at every milking. In the United States, regulatory requirements state that teats must be sanitized and dried before milking, and farmers rapidly switched from washing udders to the process of good udder preparation (including pre-dipping and drying teats). National statistics indicate that use of premilking teat sanitation with a dip cup (or spray) followed by drying increased from 58 to 85% of farmers between 1996 (USDA, 1996b) and 2014 (USDA, 2016). Although geographical differences exist in adoption of pre-dipping and other premilking procedures, it is likely that processor preferences for milk with little bacterial contamination, sediment, or residues will continue to encourage adoption of increasingly stringent teat preparation practices.

Other Important Preventive Strategies

Genetic Selection for Mastitis Resistance. The ability to use genetic selection to reduce mastitis has gradually evolved. As part of their pioneering work, Murphy et al. (1944) observed differences in the rate of IMI among separate cow families of equal productivity within a single herd and noted that heritable differences in susceptibility may contribute to development of IMI. Early estimates of heritability of mastitis ranged from 0.27 (Legates and Grinnells, 1952) to 0.38 (Lush, 1950), but progress toward selection of mastitis resistance was impeded by differences in definition of the disease and by the lack of testing programs. Advancements in genetic selection for mastitis resistance were not possible until widespread adoption of SCC testing in DHI programs. Selection for mastitis resistance was encouraged because genetic increases in milk yield were shown to be correlated with increased susceptibility to mastitis (Shook and Schutz, 1994). Somatic cell scores (Ali and Shook, 1980) were incorporated into US selection indices in 1994 (Schutz, 1994). Although improving mastitis resistance has not been the highest priority of US dairy farmers, considerable progress has occurred in other countries (Heringstad et al., 2008), and future innovations in genomic selection technologies will likely be used to accelerate genetic gains in resistance to mastitis (Vukasinovic et al., 2017).

Supplementation with Vitamin E and Selenium. The role of nutritional management in development of mastitis has long been controversial and difficult to separate from other confounding effects. Plastridge (1958) erroneously suggested that feeding high-concentrate diets was a risk factor for mastitis but direct effects of nutrition on mastitis were not reported until Smith and coworkers (1985) performed experiments that demonstrated that dietary deficiencies of selenium and vitamin E increased incidence and duration of clinical mastitis. Initial experiments were supported by later field studies (Erskine et al., 1987; Weiss et al., 1990) that demonstrated increased subclinical and clinical mastitis in selenium-deficient herds. Researchers performed experiments that demonstrated the essential role of these nutrients in maintaining effective neutrophil function (Grasso et al., 1990; Hogan et al., 1990b, 1993). The important role of vitamin E and selenium in maintaining udder health are now well established and this work contributed to both dietary modification and important knowledge about neutrophil function.

Immunization. The development of effective vaccines to protect cows from developing new IMI has been a goal of numerous mastitis workers. Although vaccines have been used to effectively control other bacterial diseases of dairy cows, the nature of mastitis poses numerous challenges to their success. Mastitis is caused by a variety of evolving bacterial pathogens with strains that vary among farms and over time. The site of IMI within the mammary gland, virulence

characteristics, and immunogenic capabilities all vary among pathogens. Initial vaccine research was directed toward development of vaccines against *Strep. agalactiae* and *Staph. aureus* and although potential efficacy was shown in laboratory experiments, early field trials failed to demonstrate that immunization could reduce new IMI (Oehme and Coles, 1967; Mellenberger, 1977). While several *Staph. aureus* vaccines have been commercialized, successful control of these organisms has been achieved in many regions without use of immunization based on adoption of the well-known principles first described by Dodd et al. (1964).

In contrast to vaccines directed at gram-positive pathogens, experimental challenges and field trials were able to demonstrate acceptable efficacy of a gram-negative core-antigen vaccine (Hogan et al., 1990a, 1992a,b, 1995), and several vaccines are marketed to help dairy farmers control symptoms of mastitis caused by gram-negative bacteria. Gram-negative vaccines are based on a highly conserved core antigen of lipopolysaccharide, thus avoiding the problem of variation in bacterial strains among farms. Similar to vaccines directed at gram-positive pathogens, vaccination with gram-negative vaccines does not have a large effect on reducing new IMI but does significantly reduce the development of clinical signs. In contrast to mastitis caused by *Staph. aureus*, most IMI caused by coliform bacteria develop clinical signs that account for most of the economic and welfare losses associated with these infections. The ability of vaccinated cows to more rapidly clear infections and prevent progression to the clinical state has resulted in widespread usage of these vaccines. The quest for efficacious vaccines continues to be a research priority (Piepers et al., 2017), and contemporary researchers are using advances in immunology to test new vaccines against *Staph. aureus*, environmental streptococci, and other pathogens.

Mastitis in Primigravid Heifers. Until recently, primigravid heifers were not considered affected by mastitis. Although Schalm (1942) recognized that intersucking among calves increased risk of postcalving mastitis caused by *Strep. agalactiae*, almost no attention was placed on IMI in heifers until Oliver and Mitchell (1983) reported results of a small study in which they recovered a high frequency of staphylococci from mammary secretion collected in the prepartum period. Subsequent field surveys indicated wide geographical differences in prevalence and type of pathogen based on region and time of sampling (Fox et al., 1995). A high prevalence of IMI caused by *Staph. aureus* was initially reported for prepartum heifers in the southern United States (Trinidad et al., 1990), and led to experiments to identify appropriate interventions. Nickerson et al. (1995) summarized experiments conducted to define prevalence and control of IMI in dairy heifers. In contrast to that in mature cows, the use of antimicrobial therapy in prepartum heifers was found to be highly efficacious in reducing IMI caused by *Staph. aureus*, and this strategy remains a tool for herds experiencing significant problems with this issue.

More recently, a comprehensive review about mastitis in dairy heifers was published (De Vliegher et al., 2012). In that paper, prevalence studies conducted from around the world were summarized, indicating that although primigravid heifers have a relatively low prevalence of infection with major pathogens, many are colonized by CNS (De Vliegher et al., 2012). Interestingly, IMI in dairy heifers caused by CNS have a high rate of spontaneous cure and do not usually have a negative effect on productivity, making the use of prepartum antibiotic treatment unnecessary in most herds. The authors of that review recommended prevention-based measures such as fly control, avoidance of inter-sucking, and assurance of hygienic and comfortable housing areas. Although considerable progress has been made in defining heifer mastitis, future research is needed to define epidemiological characteristics and to better understand the effect of IMI caused by CNS.

SUMMARY AND FUTURE DIRECTIONS

During the last century, researchers have characterized the nature of IMI, determined mechanisms of the inflammatory response, developed effective mastitis control programs that have been widely adopted throughout the world, and, in many regions, have virtually eradicated the pathogen (*Strep. agalactiae*) that was responsible for the vast majority of mastitis in the first half of this century. The effects of mastitis on productivity, reproductive performance, and product quality have been quantified. Diagnostic tools (such as SCC testing) have been developed that allow producers to identify subclinically infected cows and use targeted management strategies to reduce spread of contagious pathogens. As herd sizes grew and management intensified, researchers recognized emergence of opportunistic pathogens that often result in clinical cases. Tremendous advances in milking machines and milking management have resulted in wide adoption of highly functioning milking systems and standardized milking procedures. Limitations of antimicrobial therapy have been recognized but use of antibiotics to treat cows affected with some pathogens remains an important tool for mastitis control. During the period that this review covers, the effect that mastitis researchers have had on improving milk quality and dairy farm productivity is truly remarkable.

In 1958, Plastridge published a review of bovine mastitis in JDS (Plastridge, 1958) that summarized current mastitis research and included the following disclaimer: "A complete review is beyond the scope of this communication..." That disclaimer is even more applicable to the current review. An enormous volume of important research has been conducted in the 58 years since that statement was made. In the century covered by this review, numerous researchers have contributed to progress in controlling mastitis. I have attempted to summarize advances in the detection, prevention, and management of bovine mastitis and I have focused on papers published in JDS that helped illustrate how our understanding of mastitis has evolved. Important research has necessarily been excluded, simply due to the constraints of space. I encourage current mastitis researchers to reacquaint themselves with the historical research that has strengthened our knowledge and ability to manage this important disease.

In spite of tremendous progress, in most regions, mastitis remains the most economically significant bacterial disease of dairy cattle, and continued advances in mastitis control are necessary to ensure sustainability of dairy farming worldwide. Most countries have eliminated production controls and globalization has had a tremendous impact on quality standards. The ability to participate in global dairy trade is increasingly dependent on production of milk that meets stringent quality standards that are defined by milk processors rather than government regulators. In emerging dairy regions, there is a need to provide infrastructure and training to help farmers efficiently adopt proven management strategies that minimize development of new IMI and result in production of high quality milk. Investments in defining mastitis control strategies for minor dairy species (such as dairy sheep, goats, and buffalo) are also needed.

In developed dairy regions, intensification of herd management has resulted in new challenges for producers. Studies are needed to fully define risk factors and control strategies for emerging pathogens (such as *Prototheca*, *Mycoplasma bovis*, and others). Research using new diagnostic methods and molecular technologies is needed to fully understand the ecology and control of microbes that reside in the dairy ecosystem and are potential etiologic agents for mastitis. The issue of antimicrobial resistance and societal pressures to reduce antimicrobial therapy on dairy farms will grow in importance, and research defining appropriate use of antimicrobials is a high priority. Standardization of methods used to evaluate efficacy of mastitis treatments is needed to identify when antimicrobial usage is truly beneficial. Continued investment in research to develop alternatives to antimicrobials is required and more emphasis should be directed at methods of improving mastitis resistance.

Limitations in labor supplies have already contributed to increased use of automation, and this trend will likely accelerate. Increased use of automatic milking systems and incorporation of automation into milking parlors requires research on optimization and effective use of data originating from these systems. All of these research priorities require effective means to communicate and persuade farmers of their utility, thus continued development of mechanisms for knowledge transfer are necessary to fully capture the value of future research gains.

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APPENDIX

Table A1. Timeline of significant advances in detection, management, and prevention of mastitis

Date	Milestone	Reference
1917	Streptococci from infected udders are identified as cause of high bacterial counts in milk.	Breed and Brew, 1917
1927	"Flaky" milk appearance is associated with bacterial inflammation caused by staphylococci and streptococci.	Jones and Little, 1927
1937	Occurrence of subclinical mastitis is shown to reduce milk yield.	White et al., 1937
1942	Anatomic and physiologic aspects of teat sphincter are described.	Espe and Cannon, 1942
1945	In vitro study demonstrates the efficacy of penicillin against gram-positive mastitis pathogens.	Seeley et al., 1945
1950	Heritability of susceptibility to mastitis is estimated.	Lush, 1950
1953	Leukocyte count of bulk milk is shown to predict prevalence of subclinical mastitis in herd.	MacLeod et al., 1953
1956	Pathogen-specific characteristics of infection and disease presentation are first defined.	Murphy, 1956
1957	Development of California Mastitis Test.	Schalm and Noorlander, 1957

Continued

Table A1 (Continued). Timeline of significant advances in detection, management, and prevention of mastitis

Date	Milestone	Reference
1961	National Mastitis Council is formed to unify recommendations for mastitis control.	
1967	First US limit on bulk tank SCC is set at 1,500,000 cells/mL.	
1969	Seminal works on control of *Streptococcus agalactiae* and *Staphylococcus aureus* through hygiene and management are published.	Dodd et al., 1969; Neave et al., 1969
1971	Use of comprehensive antibiotic treatment at dry off is promoted.	Natzke, 1971
1982	Linear relationship between SCC and milk yield loss is demonstrated.	Ali and Shook, 1980
1984	Epidemiology and control of environmental mastitis is defined.	Smith et al., 1985
1992	Efficacy of *Escherichia coli* core antigen vaccine is demonstrated.	Hogan et al., 1992a,b
1994	Mastitis is included in genetic selection indices in United States.	
1995	IMI in prepartum heifers is recognized.	Nickerson et al., 1995
1998	Mastitis shown to reduce fertility.	Barker et al., 1998
1998	Efficacy of internal teat sealants in preventing mastitis is demonstrated.	Woolford et al., 1998
2001	Era of molecular diagnostic tests begins.	Phuektes et al., 2001
2002	Antibiotic usage on farms and possible linkages with antibiotic resistance emerges as an important issue.	Erskine et al., 2002; Pol and Ruegg, 2007a,b
2012	Most US producers are required to meet European Union SCC standard of 400,000 cells/mL.	

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A 100-Year Review: Stress physiology including heat stress[1]

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ABSTRACT

Stress is an external event or condition that places a strain on a biological system. The animal response to a stress involves the expenditure of energy to remove or reduce the impact of the stress. This increases maintenance requirements of the animal and results in loss of production. The biological response to stress is divided into acute and chronic phases, with the acute phase lasting hours to a few days and the chronic phase lasting several days to weeks. The acute response is driven by homeostatic regulators of the nervous and endocrine systems and the chronic phase by homeorhetic regulators of the endocrine system. Both responses involve alterations in energy balance and metabolism. Thermal environment affects all animals and therefore represents the largest single stressor in animal production. Other types of stressors include housing conditions, overcrowding, social rank, disease, and toxic compounds. "Acclimation" to a stress is a phenotypic response developed by the animal to an individual stressor within the environment. However, under natural conditions, it is rare for only one environmental variable to change over time. "Acclimatization" is the process by which an animal adapts to several stressors within its natural environment. Acclimation is a homeorhetic process that takes several weeks to occur and occurs via homeorhetic, not homeostatic, mechanisms. It is a phenotypic change that disappears when the stress is removed. When the stress is severe and not relieved by acclimatization or management changes, the animal is considered chronically stressed and is susceptible to increased incidence of disease and poor health. Milk yield and reproduction are extremely sensitive to stress because of the high energy and protein demands of lactation and the complexity of the reproductive process and multiple organs that are involved. Improvements in protection of animals against stress require improved education of producers to recognize stress and methods for estimating degree of stress on animals.

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INTRODUCTION

Hans Selye (1936) first introduced the concept of stress as 'the non-specific response of the body to any demand," and many attempts have been made to refine its meaning (Friend, 1991; Appendix Table A1). However, the definition remains ambiguous and the word is used differently in different contexts. As pointed out by Schulte (2014), the variability in the definition of stress may stem from the fact that stress research has developed relatively independently across several fields of biology, with substantial gulfs between those interested in stress from a biomedical perspective and those interested in the effects of stressors in natural populations (Bijlsma and Loeschcke, 2005; Boonstra, 2013).

For the purpose of consistency, we will define stress as an "external event or condition." We will further define a stressor as the component of the environment that places a strain on a biological system. Examples of stressors are shown in Table 1 and include thermal environment, management, social interaction, environmental contaminants, and disease, to name a few. Stress is a threat to homeostasis because it always increases the maintenance requirements of domestic animals. Because energy demands alter animal production, we are restricting this review to "external challenges that require a change in maintenance output to meet the challenge." One of the earliest estimates of maintenance energy requirements in cattle was published by Washburn (1938). Brody (1956) reviewed the effects of thermal environment on basal metabolism. The increase in maintenance requirement by stress is of real concern to production animal systems because it increases costs, reduces efficiency, and leads to lower profitability of an animal enterprise. The reason a stress increases maintenance cost is because energy must be expended to return the animal to homeostasis of body function. This energy must come from net energy for production because it is not physically possible to remove it from net energy for maintenance. The environmental temperature below which the body produces extra heat to meet the thermostatic heat requirement is termed the "lower critical temperature" and was first estimated

Table 1. Partial list of types of stressors and biological responses to them[1]

Stressor	Symptom	Physiological system activated or inhibited
Heat	Elevated body temperature	Heat loss mechanisms increased; decreased phagic drive
Cold	Reduced body temperature	Heat gain mechanisms enhanced; heat loss mechanisms reduced; feed intake enhanced
Infection	Elevated body temperature	Immune system activated; decreased phagic drive; hypothalamic body temperature setpoint altered
Poor housing	Increased lameness	Hypothalamic-pituitary-adrenal axis activated; reduced phagic drive
Poor nutrition	Deficiency symptom varies with nutrient	Mobilization of nutrient reserves; activation of pituitary-adrenal axis; altered behavior
Environmental	Hepatotoxicity	Liver function reduced; multiple organ damage; hypothalamic-pituitary-adrenal axis activated
Social	Altered behavior	Feed intake reduced; hypothalamic-pituitary-adrenal axis activated

[1]References: Bauman and Currie, 1980; Collier et al., 1982b; Friend, 1991; Coulombe, 1993; Bauman (2000); Collier and Gebremedhin, 2015; Chebel et al., 2016.

by Kleiber (1961) and confirmed by Hamada (1971). The environmental temperature above which the body starts storing heat and losing milk yield was established by Berman et al. (1963, 1985). For other stressors, we do not yet have specific end points by which to measure the relationship between the stress and loss in productivity of cattle. A key opportunity for the future will be to improve our ability to identify and measure levels of various stressors and their impact on animals in order to develop strategies to reduce the effects of those stressors on dairy animals (Appendix Table A1).

STRESS RESPONSE

Animals mount a response to a stress that involves behavioral, metabolic, and physiological changes at multiple levels of vertebrate organization from subcellular to the whole animal (Selye, 1936; Collier and Gebremedhin, 2015). The stress response is divided into 2 phases: acute and chronic (Friend, 1991). Acute stress responses last from a few minutes after the beginning of the stress to a few days (Horowitz, 2001). Activation of the acute response to stress is initiated by various receptors that respond to changes in the environment (Collier and Gebremedhin, 2015; Figure 1). The afferent pathways for the stress transmit this information to the central nervous system, including the thalamus and hypothalamus, where setpoints are controlled, and to the cortex for perception (Figure 1). These centers then activate various efferent pathways to effect a response to the environment (Figure 1). The acute response is driven by the autonomic nervous system promoting release of catecholamines and glucocorticoids, which alter metabolism and activate transcription factors involved in the acute response. The chronic response to stress is driven by the endocrine system and is associated with altered receptor populations, changing tissue sensitivity to homeostatic signals and resulting in a new physiologic state (Bligh, 1976; Bauman and Currie, 1980). Selye (1946) coined the term "heterostasis" to describe the process of achieving a new equilibrium state following exposure to a stressor (Fink, 2009). The term "rheostasis" was introduced to emphasize that the setpoints for homeostatic regulation may vary across environments or seasons (Mrosovsky, 1990), and the term "enantiostasis" was coined to refer to a situation in which multiple physiological variables are varied to maintain the overall functionality of a system (Mangum and Towle, 1977). These concepts emphasize the idea that maintaining functional homeostasis may require dynamic changes in a variety of parameters. Acclimation to a stress is a phenotypic response developed by the animal to an individual stressor within the environment (Fregley, 1996). However, under natural conditions, it is rare for only one environmental variable to change over time. Acclimatization is the process by which an animal adapts to several stressors within its natural environment (Bligh, 1976). Acclimation and acclimatization are not therefore evolutionary adaptations or natural selection, which are defined as changes allowing for preferential selection of an animal's phenotype and are based on a genetic component passed to the next generation. The altered phenotype of acclimated animals will return to normal if environmental stressors are removed, which is not true for animals that are genetically adapted to their environment (Collier et al., 2004). Acclimatization is a process that takes several weeks to occur, and close examination of this

process reveals that it occurs via homeorhetic and not homeostatic mechanisms. As described by Bligh (1976), there are 3 functional differences between acclimatory responses and homeostatic or "reflex responses." First, the acclimatory response takes much longer to occur (days or weeks versus seconds or minutes). Second, acclimatory responses generally have a hormonal link in the pathway from the central nervous system to the effector cell. Third, the acclimatory effect usually alters the ability of an effector cell or organ to respond to environmental change.

These acclimatory responses are characteristic of homeorhetic mechanisms as described by Bauman and Currie (1980) and revisited by Bauman (2000) and the net effect is to coordinate metabolism to achieve a new physiological state. Thus, the seasonally adapted animal is different metabolically in winter than in summer. Bauman and Currie (1980) incorporated these characteristics of acclimation into the concept of homeorhesis, which is defined as "orchestrated changes for priorities of a physiological state" (Bauman and Currie, 1980). The concept originated from considering how physiological processes are regulated during pregnancy and lactation (Bauman and Currie, 1980), but application of the general concept has been extended to include different physiological states, nutritional and environmental situations, and even pathological conditions. Key features of homeorhetic controls are its chronic nature (hours and days versus seconds and minutes required for most examples of homeostatic regulation) and its simultaneous influence on multiple tissues and systems that results in an overall coordinated response, which is mediated through altered responses to homeostatic signals (Bauman and Elliot, 1983; Vernon, 1988; Bell and Bauman, 1997).

ENERGY BALANCE

Stress does not uniformly affect energy balance, which is the difference between energy intake and energy expenditure. Dependent on the stress, phagic drive may be increased (pregnancy, lactation, cold) or decreased (heat, social, immune, calving). Energy expenditure is both increased (pregnancy lactation, cold, immune stress) and decreased (fasting and heat) by stressors. Herein, we review the role of stress on feed intake and energy expenditure in the dairy cow.

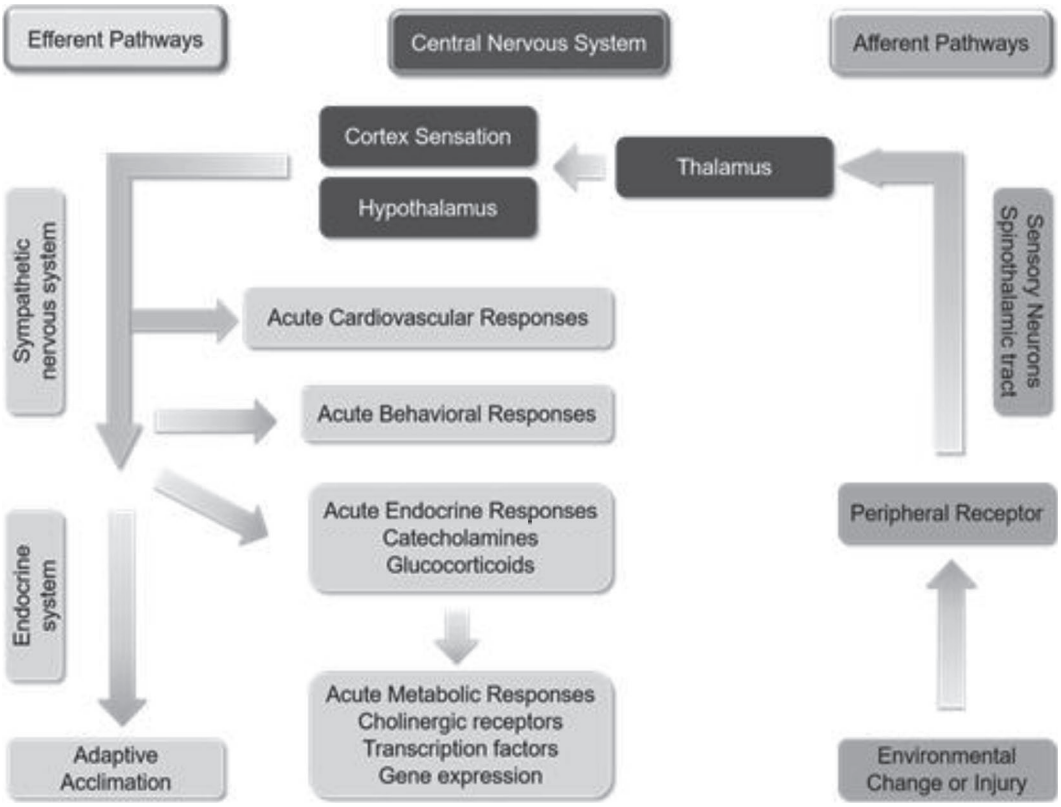

Figure 1. Schematic representation of pathways of stress response.

Pregnancy and Parturition

Pregnancy is a primary metabolic requirement in the dairy cow. During the first 180 d of pregnancy, the metabolic energy requirements (Mcal/kg$^{0.75}$) increase by approximately 16%. Over the next 105 d, approximately the last one-third of gestation, metabolic demand increases to 175% of that observed in nonpregnant cows at parturition (Moe and Tyrrell, 1972). In line with this estimate, Brody (1956) showed that heat production increased 59% during the final 200 d of pregnancy. On the day of calving, there is a consistent and robust decrease in feed intake (Marquardt et al., 1977; Goff et al., 2002; Huzzey et al., 2007; Lukas et al., 2008). The decrease in feed intake at calving is coincident with a decrease in rumen contractility at calving (Marquardt et al., 1977). Calving is associated with a rise in serum cortisol that is exacerbated in cows that have depleted serum Ca^{2+} (Horst and Jorgensen, 1982). This may be an adaptive positive feedback response because cortisol increases serum Ca^{2+}. However, the decrease in feed intake is not dependent on a lactation-induced depletion of serum Ca^{2+} or an increase in serum cortisol, because calving more robustly decreases feed intake in mastectomized cows, which respond to calving with a smaller decrease in Ca^{2+} and increase in cortisol (Goff et al., 2002). In fact, feed intake remains lower in mastectomized cows than in control cows for the first 4 d after calving. This suggests that the metabolic demands of lactation are driving the rapid restoration of feed intake postcalving.

Lactation

The energy demands of lactation often result in a negative net energy balance that remains until 16 wk of lactation (Bauman and Currie, 1980). Brody (1956) reported that at peak lactation, heat production was twice that observed in the dry cow. The increased heat production is partly a result of the increase in energetically expensive organ mass that is required for lactation (Smith and Baldwin, 1974). Obviously, the mammary gland increases in size with lactation (73%), but the liver, heart, and lungs are also 22 to 25% larger in lactation than in dry animals. Indeed, the metabolic demands of the liver increase by 25%. In addition, lactation increases digestive tract mass and energy expenditure by 29 and 28%, respectively. To meet the increased maintenance and lactation energy demands, dairy cows increase feed intake. In fact, DMI more than doubles from precalving to 2 wk postpartum and nearly triples by 7 wk postpartum, reaching maximal values at 12 wk of lactation (Bauman and Currie, 1980; Silanikove et al., 1997). This increase in feed intake is associated with an increased number of meals and simultaneous decrease in total feeding time (Huzzey et al., 2005).

Environmental Cold Exposure

Cold exposure increases the energy expenditure necessary for the cow to maintain homeothermy and, in turn, drives increases in both energy expenditure and feed intake. Brody (1956) defined the "comfort zone" as the temperature at which no demands are made on the temperature-regulating mechanism and identified this to range between −1 and 15°C (30 and 60°F) in European cattle (Brody, 1956). Kibler and Brody (1949) demonstrated that a decrease in environmental temperature from 10 to −15°C (50 to 5°F) increased heat production 30 to 35% in lactating Jersey cows and 20 to 30% in lactating Holstein cows. The more robust increase in energy expenditure in the Jersey cow may be a result of smaller body size or decreased heat of lactation. The metabolic stimulator thyroxine (**T4**) increases in response to cold exposure and decreases with an increase in temperature (Yousef and Johnson, 1966). This increase in T4 is a mechanism to enhance endothermy. Cows that have higher milk production have a less robust increase in T4 at 15°C, which suggests that high-producing dairy cows with high levels of body heat production are not as reliant on T4 for stimulation of metabolic rate in response to a cold environment (Johnson and Vanjonack, 1976). Of note, if cold-exposed cows are not allowed to become hyperphagic in response to a decrease in environmental temperature, the body adapts with high levels of thyroxine to further stimulate metabolic rate. The increase in metabolic rate associated with cold exposure is evident in the metabolic data from calves reared in a cold environment, which have increased serum nonesterified fatty acids and decreased serum glucose, despite consuming more feed.

Environmental Heat Exposure

Heat has been one of the primary stressors evaluated in dairy science, as the high metabolic demand and associated endothermy makes high-producing dairy cows uniquely sensitive to heat-induced depression of production. In fact, a temperature-humidity index (**THI**) as low as 78 decreases DMI (Cowley et al., 2015). The decrease in feed intake with heat exposure is likely an adaptive mechanism to decrease heat of digestion and the heat of metabolism (Thompson et al., 1963; Abilay et al., 1975; Magdub et al., 1982). The decrease in feed intake depends on the duration and severity of heat

exposure (Kibler and Brody, 1949; Brody, 1956; Ominski et al., 2002). Ominski et al. (2002) showed that 5 d of heat exposure decreased DMI during heat exposure and continued to suppress feed intake for 5 d under thermoneutral conditions. Interestingly, the THI 2 d prior appears to most robustly affect feed intake (West et al., 2003), which is in agreement with a previous report by Collier et al. (1981a), that afternoon black globe temperature 2 d prior had the greatest effect on reduced milk yield. Although the decrease in feed intake associated with heat exposure has been reported to be 60 to 70% of that in thermoneutral cows (McDowell et al., 1969; Tao and Dahl, 2013), the hyperthermia experienced by the cow is best able to predict the degree of hypophagia. In fact, feed intake is negatively correlated with rectal temperature (Maust et al., 1972; Abilay et al., 1975). This decrease in feed intake plays a primary role in the depression of milk production during heat stress (Wayman et al., 1962; Clark et al., 1972). Accordingly, researchers have focused on physiological adaptations and cooling strategies that may restore feeding.

In response to heat exposure, cows physiologically adapt to depress their heat production and increase heat loss. We have already described how a decrease in feed intake can decrease the heat of digestion. Additionally, because decreasing feed intake depresses milk production, this adaptation also serves to depress the heat of lactation. Heat-stressed cows also upregulate the production of β-hydroxybutyrate, which binds to GPR109a, the niacin receptor, to induce peripheral vasodilation and enhance evaporative heat loss (Di Costanzo et al., 1997). A decrease in T4 with environmental heat exposure limits the metabolic rate stimulation and heat production of heat-stressed cows (Thompson et al., 1963; Magdub et al., 1982). Finally, the cow increases respiration rate to encourage evaporative heat loss (McDowell et al., 1969; Collier et al., 1981a). This increase in respiration rate is among the most sensitive phenotypic indicators of heat stress; a respiration rate >60 breaths per minute is an indicator of heat stress in lactating dairy cows (Shultz, 1984; Berman et al., 1985).

In heat-stressed animals, milk energy output decreases twice as much as digestible energy intake (McDowell et al., 1969). This was confirmed by Wheelock et al. (2010), who demonstrated that only half of the decrease in milk yield could be accounted for by decreases in feed intake. Although milk yield is decreased, the basal metabolic requirements are increased during thermal stress despite a decrease in T4 (Kibler and Brody, 1949, Brody, 1956)

The physiological consequences of heat exposure can be mitigated through management practices that directly cool the dairy cow (Berry et al., 1964). Cooling heat-exposed cows increases feed intake (do Amaral et al., 2009; Tao et al., 2012a). Cooling dry cows increases the gestation length and calf weight at birth and weaning (Tao et al., 2012b). Many strategies to cool dairy cows efficiently have been investigated. Cooling only the head and neck improves feed intake while decreasing body temperature and respiration rate (Roussel and Beatty, 1970). Similarly, cooling the bedding to encourage conductive cooling when the cow is lying down decreases temperature and respiration rate while increasing DMI (Perano et al., 2015; Ortiz et al., 2015). In fact, simply providing bedding materials that remain cooler increases feed intake and time spent resting (Ortiz et al., 2015). Sprinklers that wet the animal, along with ventilation to increase airflow, encourage evaporative cooling and increase feed intake while decreasing water intake (Flamenbaum et al., 1995; Schütz et al., 2011). Although sprinkling with water is effective at lowering body temperature, cows prefer shade (Schütz et al., 2011). Shaded heifers have higher feed intake and better ADG than those that were sprinkled for cooling (Marcillac-Embertson et al., 2009). In cows on pasture, shade availability increases the time spent grazing and the time spent lying (Palacio et al., 2015). Of note, rumen contractions are increased in shaded animals, which may explain the improved feed intake associated with each of these cooling strategies (Collier et al., 1981b).

Health Challenge

Immune challenges are well established to depress feed intake across species. A lipopolysaccharide-induced immune challenge dose-dependently decreases feed intake in the dairy cow up to nearly 50% (Waldron et al., 2003). In the dairy cow, mastitis and metritis are immune challenges that occur at high incidence. Mastitis occurs in 10 to 24% of dairy cows, whereas clinical metritis has been reported to occur in 5.3 to 12.7% of dairy cows (Wittrock et al., 2011; Ribeiro et al., 2013; Levison et al., 2016). Inclusion of subclinical metritis increases the incidence of metritis to over 40% (Wittrock et al., 2011). Both mastitis and metritis decrease DMI in relation to severity of infection (Huzzey et al., 2007; Lukas et al., 2008). Severe metritis can depress feed intake by up to 40%, whereas mild metritis results in an 18% reduction in feed intake (Huzzey et al., 2007). The decrease in DMI may be related to a decrease in feeding time observed in metritic cows (Huzzey et al.,

2007). Lameness, another common malady in dairy cows, similarly decreases feeding time and DMI when lameness scores meet or exceed 4 on a scale of 5 (Norring et al., 2014).

Social Stress

Social stresses in herd animals such as cattle may include isolation, introduction to a novel group, or limited feed bunk availability. A detailed review of stresses due to housing and other welfare-related situations can be found in this issue (von Keyserlingk and Weary, 2017). Putting a calf into a new group of calves decreases milk intake on the day of introduction (O'Driscoll et al., 2006). However, this effect is acute, as feed intake is not affected by this change in social interaction in the 3 d following mixing. The decrease in milk intake is associated with a decrease in the number of meals per day and the time on teat. However, there is some compensatory response to the decreased number of meals: intake/meal and feeding rate (kg/h) are increased. Similarly, putting a cow into a novel group depresses DMI on the day of introduction but not thereafter (Schirmann et al., 2011). This decrease in feed intake is not a result of decreased time at the feed bunk (von Keyserlingk et al., 2008). Feed bunk availability may also affect time spent at the feed bunk. In fact, decreasing feed bunk space from 1 to 0.5 m/cow decreased the total time spent feeding by 14% and increased the number of aggressive interactions. Furthermore, decreasing bunk space limits the time spent feeding immediately after fresh feed provision by 24% (DeVries et al., 2004; Huzzey et al., 2006). A more severe reduction in bunk space results in a further decrease in time spent at the bunk and a more significant relationship between dominance and time spent at the bunk (Friend et al., 1977; Huzzey et al., 2006). Despite these changes in feeding behavior, only one study has shown that limiting bunk space depresses daily DMI and that occurred only when cows were provided 0.1 m of bunk space/cow (Friend et al., 1977). Studies with direct competition for a single feeding space have shown that sharing a feeding space increases feeding rate without altering daily DMI (Olofsson, 1999; Hosseinkhani et al., 2008). This suggests that cows adapt their feeding behavior to accommodate a limiting feeding space until the restriction is so severe that it limits feed access throughout the day. Social stressors do alter feed intake in both calves and cows. However, these effects appear to be acute and are not likely to robustly affect production. Social dominance has also been shown to affect feeding behavior, growth rate, metabolic status, and age at onset of puberty (Fiol et al., 2017).

EFFECTS OF STRESS ON METABOLISM

Several potential metabolic health problems and related interactions of stress and nutrition can arise from physiological responses to stress. Energy and nutrient deficits, respiratory alkalosis, ketosis, and ruminal acidosis are some examples of alterations in metabolism associated with heat stress–induced alterations in nutrient metabolism and balance. Nutritional metabolic changes include alteration in feed consumption and energy and protein metabolism (Baumgard and Rhoads, 2013), water balance, metabolism of electrolytes and associated acid–base imbalance (Beede and Collier, 1986), and endocrine status (Collier et al., 1982b; Rhoads et al., 2009, 2010; Baumgard et al., 2011). Dale and Brody (1954) conducted 2 experiments to study the impact of reduced feed intake during thermal stress on metabolic state. In the first experiment, feed consumption of cows maintained at environmental temperatures above 27°C was depressed, thus setting the metabolic circumstances for body fat catabolism. However, these cows did not develop ketosis or even ketonuria. Blood ketone concentrations were actually lower as environmental temperatures increased from 7 to 27°C, and total urine ketone bodies were not affected. In a second experiment, 4 cows were fasted for 5 d at 18°C (thermal comfort). In contrast to results of the first experiment, blood and urine ketone concentrations increased dramatically after the second day of fasting, suggesting increased fat depot mobilization and incomplete fat oxidation. These 2 experiments emphasized an apparent difference in energy metabolism of animals experiencing similar dramatic reductions in feed intake and carbohydrate oxidation but responding differently, possibly because of differences in general metabolic and physiological state (thermal stress vs. starvation). Later work by Wheelock et al. (2010), Baumgard et al. (2011), and Rhoads et al. (2009, 2011) confirmed these observations and extended these differences to alterations in insulin secretion and clearance and gluconeogenic genes in liver of heat-stressed cows. They proposed that elevated insulin during periods of thermal stress suppresses adipose tissue fatty acid mobilization and is associated with increased catabolism of protein for glucose (Baumgard and Rhoads, 2013). O'Brien et al. (2010) extended these observations to growing cattle, demonstrating that heat stress alters postabsorptive carbohydrate (basal and stimulated) metabolism, characterized primarily by increased basal insulin concentrations and insulin response to a glucose challenge. However, heat stress–induced reduction in feed intake appeared to fully explain decreased ADG in Holstein bull calves. There is currently no mechanism

to explain increased insulin secretion during heat stress in growing and lactating cattle. Recently, Baumgard and Rhoads (2013) proposed that heat stress impairs gut integrity, leading to influx of endotoxins that could cause some of the metabolic effects detected in heat-stressed dairy cows. Additionally, there is scant information on effects of other stressors on metabolism.

IMPACTS OF STRESS ON REPRODUCTION IN DAIRY CATTLE

Cattle, like most other mammals, have to be bred and deliver calves to initiate and maintain normal lactation. Keeping ideal reproductive performance is important for maximizing the efficiency of milk production in dairy cows. The reproductive processes include production of sperm in males, production of oocytes in females, and embryonic and fetal development. These processes are susceptible to stressors such as heat, cold, handling, and so forth. Heat stress is the best characterized stress and has the most severe impacts on reproductive performance in dairy cattle among all the physiological stressors.

Fertility

In early studies, the first identified stressor affecting fertility of dairy cows was considered the effect of season. Morgan and Davis (1938) reported the first study on seasonal effects on breeding efficiency in dairy cattle. They went through records of the University of Nebraska dairy herd from 1896 to 1934. The breeding records covered 2,090 cows from 5 dairy breeds, and the ages of cows and bulls ranged from <2 to 18 yr. They found that more services were required for conception during the summer season (May to October) than the winter season (November to April) for services between bulls of all ages and cows of all ages. Conception rates were lower during August and September than in other months. Erb et al. (1940) investigated breeding records of the Purdue University dairy herd from 1920 to 1940, and found that the herd had the lowest breeding efficiency during August and highest efficiency in May. Seath and Staples (1941) at Louisiana State University also demonstrated that both of the north and south Louisiana herds required more services per conception in summer than that in other seasons. However, the study conducted by Mercier and Salisbury (1947a,b) at Cornell University demonstrated that the poorest season for breeding dairy cows in eastern Canada and New York State was winter, indicating the presence of adverse effects of cold stress on fertility. These early studies and a comprehensive review (Vincent, 1972) suggested that the impacts of heat and cold stress on fertility of dairy cattle might associate with climates of the region. It may also represent poor ability to detect estrus in stanchion barns during winter months when movement of cows was greatly restricted.

Instead of using seasons to define environmental conditions, Stott and Williams (1962), from the University of Arizona, presented that a lengthened estrous cycle and lowered breeding efficiency in dairy cows were associated with the high temperature and humidity in summer. Ingraham et al. (1974), from Iowa State University, evaluated conception rates of dairy cows in a subtropical climate using THI as an indicator. They reported that conception rate decreased from 55 to 10% as the THI of the second day before breeding increased from 70 to 84. Gwazdauskas et al. (1975), from the University of Florida, selected the 5 most important factors affecting conception rate from 21 climatological parameters: maximum temperature day after insemination, rainfall day of insemination, minimum temperature day of insemination, solar radiation day of insemination, and minimum temperature day after insemination. These defined environmental indices or factors were helpful not only in recognizing the magnitude of environmental stress on fertility of dairy cows, but also in developing strategies for alleviating the impacts. To that end, scientists in the field began to identify which reproductive processes were affected by these stressors and how.

Sperm Production in Dairy Bulls

To dissect effects of environmental stress, researchers initially focused on investigating these impacts on fertility of bulls, and Anderson (1941) reported low volume and sperm motility of semen collected from 5 breeds of bulls from May to August in Kenya. Erb et al. (1942) systematically investigated effect of season on semen quality of the dairy bulls. They found that semen volume, sperm concentration, sperm motility, and sperm survival were the lowest and the abnormal sperm number was the greatest in summer months. Their studies indicated that semen production in dairy bulls was impaired during summer. In an attempt to test whether environmental temperatures were involved in the seasonal impacts on semen quality, Casady et al. (1953) studied the reproductive activity of dairy bulls exposed to high ambient temperatures using climate-control chambers. When ambient temperature in the chambers increased to 32.2°C or higher, they found that bulls showed typical stress symptoms, such as increase in respiration, decrease in body weight, restlessness, and excess salivation. Continuous exposure to high ambient

temperatures led to decreases in sperm concentration, total count, and motility in the collected ejaculates and adversely affected sperm production in young bulls. With the aid of climate chambers, de Alba and Riera (1966) demonstrated that high ambient temperature lowered semen quality and delayed puberty in Jersey calves. These studies suggested that high temperatures in summer could cause heat stress and impair semen quality. With the emergence of AI, frozen semen techniques, and use of climate-controlled barns for bulls, these negative effects of stress on sperm production in bulls were eliminated in AI semen. However, these effects are still a major issue when bulls are used on farm.

Estrus, Reproductive Hormone Secretion, and Follicle Dynamic in Dairy Cows

Environmental stress is also deleterious to reproductive performance in females, perhaps even more severe than in males. Stott (1961) studied 2 sets of breeding records: (1) Jerseys, Holsteins, and Guernseys inseminated in Arizona with semen collected in California, Ohio, and Arizona; and (2) cows inseminated inside or outside Arizona at the same time of summer with semen from the same bull in California. He found that, regardless of the source of semen, the Arizona cows demonstrated a substantial decrease in breeding efficiency during summer months; moreover, cows bred outside Arizona had a higher fertility level than those bred in the state using the same semen. He concluded that heat stress effects on females were the major contributor to depressed fertility in summer.

Maintaining normal estrous cycles in dairy cows is essential to control calving intervals, which eventually affects days in milk. Estrus could also be negatively affected by environmental stressors. Hall et al. (1959) observed 1,460 estrous periods of 270 cows and heifers in a 15-mo period. By comparing the observations reported from the temperate regions, they found lengthened estrous cycles, shortened estrus duration, and decreased estrus intensity in dairy cattle under Louisiana's hot and humid climate. This environmental effect was later confirmed under climatic controlled conditions (Gangwar et al., 1965).

Estrus is driven by reproductive hormone fluctuations, and hormonal activity regulates follicular waves in ovaries during each estrous cycle. Adverse effects of environmental stressor such as elevated ambient temperature on reproductive hormones and follicular dynamic were described in dairy cows as early as the 1970s. Madan and Johnson (1973) investigated effect of thermal stress on the pattern of plasma luteinizing hormone (LH), which is involved in follicle development and ovulation. Guernsey heifers with synchronous estrous cycles were placed in 2 climatic controlled environments (control and heat stress) for 2 consecutive estrous cycles in each environment. The LH in plasma was monitored daily, and the results showed distinctive depression in LH at the LH surge phase of both estrous cycles when heifers were exposed to heat stress conditions. Later, the same laboratory published that heat stress elevated progesterone levels of the first heat-exposed estrous cycle but not the second one in heifers (Abilay et al., 1975). Gwazdauskas et al. (1981) induced synchronized estrus in Holstein heifers with prostaglandin $F_{2\alpha}$, and then measured their hormonal patterns when they were placed in environmental chambers with either thermoneutral or heat stress conditions. The results demonstrated that a decrease in plasma estradiol levels was associated with the heat stress exposure; however, the progesterone in heat-stressed heifers was comparable to that in thermoneutral heifers. William Thatcher's group from the University of Florida described the connections between altered hormonal secretion and follicular development during summer heat stress (Badinga et al., 1993). Lactating cows were assigned to shade with sprinkler-fan cooling system or no shade management system after estrous synchronization. The cows with no shade had smaller first-wave dominant follicles and less follicular fluid in volume at d 8 of the estrous cycle. In contrast, the subordinate follicles were larger and contained more fluid in cows with no shade, which suggested altered follicular dominance in heat-stressed cows. Estradiol in plasma and the first-wave dominant follicle fluids were decreased from the beginning of summer (July) to the end (September) in the study. Research teams led by M. C. Lucy from the University of Missouri conducted a series of studies focusing on effects of heat stress on serum estradiol levels and the second follicular wave in lactating cows and heifers (Wilson et al., 1998a,b). When comparing lactating cows and heifers in a thermoneutral chamber, heat-stressed animals showed decreased estradiol from d 11 to 21 of the estrous cycle, delayed corpus luteum regression in the proestrus period, and decreased ovulation rate of the second-wave dominant follicles. Collectively, these studies suggested a regulatory axis that heat stress impaired estradiol secretion, which hindered luteolysis, and eventually altered follicle dominance and disrupted ovulation.

Effect of heat stress on other follicular-development-associated hormones, such as inhibin, FSH, and androstenedione, were also addressed. An experiment conducted in Israel by Wolfenson et al. (1995) demonstrated that heat stress tended to depress plasma inhibin in heat-stressed lactating cows, and also altered

the dominance of the first- and second-wave dominant follicles. To evaluate effect of heat stress on follicular functions, Wolfenson et al. (1997) determined hormone production of follicular theca and granulosa cells collected from follicles of the cows in summer, autumn, and winter under thermoneutral and heat-shock culture conditions. Results indicated that estradiol in follicular fluid was lower during fall and winter in cows that were stressed during summer months but was higher in fall and winter in cows that were housed under thermoneutral conditions during summer months. The capacity to produce androstenedione had the same pattern in the theca cells isolated from follicles in different seasonal or heat stress conditions. This study demonstrated adverse effects of heat stress on steroidogenic capacity of follicles, but also suggested delayed effects of heat stress on ovarian functions in dairy cows. Subsequently, the delayed heat stress effect was observed on follicle dynamics in a later study (Roth et al., 2000), and the authors suggested that FSH secretion was upregulated because of downregulated inhibin in plasma in heat-stressed cows, resulting in altered follicular dominance and increased incidence of twinning.

Handling related stressors can also cause alterations in reproductive hormones. Thun et al. (1998) investigated hormonal responses of 5 lactating cows under restraint stress, and found that plasma progesterone and LH were increased by a 2-h immobilization. Mann (2001) reported that stress induced by AI and blood collection increased plasma estradiol at the end of follicular phase and reduced pregnancy rates in dairy cows. However, Szenci et al. (2011) showed that 2 h of restraint of pregnant heifers at d 30 to 40 of gestation did not affect progesterone levels. Therefore, effects of handling stressors may vary due to type of stress and physiological status of dairy cows.

Oocyte Maturation, Fertilization, and Embryonic and Fetal Development in Dairy Cattle

Because follicular function could be negatively influenced by stress as described above, impairments on oocyte development and maturation within the affected follicle could be expected. In vivo studies demonstrated that extreme hot or cold conditions during the days before or after insemination led to reduced conception rates in dairy cows (see reviews: Ulberg and Burfening, 1967; Gwazdauskas, 1985), suggesting that oocyte maturation, fertilization, and early embryonic development could be all impaired by environmental stressors. Researchers led by N. L. First (Lenz et al., 1983) examined in vitro maturation and fertilization of oocytes exposed to different temperatures. Oocytes incubated at high temperature (41°C) had lower nuclear maturation rates, which was likely associated with impaired cellular function of cumulus cells surrounding the oocytes. High (41°C) or low (35 or 37°C) temperature at maturation also decreased the probability of the oocyte being fertilized, and the fertilization process per se was temperature sensitive. Moreover, this study and a later study reported by Peter Hansen's group (Monterroso et al., 1995) from the University of Florida provided evidence that exposure to elevated temperatures in vitro had adverse effects on sperm collected from bovine epididymis or frozen semen, including impaired viability and motility. Collectively, these studies demonstrated that core temperature variation caused by heat or cold stress in dairy cows could be deleterious to fertility of inseminated sperm and oocyte maturation and fertilization.

To reproduce, a mature oocyte has to be fertilized by a sperm to become an embryo. It has been proven that embryonic development in dairy cows is also susceptible to environmental stress. Putney et al. (1988) performed superovulation in Holstein heifers followed by AI and then assigned heifers to either thermoneutral or heat stress chambers. The embryos recovered from heat-stressed heifers had lower percentage of normal quality and higher incidence of abnormal and delayed development compared with those from thermoneutral animals. Just like embryos of some other mammalian species, however, bovine embryos can gain the capacity to resist the effect of maternal heat stress during early development. Ealy et al. (1993) exposed Holstein cows at an unshaded lot in University of Florida Dairy Research Unit to induce heat stress on d 1, 3, 5, or 7 of pregnancy. Embryonic survival analysis of the embryos recovered on d 8 showed that maternal heat stress had a distinctive negative effect on d 1 of pregnancy but not on days after d 3, suggesting that susceptibility of bovine embryos for heat stress was specific to the developmental stage. This gain of thermotolerance during early development was confirmed by a later in vitro study (Ealy and Hansen, 1994). Taking advantage of the in vitro culture system, the Hansen group demonstrated anti-apoptotic roles of IGF-1 and colony-stimulating factor 2 in increasing survival rates of heat-shocked embryos (Bonilla et al., 2011; Loureiro et al., 2011).

Heat stress during gestation reduced birth weights in rats and sheep (Cartwright and Thwaites, 1967; Benson and Morris, 1971; Brown et al., 1977). Heat stress during gestation also reduced placental weight (Alexander and Williams, 1971) and uterine blood flow (Oakes et al., 1976). The reduction in placental mass or placental function has significant implications for the maternal system (Tao and Dahl, 2013). Altered placental func-

tion or size may result in altered endocrine dynamics during pregnancy. Several studies have related placental mass or hormone secretion by the placenta to mammary growth or postpartum milk yield (Desjardins et al., 1968; Bolander et al., 1976; Eley et al., 1981). Thus, altered placental function may influence the extent of mammary growth. Pregnant rats subjected to heat stress during the last two-thirds of gestation gave birth to pups of reduced weight and exhibited impaired lactation (Benson and Morris, 1971). Collier et al. (1982b) assigned lactating Holstein cows and heifers to shade or no shade treatments during the dry period beginning in June. Calves born from no shade cows weighed less than those born from cows with shade, and shaded cows produced more milk in the next lactation. Hormonal analysis of the plasma collected from cows during treatment period demonstrated that concentrations of estrone sulfate were lowered by heat exposure. These data demonstrated that heat stress exerted adverse effects on fetal and mammary development by disrupting placental function during pregnancy. Effects of heat stress effects during pregnancy on reproductive performance in the same study were also reported (Lewis et al., 1984). The effects of heat stress during the dry period on postpartum performance of the dam were confirmed by several investigators (reviewed in Tao and Dahl, 2013). Dahl and coworkers have extended the known impacts of heat stress during pregnancy to include effects on growth and survival of the neonate as well as lifetime performance (Tao and Dahl, 2013).

LOOKING AHEAD

It is clear that producers and consumers are increasingly concerned about the welfare of food animals, and producers know that stressing animals reduces the profitability of their operations. Thus, there will be growing interest in identifying and reducing stressors on dairy farms. Key to continued progress in managing stress on farms will be identification of improved measures of stress that can be applied under practical commercial farming conditions. For example, improvement of the THI to contain specific physiological endpoints for various levels of thermal stress is one example. Biological endpoints such as changes in milk composition may also offer the opportunity to identify impending health issues before their appearance, permitting preventative health interventions. Identifying animal behaviors that are associated with stress responses are also of critical importance. Education of producers to recognize these endpoints will help them to adjust management conditions appropriately to improve welfare of animals on their operations. A better understanding of the regulatory mechanisms that are responsible for metabolic responses to stress will also lead to improved nutritional management of dairy animals.

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APPENDIX

Table A1. Timeline of key events in stress physiology

Date	Milestone	Reference
1938	Fasting energy metabolism during lactation established.	Washburn, 1938
1944	Seasonal variation in semen quality identified.	Swanson and Herman, 1944
1953	Heat stress adversely affects spermatogenesis in the bull.	Casady et al., 1953
1954	Heat-stressed cows in negative energy balance fail to develop ketosis.	Dale and Brody, 1954
1956	Breed differences in response to climate reported.	Brody, 1956
1958	Effect of certain acute stress conditions on the plasma levels of 17-hydroxycorticosteroids.	Robertson et al., 1958
1961	Lower critical temperature for dairy cattle established.	Kleiber, 1961
1961	Fire of Life published.	Kleiber, 1961
1961	Upper critical temperature for dairy cattle published.	Berman et al., 1963
1962	Causes of low breeding efficiency in dairy cattle are associated with seasonal high temperatures.	Stott and Williams, 1962
1963	Recognition of management relationship to stress in animals.	
1964	Temperature-humidity index (THI) established.	Berry et al., 1964
1980	Concept of homeorhesis defined.	Bauman and Currie, 1980
1982	Heat stress during dry period reduces calf birthweight and postpartum milk yield of the dam.	Collier et al., 1982b
1991	Behavioral aspects of stress are defined.	Friend, 1991
1994	THI categorized into mild, moderate, and severe.	Armstrong and Wiersma, 1994
1994	Effects of heat stress on embryo development are elucidated.	Ealy and Hansen, 1994
2010	Carbohydrate and fat metabolism are altered during heat stress,	Wheelock et al., 2010
2013	Dry period heat stress effects reported to extend to neonates.	Tao and Dahl, 2013
2013	Effects of heat stress on gut integrity and metabolism are shown.	Baumgard and Rhoads, 2013

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A 100-Year Review: Regulation of nutrient partitioning to support lactation[1]

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ABSTRACT

We have seen remarkable advances in animal productivity in the last 75 years, with annual milk yield per cow increasing over 4-fold and no evidence of nearing a plateau. Because of these gains in productive efficiency, there have been dramatic reductions in resource inputs and the carbon footprint per unit of milk produced. The primary source for the historic gains relates to animal variation in nutrient partitioning. The regulation of nutrient use for productive functions has the overall goal of maintaining the cow's well-being regardless of the physiological or environmental challenges. From a conceptual standpoint, it involves both acute homeostatic controls operating on a minute-by-minute basis and chronic homeorhetic controls operating on a long-term basis to provide orchestrated adaptations that coordinate tissues and body processes. This endocrine regulation is mediated by changes in circulating anabolic and catabolic hormones, hormone membrane receptors and intracellular signaling pathways. The coordination of tissues and physiological systems includes a plethora of hormones, but insulin and somatotropin are 2 key regulators of nutrient trafficking. Herein, we review the advances in our understanding of both conceptual and actual regulation of nutrient partitioning in support of milk synthesis and identify examples of the challenges and future opportunities in dairy science.
Key words: homeorhesis, homeostasis, somatotropin, insulin, metabolic regulation

INTRODUCTION

Domesticating dairy animals played a critical role in the development of human society. Dairy products were recognized as nutritious foods as early as 4,000 BC, and today milk and dairy products are key components of dietary recommendations by governmental agencies and public health organizations around the world. Cow's milk contains more of the essential vitamins and minerals required by humans than any other single food (Patton, 2004). Advances in lactation physiology during the last century have increased our understanding of the biological processes that allow dairy cows to use feed nutrients for the biosynthesis of milk (Appendix Table A1). Annual milk yield per cow was relatively constant over the first part of the 20th Century. However, beginning in the early 1940s, the application of scientific principles to nutrition, management, and genetics initiated a progressive improvement in milk production that continues to this day. Whereas annual milk per cow averaged about 2,000 kg from the 1920s through the early 1940s, the US dairy herd currently annually averages over 10,000 kg (Figure 1). Indeed, the annual herd average on some US dairy farms is >14,000 kg of milk per cow, and the current US record holder is a Wisconsin cow named Ever-Green-View My Gold-ET ("My Gold"), who had a 365-d milk production of 35,144 kg (906 kg of fat, 934 kg of true protein; http://www.dairyherd.com/news/industry/new-national-milk-production-record-set). Equally impressive, the Guinness World Records recently recognized a 15-yr-old Canadian cow, Guillette E Smurf, as the lifetime record holder in milk production; Smurf's production of 216,891 kg in 10 lactations represents an impressive average of over 38 kg of milk for every day of life (http://www.guinnessworldrecords.com/world-records/greatest-milk-yield-by-a-cow-%E2%80%93-lifetime).

Historic gains in milk yield originate partly from selection and genetic improvement (50–66%) and the remainder from advances in nutrition and management (VanRaden, 2004; Shook, 2006). Progress from applying genetic selection requires sound practices in nutrition and overall management for a cow to achieve her production potential. Likewise, production advances resulting from technology developed from basic dairy cow biology require an adequate genetic base. Examples explaining the historic gains include a better understanding of nutrient requirements, improvements in diet formulation and mixing, utilizing AI and

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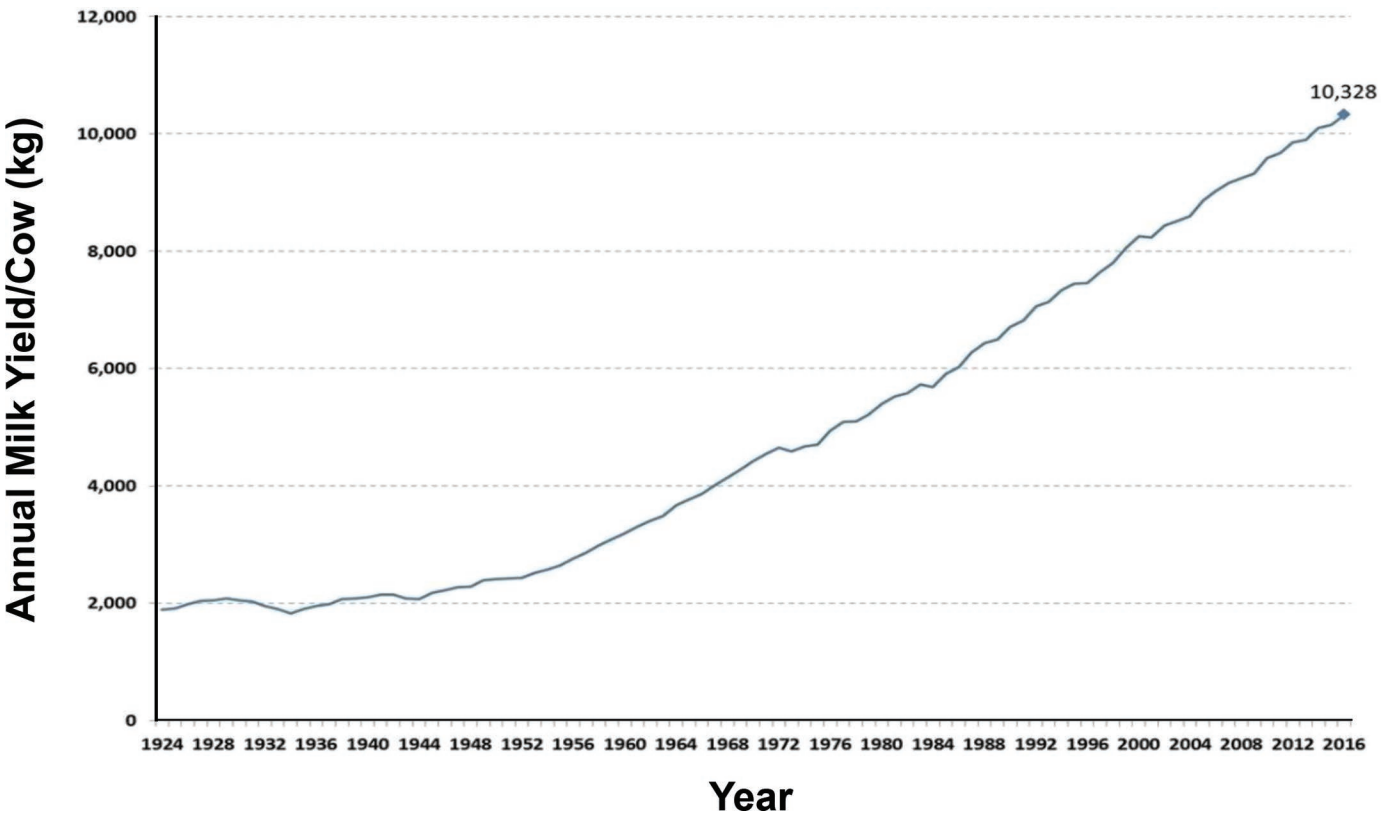

Figure 1. Average annual milk production in the United States per cow (https://quickstats.usda.nass/gov/; accessed February 20, 2017). Color version available online.

applying more accurate genetic selection methods, improved milking management practices and mastitis control, and the effective use of herd health programs to prevent disease (Collier et al., 2005). Furthermore, new technologies and management tools such as estrus synchronization, pregnancy detection, and bovine somatotropin have enhanced the production potential of dairy cows and allowed them to more nearly achieve their genetic capacity.

Lactation represents a substantial reorganization in the hierarchy of nutrient partitioning, and a dairy cow's metabolism is exquisitely coordinated to support the metabolic demands of milk synthesis. The mammary gland's synthetic capacity is so impressive that in the context of nutrient use and metabolism, Brown (1969) proposed the cow should be considered as an appendage to the udder, rather than vice versa. Herein, we will consider the cycle of life and advances in productive efficiency, review the broad concepts of regulation, and characterize specific tissue adaptations and mechanisms to support lactation (Appendix Table A1). Finally, we will speculate on upcoming challenges and opportunities for future discoveries in productive efficiency in dairy cows.

PRODUCTIVE EFFICIENCY

The continued increase in dairy cow productivity is a key component for the sustainability of the global dairy industry. Productivity or productive efficiency, defined as milk output per resource input, is improved as milk yield increases (Bauman et al., 1985; VandeHaar and St-Pierre, 2006). When the increase in milk yield/cow during the last 75 years is combined with improved crop productivity, feed use by the US dairy herd per unit of milk produced has been reduced by about 80% and carbon footprint has decreased by two-thirds (Capper and Bauman, 2013). Animal biology includes a series of processes in which dietary feed components are transformed and used to support body tissues and activities (Kleiber, 1961). Feed is consumed and digested products are assimilated and partitioned in a process governed by a physiological echelon; meeting maintenance requirements is top priority and secondary uses of absorbed nutrients are for productive functions such as milk synthesis or fetal development. Further, on a short-term basis, body reserves can be replenished or mobilized to support the hierarchical goals of nutrient trafficking.

Sources of Variation

What are the sources of variation that have allowed for the improvements in productive efficiency over the last century? Which "cycle of life" processes have been altered by the remarkable increase in productive efficiency of dairy cows? Evaluating the processes of digestion and nutrient absorption, maintenance requirements, and the partial efficiency of nutrient use for productive functions (e.g., milk synthesis) indicates that these biological processes are only minor sources of the variation among animals, and alterations in them have contributed little to the annual gains in performance and productive efficiency (Bauman et al., 1985; Gordon et al., 1995; Agnew and Yan, 2000; Reynolds, 2004; Moraes et al., 2015). In contrast, differences in nutrient partitioning represent the primary reason for cow-to-cow variation and the major source of annual improvement in milk production. High-yielding cows direct a greater portion of absorbed nutrients to the mammary gland for milk synthesis and, associated with this, they have a greater voluntary feed intake. Low-yielding cows have a lower feed intake: if they do consume more feed, they use it for excessive body fat accretion rather than milk synthesis. This nicely illustrates that increased nutrient consumption is the result of enhanced productivity (i.e., increased milk synthesis drives feed intake). Thus, selection for high milk production results in dairy cows that not only utilize more nutrients for milk synthesis but also voluntarily consume more feed to support the demands of copious milk production (Bauman et al., 1985; Reynolds, 2004). To reiterate, during the last century, we have improved our understanding and definition of nutrient requirements, but only minimal changes have occurred in the extent of digestibility, the actual requirements for maintenance, or the efficiency of synthesizing a unit of milk.

High-producing cows have a greater productive efficiency because a larger portion of total nutrient intake is used to synthesize milk. Nutrient use for maintenance represents a fixed cost, and the effect of this on productive efficiency is illustrated by calculations based on historical milk yield. In 1944, about 69% of the average cow's ME requirement was used for maintenance and approximately 31% used for milk synthesis. This contrasts to 2016 where the proportion used for maintenance has declined to about 35% of the ME requirement and the amount used for milk synthesis has more than doubled (~65%). Even more impressive, during her record lactation, "My Gold" (the current world-record holder) utilized about 16% of her ME requirement for maintenance and over 84% for milk synthesis. Thus, as milk yield per cow increases, the fixed cost of meeting the cow's maintenance requirement is proportionally reduced (Bauman et al., 1985; VandeHaar and St-Pierre, 2006; Gerber et al., 2011). This is a phenomenon generally referred to as "dilution of maintenance" and is usually considered in terms of feed resources per unit of milk, but the sustainability advantages of diluting maintenance also apply more broadly to all input costs of producing milk, including renewable and nonrenewable resources as well as the costs for facilities and labor. Consequently, diluting maintenance costs is a key component to the annual improvements in milk production, sustainability of the dairy industry, and farm profitability.

Productivity and Stress

With each advance in dairy production over the last century, some have expressed concern that cows are being "pushed too far," thereby causing metabolic stress and compromising cow health and well-being. Over 50 years ago, Sir John Hammond evaluated this claim in his review of lactational physiology and found no support for the concern. Hammond concluded, "the physiological limits to intensive milk production are... only limited by our knowledge concerning the specific nutrients required for milk production" (Hammond, 1952). Subsequent re-evaluations of the biological limits of milk production by Bauman et al. (1985), Knight et al. (2004), and Reynolds (2004) reached conclusions similar to those of Hammond and, indeed, productivity has increased at a consistent annual rate of about 140 kg/cow during the last half-century. Ostensibly, a plateau exists where the biological controls and management practices supporting lactation are maximized, but there is no evidence that we are nearing it (Figure 1). The current world record of >35,000 kg of annual production indicates the potential upper limit and suggests that future herd averages may be >3-fold higher than today's average.

Two main arguments are often proposed for reducing the rate or even stopping gains in farm animal efficiency: (1) that high production and increased productive efficiency are contradictory to the concept of "sustainable" agriculture; and (2) that increasing milk yield is contrary to cow welfare and well-being. For some, the concepts of food production efficiency and environmental sustainability are perceived to be mutually exclusive, as discussed by Roche and Edmeades (2004). Similarly, in a review of dairy cow welfare, some have argued that it is necessary to stop using genetic selection and conventional nutritional management and nutrition practices to increase milk yield because these approaches have resulted in stressed cows, in which normal biological controls are overtaxed (Broom, 1999; Oltenacu and Broom, 2010). When these arguments

are examined more closely, it is apparent that in fact the opposite is true. First, life-cycle analysis of dairy farms has demonstrated that increasing productivity is a major component of improving both sustainability and profitability (Gerber et al., 2011; Capper and Bauman, 2013; FAO, 2013). Second, advancements in the application of genetic selection techniques and management improvements are successful and sustainable because they have altered the biological controls in a coordinated manner (Hammond, 1952; Bauman et al., 1985; Knight et al., 2004; Reynolds, 2004). For any system to be truly sustainable it must, by definition, be efficient, and today, dairy herds with high production also have excellent fertility, low somatic cell counts, and minimal metabolic problems (Santos et al., 2010; Bauman and Capper, 2011; Ferguson and Skidmore, 2013). Rather than the biological controls of high-producing cows being at discord with increased performance, it is the improvements in biological control systems and management practices and the presence of minimal stress that allow for the increases in milk yield and gains in productive efficiency. In fact, it is only when the coordination of nutrient use is inadequate or an imbalance occurs that animal well-being and performance are compromised. The claim that "high production is stressful" is an oxymoron; optimal and efficient milk production can be achieved only when stress is minimal or absent, whereas the presence of stress prevents maximal production because it increases maintenance costs and compromises well-being.

CONCEPTS OF REGULATION

The primary goal in the regulation of physiological processes is to maintain an animal's well-being regardless of the physiological situation or environmental challenges. Lactation is a physiological state in which regulation is of special importance because the mother must partition nutrients to support synthesis of milk, thereby ensuring the survival of her nursing neonate, and to maintain her own health and well-being. Thus, the control of nutrient partitioning and coordination of metabolism during lactation allows for the production of copious milk while also preserving dam well-being. A cow's well-being is dependent on acute regulation of nutrient use and biological processes every second. During lactation, there must also be a chronic level of regulation to ensure the mammary glands are provided an adequate supply of nutrients for milk synthesis. From a conceptual basis, homeostasis and homeorhesis represent the 2 types of regulation.

The concept of homeostasis was first crystallized by Cannon (1929) as the condition of relative uniformity that results from organismal adjustments to environmental changes. He emphasized that dynamic regulation and coordination were key features of homeostasis. From an operational standpoint, homeostatic controls operate on an acute moment-to-moment basis so that, despite challenges from the external environment, different tissues and organs are "all working cooperatively" to maintain physiological equilibrium. Numerous examples of the multiple compensatory mechanisms functioning to maintain physiological symmetry have been described in organisms, especially mammals. Homeostatic mechanisms include the regulation of vital functions such as maintenance of body temperature, blood pH, and body defense mechanisms. Many homeostatic mechanisms involve metabolic coordination, and examples include maintaining steady-state concentrations of key circulating nutrients, 2 of which (glucose and calcium) are especially important in lactating cows. In fact, glucose was one example used by Cannon (1929) to illustrate the concept of homeostasis. Glucose supply is of critical importance for many tissues and physiological processes, and the performance and health of the dairy cow is dependent on the maintenance of glucose homeostasis (Bell, 1995; Beever et al., 1999). Over the short term, homeostatic controls, primarily insulin and glucagon, maintain a relatively constant supply of glucose to peripheral tissues by promoting glucose storage following a meal and subsequent mobilization and catabolism from hepatic and muscle glycogen stores, respectively, during the postabsorptive period (Bauman and Elliot, 1983; Vernon and Sasaki, 1991). Thus, acute regulation of plasma glucose concentration by the reciprocal actions of insulin and glucagon ensures the proper balance in glucose supply and utilization by extra-mammary tissues in support of lactation.

The second broad type of biological regulation is homeorhesis, which was defined as the "orchestrated changes for priorities of a physiological state" (Bauman and Currie, 1980; Bauman, 2000). The concept originated from considering how physiological processes are regulated during pregnancy and lactation (Bauman and Currie, 1980), and was subsequently extended to growth (Bauman et al., 1982). Although not defined as such, Hammond (1947) clearly had this concept in mind when he emphasized how circulating nutrient utilization differs by tissue depending upon the physiological states. These physiological states represent biological situations where the orchestration involves most organs and physiological processes in the body and includes the metabolism of all macro- and micronutrient classes. Subsequently, the concept of homeorhesis has been extended to other life-cycle states and physiological situations, including pathological conditions where chronic adaptations in biological processes are required to achieve the physiological stability necessary

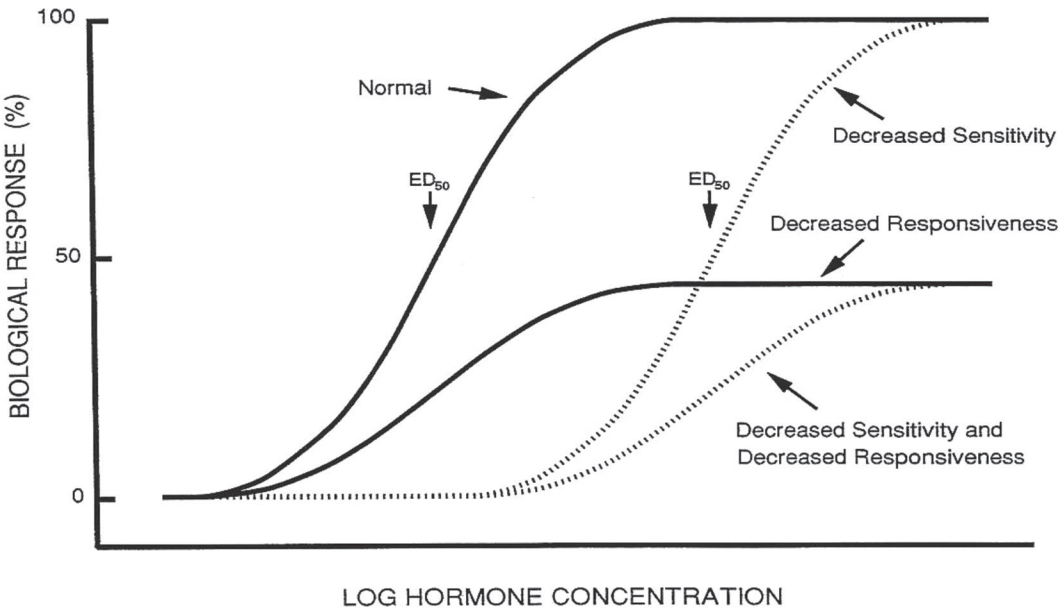

Figure 2. Schematic representation of changes in tissue response to hormonal signals, specifically changes in responsiveness (maximal response, R_{max}) or sensitivity (hormone concentration at half-maximal response, ED_{50}). Representation depicts a decrease in sensitivity, decrease in responsiveness, or decrease in both. Figure created by the authors based on a similar figure in Kahn (1978).

for survival (Bauman, 2000; Collier et al., 2005). Thus, homeorhetic regulation involves coordination of physiological processes in support of a dominant physiological state or chronic situation. Key features of homeorhesis are (1) that it is chronic in nature, involving regulation occurring over hours and days versus seconds and minutes required for most examples of homeostatic regulation; (2) its simultaneous influence on multiple tissues and systems results in an overall coordinated response; and (3) mediation involves altered tissue responses to homeostatic signals. This latter aspect represents the key to understanding the mechanistic interrelationship between homeostasis and homeorhesis (Bauman and Elliot, 1983; Vernon, 1989; Bell and Bauman, 1997).

A schematic representation of the ability of homeorhetic controls to alter tissue response to homeostatic signals is depicted in Figure 2. The interaction can involve increases or decreases in tissue responsiveness, tissue sensitivity, or both. The terms "responsiveness" and "sensitivity" were defined by Kahn (1978) to distinguish different aspects of insulin resistance in human type II diabetes. Using terminology analogous to V_{max} and K_m as applied in enzyme kinetics, whole-body dose–response relationships between insulin and insulin action involved calculation of maximal response (R_{max}) and insulin concentration at half-maximal response (ED_{50}). Thus, R_{max} represented insulin responsiveness, which Kahn (1978) characterized as an index of post-receptor metabolic capacity, and ED_{50} reflected insulin sensitivity, considered to represent an index of receptor function—number, binding affinity, or signal transduction. Thus, changes in responsiveness and sensitivity provide insight as to potential mechanisms that explain altered tissue responses to support lactation (Bell and Bauman, 1997).

ACTUALIZED REGULATION

A dairy cow's ability to regulate nutrient use is most critical at the onset of lactation. Coordination of metabolic processes is the central characteristic of the physiological adaptations that occur to support lactation and they include many, perhaps most, body tissues and involve all nutrient classes—carbohydrate, protein, fat, minerals, and vitamins. The overall net effect is that the increase in mammary metabolic rate and milk synthesis coincides with altered extra-mammary metabolism so that an adequate quantity and pattern of nutrients to support milk synthesis is ensured. A partial list of the metabolic adaptations occurring during the successful transition to lactation is presented in Table 1. Even this partial listing demonstrates the wide range of metabolic processes that are altered in an orchestrated manner. Several excellent reviews have summarized quantitative details and specific references relating to the physiological adaptations that occur to support lactation (Bauman and Elliot, 1983; Vernon, 1989; Bell, 1995; Chilliard, 1999; Drackley, 1999; McNamara, 2015).

Glucose is a particularly important metabolite during lactation, and biological adaptations prioritized to ensure its availability to the mammary gland serve as an example. At the onset of lactation, there is a marked increase in mammary glucose utilization, primarily for lactose synthesis. Total glucose turnover in a high-producing cow can exceed 3 kg/d, with up to 85% being used by the mammary glands. Lactose is the primary osmotic regulator of milk volume, and the synthesis of lactose alone utilizes 65 to 70% of the cow's total glucose turnover. If there is an imbalance between the availability and requirement for glucose, ketosis may result and well-being is compromised. To ensure an adequate glucose supply to support lactation, biological regulation involves a series of orchestrated changes that include increased hepatic rates of gluconeogenesis, decreased glucose uptake and use by adipose tissue and muscle, and a shift in whole-body nutrient oxidation so less glucose is used as an energy source (Table 1).

The role of specific homeorhetic controls in regulating these biological adaptations has been reviewed elsewhere (Vernon, 1989; Vernon and Sasaki, 1991; Bell and Bauman, 1997; Chilliard, 1999). However, an important component of the mechanism involves altered tissue set points and responses to homeostatic controls as described earlier; several examples that occur with the onset of lactation are provided in Table 2. Those related to insulin are of special importance, and hypoinsulinemia coupled with altered tissue responses to insulin is one of the best-characterized examples. At the onset of lactation, glucose-stimulated pancreatic insulin secretion is blunted (Rhoads et al., 2004). Insulin's ability to stimulate glucose disposal by muscle is also reduced and whole-body and tissue-specific dose–response relationships indicate this involves both reduced sensitivity and responsiveness (Figure 3). Insulin's ability to stimulate adipose glucose uptake is also reduced and insulin is less effective at inhibiting hepatic gluconeogenesis (Table 2). The net effect is that these glucose-related metabolic alterations are coordinated with the increase in glucose use by the mammary gland. Thus, homeostatic controls still function acutely to maintain steady-state concentrations of circulating glucose during lactation, but on a chronic

Table 1. Partial list of physiological adaptations that occur to support lactation in dairy cows[1]

Process or tissue	Response
Mammary tissue	Increased number of secretory cells Increased nutrient use Increased blood supply
Feed intake	Increased quantity
Digestive tract	Increased size Increased absorptive capacity Increased rates of nutrient absorption
Liver	Increased size Increased rates of gluconeogenesis Increased glycogen mobilization Increased protein synthesis
Adipose tissue	Decreased de novo fat synthesis Decreased preformed fatty acid uptake Decreased fatty acid re-esterification Increased lipolysis
Skeletal muscle	Decreased glucose utilization Decreased protein synthesis Increased protein degradation
Bone	Increased Ca and P mobilization
Heart	Increased cardiac output
Plasma hormones	Decreased insulin Increased somatotropin Increased prolactin Increased glucocorticoids Decreased triiodothyronine (T3) and thyroxine (T4) Decreased IGF-1

[1]Adapted from Bauman and Currie (1980), Bauman and Elliot (1983), Vernon (1989), McNamara (1991), Chilliard (1999), and Collier et al. (2005).

Table 2. A partial list of alterations in the response to homeostatic responses that occur in different tissues and processes during lactogenesis and early lactation in ruminants[1]

Process or tissue	Homeostatic control	Response to altered set-points
Feed intake	Multiple controls	↑ Appetite and satiety set-point
Adipose tissue	Insulin	↓ Lipogenesis
		↓ Uptake of preformed fatty acids
	Catecholamines	↑ Stimulation of lipolysis
	Adenosine	↑ Inhibition of lipolysis
Skeletal muscle	Insulin	↓ Glucose uptake
		↓ Protein synthesis
		↓ Amino acid uptake
		↑ Protein degradation
Liver	Insulin	↑ Gluconeogenesis
Pancreas	Insulinotropic agents	↓ Insulin release
Whole animal	Insulin	↓ Glucose oxidation
		↓ Glucose utilization by nonmammary tissue

[1]Adapted from Bauman and Elliot (1983) and Bauman (2000).

basis, homeorhetic controls have orchestrated a series of adaptations in both biological processes and extramammary metabolism to ensure the mammary gland is provided with an adequate quantity of glucose and a pattern of other nutrients to support milk synthesis (Bauman, 2000).

The management of body energy reserves provides a second example where the coordinated regulation of physiological processes is essential for a successful transition at the onset of lactation (McNamara, 1991, 2015). All mammals draw on body energy reserves to support metabolic demands during early lactation and this results in an increase in circulating nonesterified fatty acids (**NEFA**) and ketones. The use of energy reserves at the onset of lactation varies among mammalian species, with dairy cows being intermediate in the extent and duration compared with other mammals (Bauman, 2000). In high-producing dairy cows, the utilization of body energy reserves and the mobilization of body fat in the first month postpartum can be energetically equal to over one-third of the milk produced (Bauman and Currie, 1980). This period of negative energy balance (**NEBAL**; Figure 4) is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980; Bell, 1995; Drackley, 1999). Mechanisms include an attenuation in adipose tissue response to homeostatic signals (Table 2). In addition to the aforementioned reduction in insulin's ability to stimulate glucose uptake by adipose tissue, there is a reduction in insulin's ability to inhibit lipolysis. Furthermore, the lipolytic response of adipose tissue to catecholamines and adenosine, 2 key homeostatic regulators of lipid mobilization, is enhanced in early lactation (Table 2). This is illustrated by the lipolytic response to an epinephrine challenge shown in Figure 5, and by several studies demonstrating adaptations in β-adrenergic receptors, tissue responsiveness, hormone-sensitive lipase (HSL) activity, perilipin, caveolin, β-2 adrenergic receptor, and HSL message (McNamara, 2015). Detailed dose–response studies with cattle and sheep indicate the cellular mechanism may be post-receptor, based on the observed increase in R_{max} with little or no effect on ED_{50} (Guesnet et al., 1987; McNamara, 1988; Vernon et al., 1991).

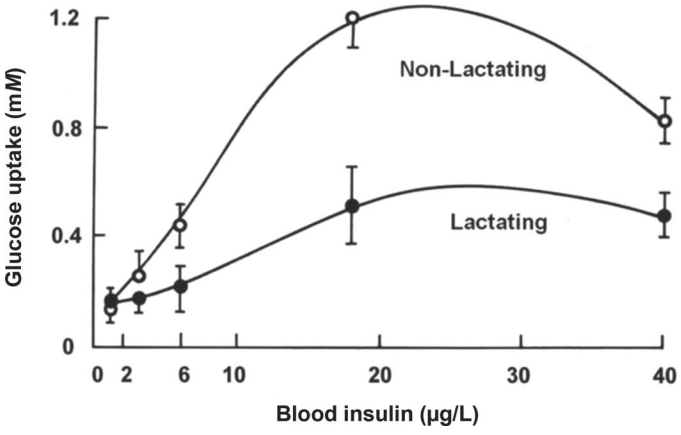

Figure 3. Effects of physiological state on insulin action in skeletal muscle. Adapted from Vernon (1986) with permission of the Hannah Research Institute (Ayr, UK).

Several hormones have been proposed to function as homeorhetic controls, including somatotropin (**ST**), prolactin, and corticoids (Vernon and Sasaki, 1991; Bell, 1995; Chilliard, 1999). In particular, the role of ST as a homeorhetic control is well established, and it plays a key role in many of the metabolic adaptations that occur with the onset of lactation (Bell and Bauman, 1997). Furthermore, the production of recombinant bST (**rbST**) has provided research opportunities to more completely characterize its mechanism as a

homeorhetic governor. Administering rbST to lactating cows increases milk production, and the mechanism includes a series of biological adaptations similar to those detailed in Tables 1 and 2 (Bauman and Vernon, 1993; Etherton and Bauman, 1998). Thus, consistent with the concept of homeorhesis, rbST both increases milk synthesis by the mammary glands and orchestrates other body processes in a manner to provide the necessary nutrients to support the increase in milk yield.

Circulating ST concentrations also increase at the onset of lactation (Figure 4) and are greater in genetically superior cows (Hart et al., 1978). During NEBAL, somatotropin promotes NEFA export from adipose tissue by increasing the lipolytic response to β-adrenergic signals (Figure 6A) and by reducing the lipogenic and antilipolytic responses to insulin (Figure 6B; Bauman and Vernon, 1993). This reduction in systemic insulin sensitivity is coupled with a decrease in circulating blood insulin levels (Figure 4). The reduction in insulin action allows for adipose lipolysis and NEFA mobilization (Bauman and Currie, 1980). Not surprisingly, reduced circulating insulin is also a key mediating factor by which high-producing cows partition nutrients away from storage and toward mammary utilization (Figure 4). Increased circulating NEFA are typical in "transitioning" and undernourished cows and represent (along with NEFA-derived ketones) a significant source of energy (and precursors for milk fat synthesis) for cows in NEBAL. The severity of calculated NEBAL is positively associated with circulating NEFA levels

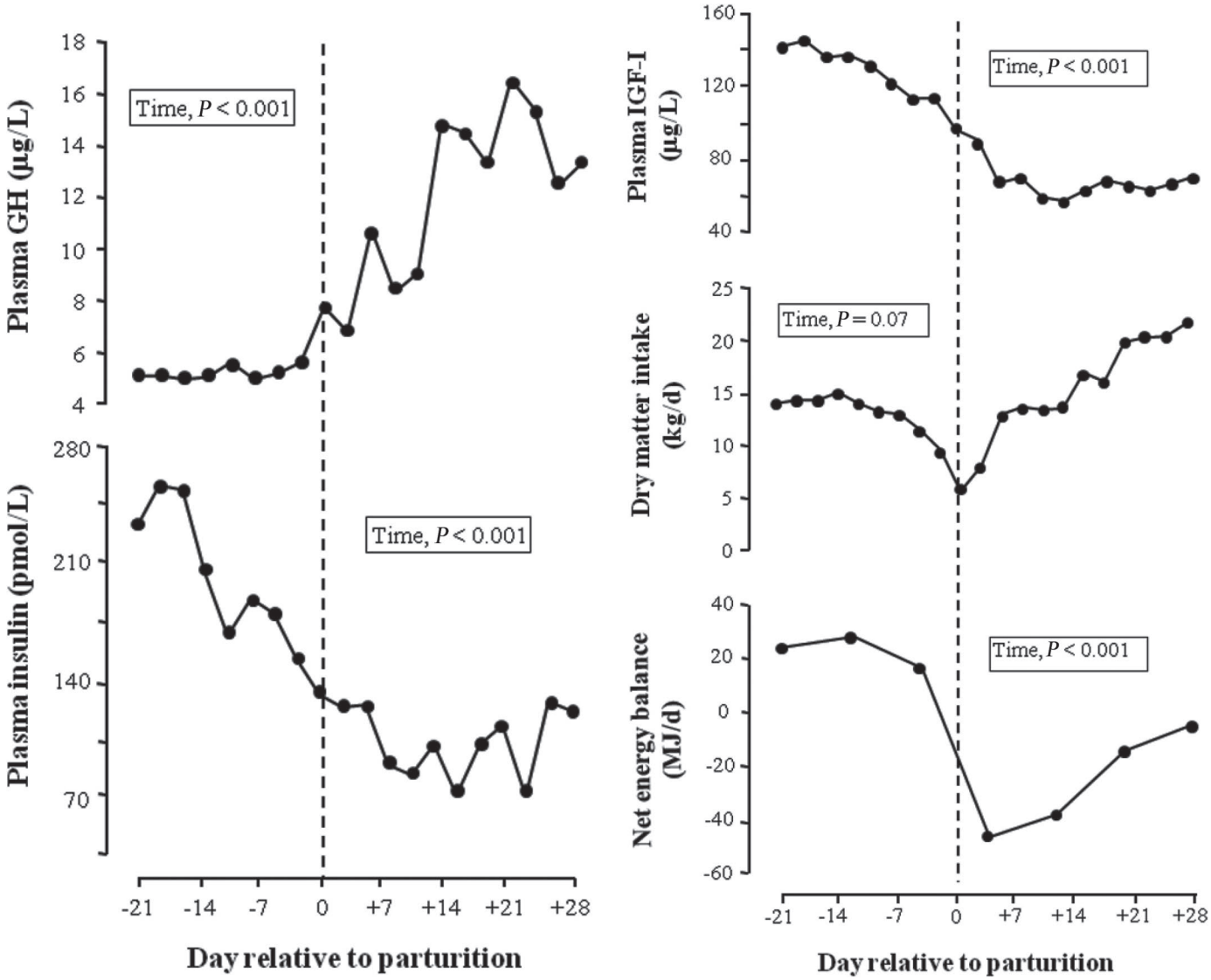

Figure 4. Temporal pattern of whole-animal energetics and key hormones responsible for nutrient partitioning in transitioning lactating Holstein cows. Reproduced from Rhoads et al. (2004) with permission of the American Society for Nutrition.

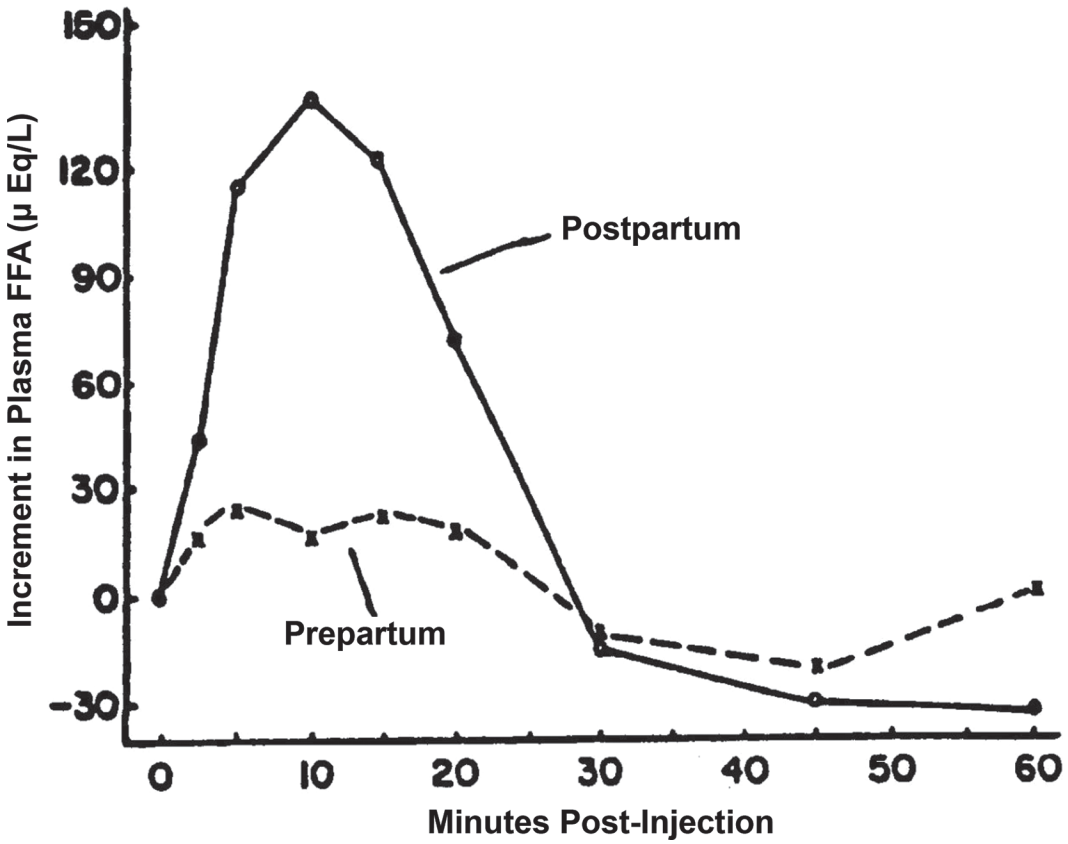

Figure 5. Effects of parturition on lipolytic response (plasma nonesterified fatty acids) to an epinephrine challenge administered intravenously at 6, 4, and 2 wk prepartum and 2, 4, and 7 wk postpartum in dairy cows. Reproduced from Bauman (1984) with permission.

(Bauman et al., 1988; Dunshea et al., 1990) and it is generally thought that there is a linear relationship (concentration-dependent process) between NEFA delivery, tissue NEFA uptake, and NEFA oxidation (Armstrong et al., 1961). The magnitude of NEBAL and thus lipid mobilization explains, in large part, why cows lose considerable BW during early lactation.

Postabsorptive carbohydrate metabolism is also markedly altered by NEBAL and this is largely mediated by reduced insulin action. During early lactation

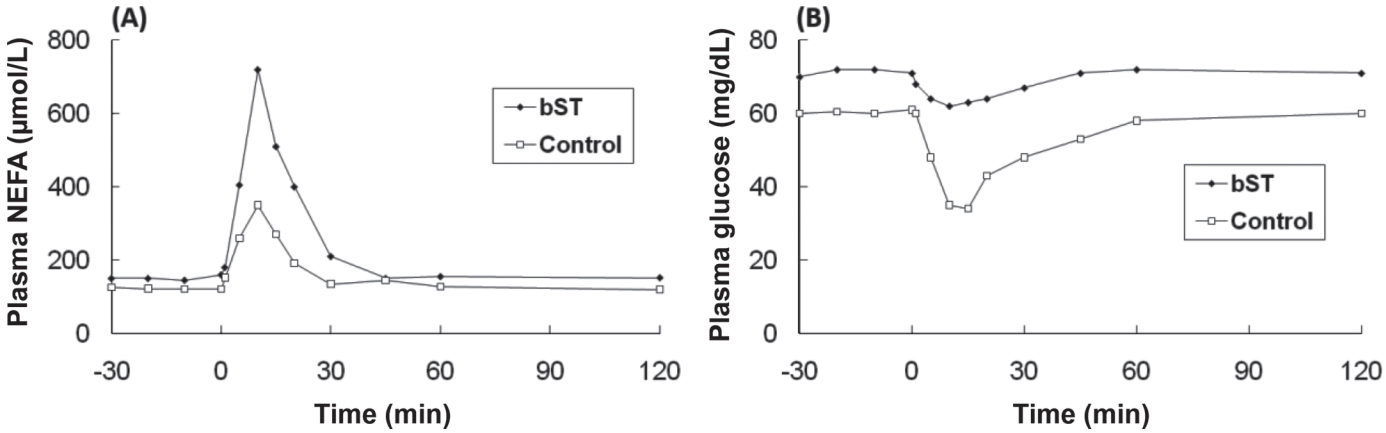

Figure 6. Effects of recombinant bST on (A) plasma nonesterified fatty acid (NEFA) response to an epinephrine challenge and (B) plasma glucose response to an insulin tolerance test in lactating Holstein cows. Reproduced from Sechen et al. (1990) with permission of the American Physiological Society.

or inadequate nutrient intake, glucose is partitioned toward the mammary gland and its contribution as a fuel source to extra-mammary tissues is decreased (Bell, 1995). This can be observed when comparing insulin's effectiveness at stimulating muscle glucose uptake in lactating and nonlactating animals (Figure 3). The early lactation or NEBAL-induced hypoglycemia likely accentuates catecholamine's adipose lipolytic effectiveness, a phenomenon observed in other species (Galster et al., 1981). This is a key "glucose-sparing" mechanism because elevated NEFA levels decreases skeletal muscle glucose uptake and oxidation and this is referred to as the "Randle Effect" (Randle, 1998). The fact that insulin simultaneously orchestrates both carbohydrate and lipid metabolism explains why there is a reciprocal relationship between glucose and NEFA oxidation. Ultimately, these are homeorhetic adaptations to maximize milk synthesis at the expense of tissue accretion (Bauman and Currie, 1980). A cow in NEBAL could be considered "metabolically flexible" because she can depend upon alternative fuels (NEFA and ketones) to spare glucose, which can be utilized by the mammary gland to support copious milk production.

CHALLENGES AND OPPORTUNITIES

World population is projected to grow to between 9 and 10 billion people by 2050 (Godber and Wall, 2014). Most of the growth is expected to occur in poor and developing countries, where income elasticity of demand for food continues to be high. This population increase, combined with moderately high growth in income, is anticipated to result in a >70% increase in demand for food and other agricultural products by 2050 (Godber and Wall, 2014). Meeting the increasing demand for food mandates continued improvements in agricultural productivity. Thus, the future presents both challenges and opportunities as shown by the following examples.

Climate Change

A significant portion of the world's domestic animal population exists in regions where climatic stressors adversely influence animal health and productivity. Even in the United States, the dairy industry loses approximately $1.5 billion annually due to heat stress (St-Pierre et al., 2003; Key and Sneeringer, 2014). Substantial progress has been made over the last 3 decades in improving the productivity of ruminant livestock exposed to adverse environmental conditions, including advances in nutritional management, selective breeding, and animal housing facilities (Collier et al., 2005). Identifying specific genes associated with thermal tolerance is in the early stages, but thermal stress has a clear gene × environment interaction, indicating that opportunities may exist to improve the thermal tolerance of dairy cattle (Collier et al., 2008).

With regard to nutrient partitioning, heat-stressed cows voluntarily decrease feed intake, but the postabsorptive metabolic changes in heat-stressed cows differ markedly from those of thermal neutral cows fed at a similar plane of nutrition (Baumgard and Rhoads, 2013). Despite being in a catabolic state, heat-stressed cows have increased basal and stimulated insulin secretion, which significantly limits adipose tissue rates of lipolysis and the mobilization of energy reserves (Baumgard and Rhoads, 2013). Consequently, heat-stressed cows have reduced NEFA and ketones and thus are unable to "spare" glucose for milk synthesis. Identifying mechanisms and basis for these postabsorptive strategies may allow the development of approaches (e.g., nutritional, genetic, pharmaceutical) designed to ameliorate the negative consequences of heat stress.

Immunometabolism

There is an increasing recognition of the complex and dynamic metabolic response to infection and inflammation and the role that the immune response may play in animal productivity and well-being. Immunoactivated animals voluntarily become hypophagic but have a paradoxical increase in circulating insulin (Kvidera et al., 2016, 2017). Despite the hyperinsulinemia, infection-induced decreased glucose clearance was first described over 90 yr ago (Hector, 1926) and more recently it has been confirmed that inflammatory conditions cause whole-body insulin resistance in mid-lactation dairy cows (McGuinness, 2005; Vernay et al., 2012). Specifically, peripheral insulin insensitivity occurs in skeletal muscle and adipose tissue, the 2 largest "sinks" of insulin-stimulated glucose disposal (Lang et al., 1990). This is likely a strategy to spare glucose for the immune system as leukocytes require insulin for activation and contain GLUT4 receptors (Maratou et al., 2007).

Relative to nutrient partitioning, peripheral insulin resistance is necessary to achieve optimal milk production in healthy dairy cows as discussed earlier, but this relationship is obviously uncoupled during an infection. Mammary epithelial cells have toll-like receptors (Ibeagha-Awemu et al., 2008) and are therefore able to recognize bacterial-derived antigens. Thus, infection-induced decreased milk synthesis represents a mechanism that reduces glucose use by the mammary glands, thereby allowing increased glucose availability for the immune system. Recent estimates indicate that an intensely activated immune system utilizes more than 2 kg of glucose per day (Kvidera et al., 2017), so

the implications of the immune system's demands on nutrient partitioning are obvious.

The transition period is associated with a temporal inflammatory state, and potential sources include uterine tissue damage, metritis, mastitis, and "sterile inflammation" (Bradford et al., 2015). Another more inconspicuous source of immune activation is the gastrointestinal tract. Decreased intestinal barrier function has been described in the transitioning period, heat stress, feed restriction, and rumen acidosis (Khafipour et al., 2009; Baumgard and Rhoads, 2013; Zhang et al., 2013; Abuajamieh et al., 2016). Thus, with regard to nutrient partitioning, it is not a coincidence that the metabolic, endocrine, and immune response is similar between multiple pathologies frequently observed in dairy production (Baumgard and Rhoads, 2013). Further identifying the coordination between nutrient use by the immune system and the utilization of nutrients for productive functions will likely provide novel approaches amenable to physiological manipulation.

Application of Omics Research

The burgeoning fields of genomics, proteomics, metabolomics, nutrigenomics, lipidomics, and microbiomics have provided new systems biology techniques, approaches, and data to assess key regulation in various biological processes. The ability to sequence genomes of microbes and animals has demonstrated that it is possible to improve the accuracy of selection information. Consequently, genomic selection is revolutionizing dairy cattle breeding by reducing the generation interval and increasing the accuracy of genetic selection decisions. With regards to microbiomics, recent work has shown a relationship between gut microbiota and both positive and negative aspects of animal performance and well-being. This is a largely unexplored area in ruminants, but the classic research in dietary-induced milk fat depression demonstrates its importance and potential impact on the regulation of nutrient partitioning. Recent discoveries have firmly established that ruminal biohydrogenation of polyunsaturated fatty acids results in the production of trace levels of bioactive fatty acid isomers, which can regulate postabsorptive metabolism and mammary milk fat synthesis (Bauman and Griinari, 2003; Bauman et al., 2011). Presumably, other bioactive molecules derived from gut microbial fermentation may also be absorbed and have non-nutritive roles involving metabolic regulation and overall production efficiency and well-being. Further characterization of the pathways and end-products of macronutrient fermentation (within both the rumen and large intestine) will undoubtedly contribute to our understanding of how the intestinal microbiome partially regulates postabsorptive nutrient trafficking and ultimately animal productivity and well-being.

Genomics is a developing technique in dairy science, and "omics" research to date has generated massive quantities of descriptive data that provide excellent baseline information. Application of these data will be especially challenging because we often have inadequate knowledge of the role and functionality of specific genes and proteins. Regulation of nutrient partitioning is complex, involving both acute and chronic orchestration of multiple tissues and physiological processes. The large amount of data harvested by "omics" techniques are noncausal, and accurately interpreting the correlated changes requires an extensive appreciation for biological principles. The challenge is applying the descriptive information gained from "omics" research to demonstrate functionality and develop a more comprehensive understanding of the regulation of these physiological processes and their role in animal productivity, health, and well-being. Clearly, translation and application of this new knowledge to improve nutrient partitioning and productive efficiency represents an area of exciting opportunity.

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APPENDIX

Table A1. Major milestones in the study of nutrient partitioning to support lactation

Date	Milestone	Reference
1923	Insulin's role in milk composition is defined.	
1929	Concept of homeostasis is elucidated.	Cannon, 1929
1932	Pituitary extract injected into cows.	
1945	First National Research Council report: *Recommended Nutrient Allowances for Dairy Cattle*.	
1953	Annual milk/cow is approximately 2,500 kg.	https://quickstats.usda.nass/gov/
1960s	Role of volatile fatty acids (VFA) in dairy cow metabolism is defined.	
1960s	Mammary balance sheet of nutrient uptake and use.	
1960s	Production of VFA by rumen fermentation established.	
1963	Insulin administration is shown to decrease milk yield.	

Continued

Table A1 (Continued). Major milestones in study of nutrient partitioning to support lactation

Date	Milestone	Reference
Mid-1960s–1970s	Biochemical pathways of milk fat synthesis are elucidated.	
Mid-1960s–1970s	Biochemical pathways of milk lactose synthesis are elucidated.	
1970s	Biochemical pathways for milk protein synthesis are elucidated.	
1977	Annual milk/cow exceeds 5,000 kg.	https://quickstats.usda.nass/gov/
1980	Concept of homeorhesis is proposed.	Bauman and Currie, 1980; Bauman, 2000
1982	First use of recombinant bovine somatotropin (bST).	
1982	High-yielding cows are shown to have decreased insulin and increased somatotropin.	
1980s	Nutritional, environmental, and genetic effects on milk composition are demonstrated.	
Late 1980s	Metabolic modeling.	
1994	US Food and Drug Administration approves recombinant bST.	
Late 1990s–early 2000s	Basis for diet-induced milk fat depression is defined.	
2014	Annual milk/cow exceeds 10,000 kg	https://quickstats.usda.nass/gov/

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A 100-Year Review: Mammary development and lactation[1]

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ABSTRACT

What is old is new again—and with respect to the study of the mammary development and function in dairy animals, the expression resonates. Many of the mammary and milk production questions raised in the early years of the *Journal of Dairy Science* apply today. To be sure, scientists have filled in many details regarding, for example, identification of hormones and growth factors important in the control of mammary growth, the onset of copious milk production at calving, and maintenance of lactation. Early years focused on identification and subsequent availability of classic mammogenic, lactogenic, and galactopoietic hormones (e.g., steroids, prolactin, and growth hormone). The advent of sensitive assays to measure concentrations of these hormones and, subsequently, myriad growth factors in blood, milk, and tissues, allowed creation of multiple hypotheses to explain mammary cell proliferation and regulation of function. It is also apparent that we understand many of the fundamentals of milk removal, milking frequency, milking management, and milk ejection for successful lactation. However, some questions remain. Are the principles that were identified when cows produced markedly less milk still valid for the high-producing cows of today and the future? What mechanism(s) explain the positive effects of early increased milking frequency on subsequent milk production? Can the persistency of lactation be improved (secretory cell number vs. secretory cell function) or does early management "program" future mammary development or productivity (epigenetics, immune responsiveness, other)? The explosion of tools and techniques (Southern and Northern blots, PCR, and the "-omics" revolution) has driven an almost overwhelming evaluation of cellular and molecular functions in the mammary gland and other tissues. One key may be the discovery of a "Rosetta stone" that will allow understanding of this mass of detailed information on gene expression, cell signaling, and so on. Many scientists can now better appreciate the difficulty of the dairy farmer seeking to process DHIA or Dairy Comp 305 data, milking data, weights, feeding reports, pedometer readings, or genomic evaluations to manage their operations.

Key words: lactation, mammary development, epigenetics, galactopoiesis

INTRODUCTION

Mammary development and mammary function—could anything be more central to dairying? Indeed, without lactating animals to populate the herds of cows, goats, sheep, and other mammals, there is no dairy industry. The goal of this review is to describe what I believe are some of the mammary development research milestones that have occurred in the first 100 years of the *Journal of Dairy Science* (**JDS**). However, it is important to appreciate that my focus is on scientific advancements that have increased understanding of how the mammary gland develops and controlling factors. In addition to mammary growth, I will describe some of the key findings that increased our understanding of the onset of lactation and ongoing milk synthesis and secretion. The successful dairy, of course, depends on more than the availability of the lactating animal; other authors for this celebratory special issue will cover aspects of nutrition, health, genetics, and physiology. Finally, I have focused on highlighting papers published in JDS while understanding that many of the fundamental findings have clear parallels with scientists focused in more directly basic studies: cell biology, development biology, physiology, and, recently, the "-omics" revolution. It is also evident that dairy-interested mammary biologists and dairy scientists routinely publish in a variety of endocrine, physiology, biochemistry, reproduction, and nutrition focused scientific journals. Consequently, many important dairy-related findings appear in those journals. Therefore, this review is not broadly comprehensive but rather seeks to highlight those contributions published in JDS. Regardless, the JDS papers that I have referenced will lead interested readers to other primary materials (listed in the timeline given in Appendix Table A1).

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What becomes clear is that many of the fundamental questions regarding mammary development and lactation have changed little over time (Petersen, 1942). As examples, consider these questions:

- What explains the shape of the typical lactation curve?
- Is persistency of milk production set?
- Does the number of secretory cells in the udder or the functional capacity of alveolar cells explain the level of milk production?
- Which is more important in explaining milk production differences—genetics or management (i.e., nature vs. nurture)?
- Are the key regulators in the "modern" high-producing dairy cow the same as described in "older" less-productive dairy cows?
- Which is more important in control of mammary growth and milk synthesis—local regulators or systemic agents?
- How does milking frequency affect mammary function and milk production?
- Why does the dry period matter and how long does it need to be?

Complete answers to these and other relevant dairy questions remain elusive and evolving. However, the continuing advancement of scientific tools, devices, and techniques provides opportunities to improve our understanding of dairy cows and other dairy animals. These efforts are critical if milk and other dairy products are to remain as key nutrients to feed the human population of the next century. I should also add that the technology sequence that took us from the transistor to the personal computer to the internet and to social media is also having major impacts on science, government, and dairying. In the absence of social media and consumerism in developed countries, would the use of effective, scientifically validated bST be an issue? Would the emphasis on animal care and animal rights, organic versus traditional production, green production methods, confusion over what constitutes milk, or the hyperbole over genetically modified foods or transgenic animals be the same?

Over many years, a variety of reviews published in JDS, often in conjunction with symposium presentations held at the annual meetings of ADSA, have served as important resources. Leading scientists in lactation biology and dairy science completed many of these reviews. Of course, many excellent reviews have been published in other journals but the focus of this contribution is on JDS. It also becomes evident upon review of the first 100 years of JDS that students and colleagues from a relatively small number of laboratories and institutions have been responsible for the majority of mammary-focused papers published in JDS. At a minimum, future students should consider these reviews to find relevant research and historical references.

HOW TO MEASURE MAMMARY DEVELOPMENT? THE DEVIL IS IN THE DETAILS

On the surface, it seems an easy matter to determine mammary growth: simply measure the mass of the udder and mass of dissected tissue components (fat pad, parenchyma, skin, and teats). Of course, sacrifice of the animals makes collection of these measurements very expensive, and time-sequence data are still limited, particularly for information related to modern, high-yielding dairy cows. In addition, these simple measures do little to characterize the number or types of specific cells that compose the mammary tissue. Tissues of the developing mammary gland include the mammary parenchyma (**PAR**; the epithelial structures, the ducts and alveoli), stromal tissue (the connective tissue elements surrounding the developing epithelial structures, vascular and lymphatic network), the mammary fat pad (**MFP**), and the skin, lymph nodes, and teats. The PAR is the portion of the mammary tissue that gives rise to the mammary alveoli and associated ducts that lead to the teat or nipple in the mammary gland of the lactating cow. Because the PAR includes the lobules of alveoli that synthesize and secrete milk, this portion of the mammary tissue has received the most attention of mammary biologists. However, it is evident that the supportive stromal tissues surrounding the PAR are essential for normal development of the PAR and for maintenance of lactation in the fully formed mammary gland. Reviews describing secretory responses of mammary epithelial cells cultured on various extracellular matrix materials illustrate the importance of stromal tissue elements on cell differentiation and milk component biosynthesis and secretion (Pitelka and Hamamoto, 1977; Aggeler et al., 1988). A recent report (Stiening et al., 2008) details effects of extracellular matrix, mechanical stimulation (release of gels for cells cultured on collagen gels), and lactogenic hormones on secretory cell differentiation and gene expression in cultured bovine mammary organoids.

As outlined by Reece (1956), it was known that the amounts of secretory tissue varied in udders of cows, and the common sense conclusion was that udders of beef cows contained less secretory tissue than udders of dairy cows. Thus, the logical assumption was that higher-producing dairy cows would have udders that contain more secretory tissue than their lower-producing herdmates. But how do you quantify secretory tissue, the number of secretory cells, and supporting

cells in the udder? Related to this, dairy physiologists began to consider how to evaluate mammary growth in calves and heifers and possible relationships between development at these early stages of development and future productivity. Several early studies (Swett, 1927, 1947; Swett and Matthews, 1934) sought relationships between udder or mammary anatomy and milk production capacity. Indeed, this fundamental question continues today (Soberon et al., 2012; Geiger et al., 2016a,b; Gelsinger et al., 2016). The reasonable conclusion is that nutrition and management both before and after weaning can influence mammary development, health, immune competency, physiology, and gene activity to modify future productivity (Kahn et al., 2011). Scientifically, the fascination comes from discovering possible mechanisms and devising useful tools and techniques to alter development and future function to make animals that are healthier and more profitable and consumers who are more satisfied.

The Mammary Gland by C. W. Turner (1952) describes and illustrates many of these early studies, many of which were conducted by Dr. Turner, his students, and collaborators at the University of Missouri. Indeed, most dairy mammary biologists in the United States between ~1940 and the 1990s can trace their academic lineage to Dr. Turner.

Many early basic studies on mammary development utilized rodents because the relatively thin, flat mammary glands of mice or rats are easy to spread onto glass slides and they can be fixed, defatted, and stained to reveal the entire developing ductal structure (Macdonald and Reece, 1960). Measurement of the number, degree of branching, and appearance of alveolar buds, for example, give a quantitative assessment of mammary development. Figure 1 illustrates examples of processed murine glands and demonstrates the readily apparent changes in prepubertal ductal development. Figure 1A shows elongating mammary ducts and the distal bulbous end buds (arrows), and Figure 1B shows the development in a mouse after puberty. Thin, minimally branched ducts fill the MFP but the end buds have disappeared. Figure 1C illustrates the very compact, dense PAR development (no end buds and lack of filled fat pad) in the prepubertal ruminant. This example is a sagittal section through the entire mammary gland of a prepubertal lamb. The PAR tissue is concentrated in the area adjacent to the teat with most of the MFP being free of epithelium. Figure 1D (lower magnification) and Figure 1E show mammary ductal development in a prepubertal heifer. Many studies have evaluated the effects of stage of development, hormone ablation and replacement, and treatment with exogenous agents on mammary development. Certainly, the size and anatomy of the ruminant udder and mammary glands make a mouse-like evaluation impossible. It is also evident that cows and other dairy animals are not rodents. This statement is not flippant but rather serves to emphasize that substantial differences exist in the patterns of tissue development, physiological properties, and metabolism between rodents and ruminants. This concern is not new—consider the words of McCandlish (1918): "No attention will be given here to experimentation with the human subject, the goat, or mammalia other than the cow, for the simple reason that through much valuable work has been done with these subjects the results need not necessarily be directly applicable to the cow as there may be certain generic differences in physiological activity, and it is not that in some cases, e.g., with the human subject, variations in milk production can be brought about much more readily than in the case of the cow." Figure 2 provides an overview of bovine mammary development from puberty into pregnancy and into lactation.

Indeed, subtle differences exist even between dairy ruminant species (Ellis et al., 1998; Rowson et al., 2012; Capuco and Ellis, 2013). The same can be said of the many rodent-based studies aimed at better understanding breast cancer. This does not diminish the many important molecular, endocrine, and cellular findings derived from rodent models, but is a reminder that caution is necessary with uncritical extrapolation between species (Berryhill et al., 2016). Regardless, the review by Sheffield (1988) provides an interesting twist on these rodent techniques to evaluate the growth of bovine mammary tissues transplanted into the cleared (i.e., native epithelium removed) MFP of athymic nude mice. As recent examples, xenografts with bovine mammary cells are used to explore populations of mammary stem cells (Rauner and Barash, 2016) and responses of genetically engineered cells capable of secreting human milk proteins (Martignani et al., 2010).

As described by Akers (1985) and Tucker (2000), now classic studies with triply operated rodents (hypophysectomized, adrenalectomized, ovariectomized) and serial replacement of hormones (or hormone-containing tissue fractions) established the key roles of estrogen and growth hormone in mammary ductal development, progesterone and estrogen in lobulo-alveolar formation, and prolactin in lactogenesis. With the exception of the ovariectomy, the difficulties of surgery and the maintenance of animals for subsequent experimentation limited the use of extensive hormone ablation or replacement techniques in larger dairy ruminants. Regardless, numerous early studies published in JDS reported effects of various endocrine manipulations on mammary development and function. Indeed, a paper in one of the earliest issues of the journal (Hill, 1919) includes a discussion of factors responsible for

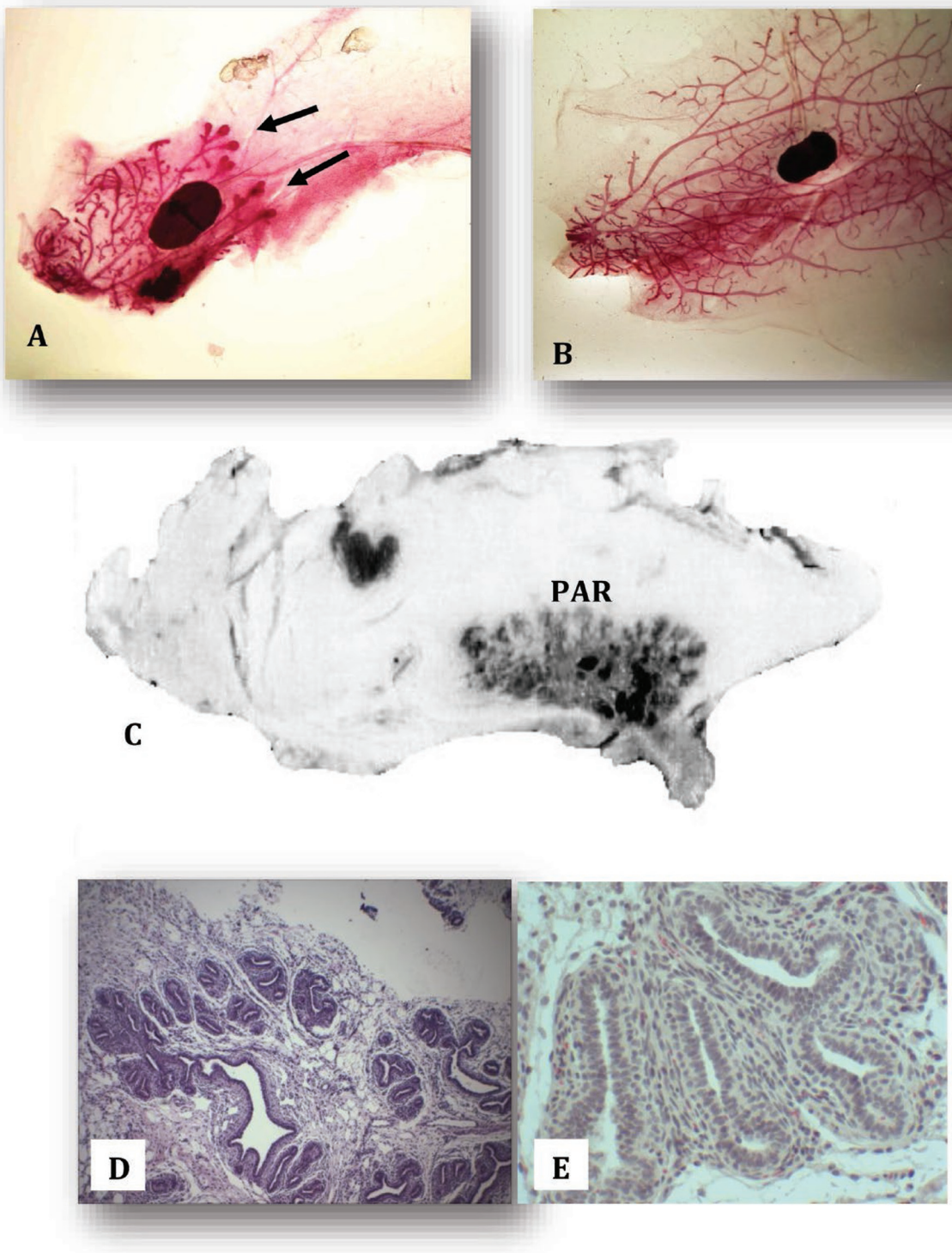

Figure 1. The appearance of mammary glands or mammary tissue at several stages of development. Panels A and B are whole mounts of mammary glands from mice. (A) Mammary growth in a prepubertal mouse at approximately 2 wk of age; the mammary ducts can be seen branching from the rudimentary tissue near teat (to the left). The arrows indicate the bulbous end buds present at the distal ends of the ducts as they progressively grow through the mammary fat pad. (B) Mammary gland of a postpubertal mouse at approximately 4 wk of age. The mammary ducts have spread throughout the mammary fat pad, and the end buds have disappeared. (C) Midsagittal section through the mammary gland of a prepubertal ewe lamb. Note the very dense development of the parenchymal tissue (PAR) in the region adjacent to the teat and gland cistern. (D) Histology of the mammary tissue from a 2-mo-old prepubertal calf. Epithelial structures are located in multiple clusters around larger ducts. (E) Cellular detail surrounding a developing epithelial cluster. Color version available online.

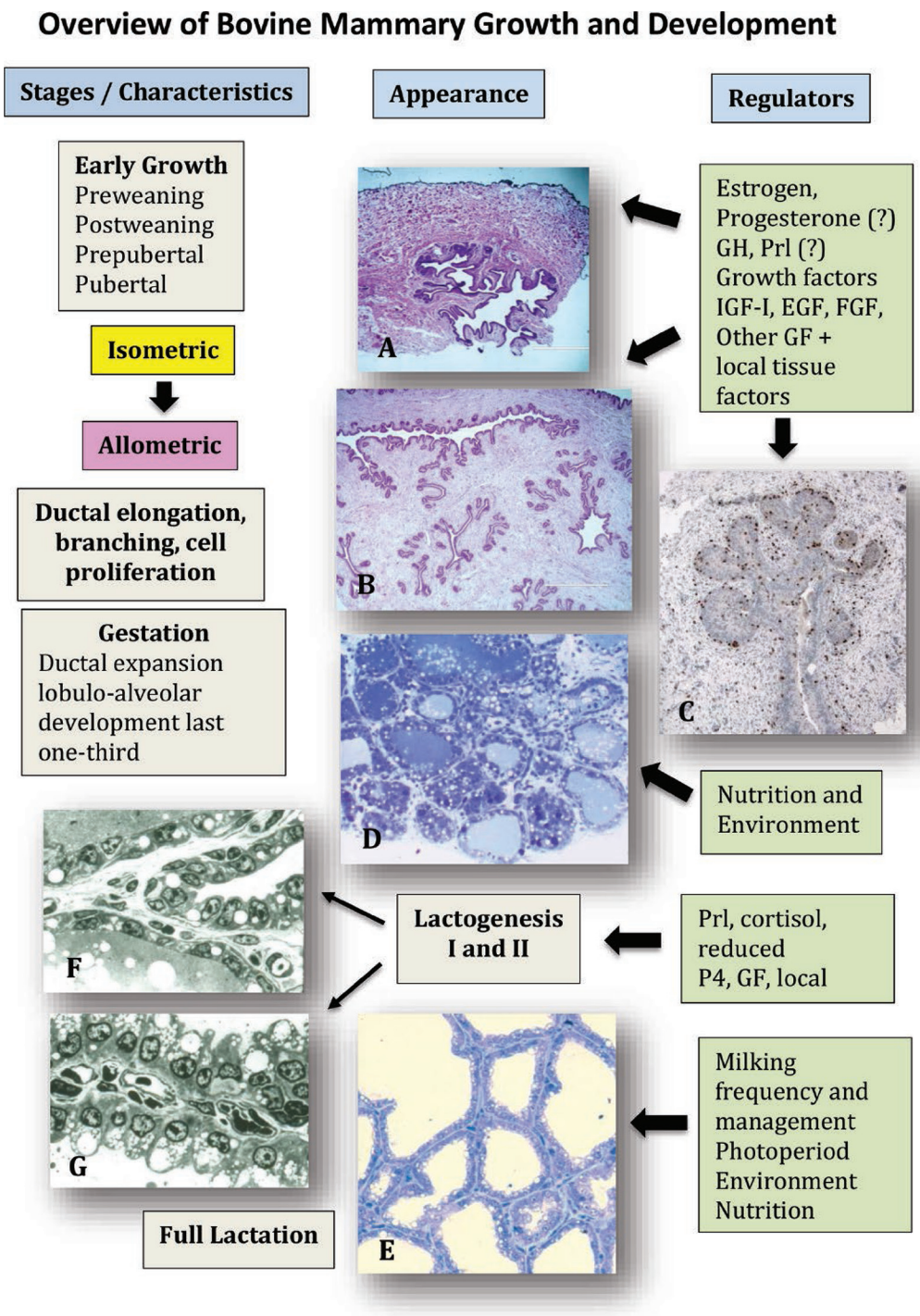

Figure 2. Overview of bovine mammary growth and development. Changes in female mammary development from the young calf to gestation and lactation are illustrated. The left column describes major development landmarks and events; the center column shows changes in histological appearance of the mammary parenchymal tissue; and the right column lists some of the regulators that are important during each of these developmental stages. (A) Rudimentary parenchyma from a very young calf. (B) Mammary parenchyma from a well-fed prepubertal calf at weaning. (C) View of a developing ductal structure from a prepubertal calf that was injected with 5-bromo-2-deoxyuridine (BrdU) 24 h before the tissue was collected. The dark spots appear in cell nuclei that have incorporated BrdU (a measure of proliferation). (D) Developing alveoli from the mammary gland of a pregnant heifer (~170 d of gestation). (E) Low-power view of cross-sectioned alveoli from a cow in established lactation. The gland was thoroughly milked before tissue collection. (F) High-resolution view of alveolar tissue ~2 wk before calving; note the minimal cellular differentiation. (G) High-resolution image of alveolar tissue ~2 wk after calving; note the dramatic polarization of the cells, rounded nuclei, abundant secretory vesicles, and lipid droplets. GH = growth hormone, Prl = prolactin, EGF = epidermal growth factor, FGF = fibroblast growth factor, GF = growth factor, P4 = progesterone. Color version available online.

mammary development and milk secretion, as well as photographs of a 4-mo-old doe kid producing milk while suckling its dam. This old example emphasizes the variation in mammary development and lactation even among well-studied dairy animals. Akers (2006) described experiments demonstrating milk component biosynthesis in mammary tissue explants from bulls stimulated by addition of prolactin and effects of genetic selection for increased milk production on these responses. There are also reports of normal lactation in males of some species (Francis et al., 1994), including goats (Asdell et al., 1936; Nair et al., 1981). It remains relevant to point out that details about mammary development and lactation are available for only a small number of the mammals known to biologists. Moreover, as the examples above demonstrate, there continue to be surprises even among well-studied dairy animals.

Experimental examples of endocrine effects on mammary development include Bergman and Turner (1940), Lewis and Turner (1941, 1942), Schultze and Turner (1933), and Sykes and Wrenn (1950, 1951). Several British studies utilizing dairy goats served to extend ablation/replacement findings from rodents to dairy animals (Cowie et al., 1952, 1964, 1966). Cowie and Tindal (1961) demonstrated that milk production impaired by hypophysectomy in lactating goats could be restored with appropriate hormone replacement therapy. An extensive and comprehensive monograph (Cowie et al., 1980) provides an excellent overview of the endocrine control of mammary development and function before 1980.

Noninvasive methods to measure mammary development are highly desirable. Some approaches include palpation, as described above, as well as use of ultrasound, X-ray, and computed tomography (**CT**) scanning. Sørensen et al. (1987) demonstrated that CT scanning of whole udders effectively measured the content of fat-free PAR tissue from mammary glands of prepubertal heifers. Sejrsen et al. (1986) showed that CT scanning data mirrored increases in mammary PAR in heifers treated with bST, as noted after tissue dissection. Despite the accuracy of CT scanning, the availability, expense, and lack of ability to scan intact animals has limited the use of CT to evaluate mammary development in farm animals. Esselburn et al. (2015) showed that ultrasound measurements could be used to measure changes in mammary PAR in young calves; at the time of slaughter, direct measures of PAR mass and ultrasound data were highly correlated.

As Tucker (1987) noted, "Before 1953, methods for quantifying mammary growth were either subjective or involved tedious histometric procedures." Measurement of tissue DNA evolved as a fundamental tool to measure the total number of cells, with the finding that the amount of DNA per cell was essentially constant. When combined with other measures (e.g., histology) to estimate proportions of cell types and structures (e.g., stromal tissue cells, blood vessels, or leucocytes), accurate estimates of udder growth—particularly growth of the PAR—became possible. One of the first studies to use DNA content evaluated rat mammary glands (Kirkham and Turner, 1953). Williams and Turner (1961) were apparently the first to report DNA content as a measure of mammary growth in the bovine. Since then, total gland DNA with ancillary biochemical measures (protein, fat) and histology have become standards in estimating both normal and experimental mammary growth. Following a long line of studies evaluating the effects of ovarian steroids on mammary development, Sud et al. (1968) quantitatively evaluated mammary development in ovariectomized heifers to determine the amounts and ratios of estrogen and progesterone needed to produce mammary development that most closely mirrored that of pregnant heifers.

Direct measures of cell proliferation include incorporation of radiolabeled thymidine via direct counting or autoradiography (Capuco et al., 1997, 2001) or bromodeoxyuridine (Capuco et al., 2001; Berry et al., 2003; Silva et al., 2005). Alternatively, expression of transcription factors, such as Ki67, linked to cell cycle progression are frequently used to evaluate mammary cell proliferation (Capuco et al., 2001; Brown et al., 2005). The study by Capuco et al. (2002) provided an elegant combination of cell labeling with 3-dimensional reconstruction of developing ductal structures in the bovine mammary gland.

As indicated above, there has been consistent interest in early mammary development and possible relationships with future productivity. Studies have generally been concentrated in 3 areas: preweaning, weaning to puberty, and puberty to gestation. A now classic JDS paper (Sinha and Tucker, 1969) was important because it provided a quantitative assessment (DNA content) of bovine mammary parenchymal development from birth to puberty and during the estrous cycle. Perhaps more importantly, it defined differences in the rate of mammary growth relative to changes in general body growth. A key finding was that mammary growth shifted to an allometric rate of development well in advance of actual puberty. This and subsequent studies (reviewed by Tucker, 1987) indicated that mammary gland growth increased for the first few estrous cycles but returned to an isometric rate until breeding. As reviewed by Sejrsen and Purup (1997), several applied field studies in subsequent years showed that diets or feeding rates producing high rates of prepubertal gain

were linked to lower future milk production. Related studies (Sejrsen et al., 1982, 1983) showed that a high feeding rate impaired mammary development in heifers and altered secretion of mammogenic hormones. A subsequent meta-analysis (Zanton and Heinrichs, 2005) confirmed the negative effects of high average daily gain on future milk production. Continuing studies (Meyer et al., 2006a,b) have provided further cellular and endocrine insights into this phenomenon. It seems likely that enhanced prepubertal feeding essentially truncates the period of allometric mammary growth and thus blunts overall mammary development. However, it remains unclear how relatively small differences in mammary mass (relative to growth during gestation) produce future effects on milk production or whether this is only a marker.

To add further complexity, as summarized by Kahn et al. (2011), enhanced feeding of calves before weaning correlates with increased future milk production. We (Geiger et al., 2016a,b) showed that enhanced feeding of calves increases mammary development compared with restricted feeding. Demonstration of divergent effects of feeding rate before and after weaning illustrates the malleable nature of mammary development and how little we truly understand about links between early management of calves and heifers and future performance (Capuco and Akers, 2010).

Work primarily with nursing piglets has demonstrated that early colostrum feeding has tremendous effects on subsequent development of the reproductive tract and ultimately the success of the animals. As reviewed by Bartol et al. (2013), these studies led to the lactocrine hypothesis—the concept that biologically active agents (e.g., growth factors, hormones, and bioactive peptides) in colostrum and milk act to program postnatal uterine development (Bartol et al., 2008). Given that mammary development (and that of the reproductive tract) also occurs primarily postnatally, it is not surprising that early colostrum and milk feeding likely influence future mammary development. These concepts are easier to explore in litter-bearing species (due to cost, similarity of littermates, and so on). Regardless, such studies underscore the seemingly forgotten idea that mammary secretions evolved to provide not just nutrition to the suckling young but also protection and likely signaling molecules to promote growth and development. A recent report by Wilson et al. (2017) shows that preweaning restricted-fed calves have impaired endometrial gland development and alterations in growth factor–related signaling molecules compared with enhanced-fed calves, suggesting that level of nutrition, components in milk replacer, or both, affect reproductive tract development in calves.

HORMONES, GROWTH FACTORS, AND MAMMOGENESIS

Just as rodents were important in basic studies of mammary development, responses of rodents as bioassays were important in attempts to evaluate the expression of mammogenic hormones in cattle. For example, Nibler and Turner (1929) collected urine from pregnant cows and developed extracts for injection into rats to determine the relative secretion of ovarian hormones in cows (based on changes in cells appearing in vaginal smears). Other bioassays included the pigeon crop assay (growth and histological response of tissue of the crop sac to local injections of pituitary preparations and purified fractions) to evaluate prolactin-like activity, ovarian and uterine responses in rodents to assay for estrogen or progesterone-like activity, and growth responses in rodent tibia to assay for growth hormone activity.

Measuring Hormones and Growth Factors and Their Receptors

Although JDS was never a major destination for papers relating the development of radioimmunoassays (RIA) or other immunoassays, several reports have described use and validation of assays to measure concentrations of hormones and growth factors in bovine blood, milk, saliva, and tissues. As with many techniques and tools, development of assays sensitive enough to measure circulating concentrations of these agents had their origins in basic and medical research (see Tucker, 1981). Since the 1970s, RIA or related immunoassays have evolved to measure a plethora of hormones and growth factors known to be or suspected of being involved in regulation of mammary growth and mammary function. It is difficult to overstate the explosion of information and relationships between concentrations of these agents and physiological status that arose from the use of these technologies. Two JDS reports described validation of an RIA to measure serum prolactin in cows after injection of oxytocin (Koprowski and Tucker, 1971) and to measure luteinizing hormone in the blood of heifers during an estrous cycle (Hansel and Snook, 1970). Malven and McMurtry (1974) subsequently reported procedures to measure prolactin in milk. Since that time, numerous papers have described changes in concentrations of mammary active hormones relative to puberty, the estrous cycle, gestation, and the periparturient period as well as responses to dietary treatments, stimulated mammary growth, milking, stage of lactation, and breed (Convey, 1974; Erb, 1977; Tucker, 1981, 2000).

Related procedures using radiolabeled hormones and competitive binding to monitor changes in expression of hormone receptors in mammary tissue have been published in JDS. Examples include glucocorticoid receptors in mammary tissue slices (Gorewit and Tucker, 1976a,b), lactogenic pituitary hormones in mammary and liver cell membranes (Akers and Keys, 1984), and progesterone receptors in mammary tissue from pregnant ewes (Smith et al., 1987). As techniques evolved, Western blotting (essentially an extension of RIA and binding assays) allowed for detection and quantitation of mammary-relevant proteins separated on polyacrylamide gels and transferred to membranes. The technique used radiolabeled agents; that is, ligand binding with IGF-I to detect various IGF-I–binding proteins (Cohick, 1998; Weber et al., 2000) or antibodies to detect and measure intracellular signaling molecules (Murney et al., 2015c).

As described by Tucker (1981, 2000), the development of new tools, the isolation and identification of mammary active steroid and protein hormones, and ultimately the production and availability of these hormones and later growth factors (IGF-I and epidermal growth factor, EGF) stimulated rapid expansion of our understanding of endocrine and growth factor stimulation of mammary development. Several JDS reviews illustrate many of these relationships. Examples include circulating hormones and physiological state (Convey, 1974; Erb, 1977), mammary tissue and cell culture responses (Heald, 1974; Pitelka and Hamamoto, 1977; Houdebine et al., 1985), and actions of hormones and growth factors (Akers, 1985, 2006; Berryhill et al., 2016). Other scientists have reminded us of how little we understand or appreciate mammary and lactation variations among mammals and how insights might allow for solutions to dairy-specific issues. These examples include local versus systemic control of mammary development and function as illustrated by the wallaby and seals (Brennan et al., 2007), evolutionary and comparative aspects of lactation (Blackburn, 1993; Oftedal, 1993), and endocrine and growth factor species variation (Forsyth, 1986, 1996). Others have described new frontiers for the mammary gland (Bremel et al., 2001; Hadsell et al., 2002), including the mammary gland as bioreactor, and transgenic animals (Karatzas and Turner, 1997; Donovan et al., 2001).

Somatotropin, Growth Hormone, and bST

Interest in the effects of growth hormone (**GH**) on mammary development (mammogenesis) and milk production (galactopoiesis) can hardly be overstated. A simple search using "bST", "somatotropin", or "GH" as key words in the title or abstract yielded 328 full articles in JDS as of February 2017. Certainly, a great deal of the interest in GH in the last 30 yr or so derives from the availability of bST from recombinant DNA technology and the exhaustively studied positive effects of exogenous bST on milk production and mobilization of nutrients to support increased milk production in cows, goats, sheep, and camels. Comprehensive JDS reviews (Bauman and Currie, 1980; Peel and Bauman, 1987; Bauman, 1992) and studies (Bauman et al., 1985, 1999) illustrate my belief that bST remains the most highly tested and studied recombinant DNA–derived product ever produced for use in dairy ruminants. Given the market and consumer forces that limit use of bST in the United States and Western Europe, despite rigorous safety assurances and Food and Drug Administration approval in 1994, it remains to be seen how attitudes of companies toward development of products to increase growth or lactation responses will fare in the future. As I noted (Akers, 2006), reasons for the debate surrounding the approval and subsequent use of bST in dairy cows were varied. It is possible to stimulate native GH secretion and associated increases in milk production but this involves use of exogenous treatment with GH-releasing factor (Dahl et al., 1991, 1993). It is interesting to realize that one of the earliest large-scale demonstrations on the effectiveness of GH (pituitary fractions in this case) to increase milk production in cows was by Russian researchers Asimov and Krouze (1936) and was published in JDS. In the context of today's political atmosphere, this seems otherworldly. Indeed, current societal conditions indicate a widespread lack of confidence, belief, or reliance on science and scientific principles by the public (Nichols, 2017; Strauss, 2017; Weiss, 2017), which does not bode well for the capacity of our citizens and citizen-leaders to make logical, well-reasoned decisions. This is regardless of whether the issues are mammary development and use of recombinant proteins, genetically modified plants and animals, or occurrence and causes of climate change.

Gene Expression and Signaling Cascades

The convergence of biotechnology, gene splicing, PCR, monoclonal antibody production, and other related technologies beginning in the 1980s ushered in many new opportunities and tools to study relationships between hormones and growth factors and mammary growth and function. McCarty and McCarty (1975) were among the first researchers in JDS to discuss possible links among binding of hormones to their receptors on (or in) mammary cells, phosphorylation events, and synthesis of RNA. Gorski (1979) subsequently provided one of the first JDS reviews to discuss the significance

of interactions between circulating concentrations of mammary active hormones, receptors, and intracellular signaling cascades relative to the mammary gland. Specific to induction of gene expression, initial studies largely depended on Northern assays. This technique required the generation of specific radiolabeled (generally ^{32}P) nucleic acid probes that were complementary to the mRNA of interest. For this analysis, mRNA was isolated from cells or tissues, separated on gels, transferred to membranes, incubated with the probe, washed, and exposed to X-ray film to detect the mRNA of interest. This effective but laborious method allowed the study of effects of hormone stimulation, dietary treatments, and other factors on induction of gene expression. One of the earliest JDS reports described increases in casein mRNA using a dot blot assay (essentially the Northern assay without separation of RNA on a gel). Choi et al. (1988) showed that bovine mammary cells incubated with prolactin had increased expression of casein mRNAs, and greater expression of casein mRNAs was observed in mammary tissue from lactating compared with 8-mo-pregnant cows. Glimm et al. (1992) reported decreased expression of IGF-I receptor mRNA in mammary tissue of lactating cows treated with bST, and Weber et al. (2000) reported effects of feeding level and bST on expression of IGF-I and IGF-binding proteins in mammary tissue of prepubertal heifers. Schanbacher et al. (1993) and Koff and Plaut (1995) noted changes in mammary expression of lactoferrin mRNA and transforming growth factor mRNA, respectively, during the lactation cycle.

The subsequent development of quantitative real-time PCR, microarrays, and recently RNA sequencing has dramatically increased the both the frequency of gene expression measurements and the array of gene targets. A JDS keyword search for [gene expression + mammary] within the title, abstract, or keywords yielded 152 papers. A similar PubMed search restricted to [gene expression, mammary, and bovine] provided 257 hits. Examples of JDS papers related to control of mammary development include Connor et al. (2007), concerning mammary gene expression regulated by ovarian steroids, and Nørgaard et al. (2008), which tracked expression of genes involved in cell turnover in the dry period and into lactation. Broader evaluations of mammary gene expression include Piantoni et al. (2012), with a study of the effects of diet on gene expression in the PAR and MFP of calves, and changes in the clock circadian regulator gene network in mammary tissue during the transition to lactation (Wang et al., 2015). Stiening et al. (2008) provide an especially interesting and through evaluation of the impacts of lactogenic hormones and mechanical stimulation on gene expression of bovine mammary cells in culture.

Although the availability of tools for more robust and global evaluation of gene transcription has provided voluminous information and unexpected relationships between signaling and metabolic pathways and gene networks, we have learned that transcription of mRNA does not necessarily mean translation of the message and creation of the mature protein. Consequently, tools to detect and measure gene products remain an important and critical element to understand regulation of mammary development and function. Therefore, continued use of tools such as Western blotting (detection and quantitation) and functional assays, and measurement of metabolites are critical to comprehensive understanding. As examples, Murney et al. (2015a,b,c) reported effects of unilateral milking frequency on cell proliferation, gene expression for milk proteins, changes in IGF-I-linked signaling molecules and prolactin receptor, and STAT3 and STAT5 signaling pathways.

Discovery of tissue and cellular localization of signaling molecules, receptors, and other mammary relevant proteins have been an important part of learning about regulation of mammary development and function. Often, these reports have depended on various combinations of immunochemistry and immunohistochemistry. Larson and Twarog (1961) illustrated the relevance of an immunological assay when they reported quantitation of β-lactoglobulin in solution via a simple immunoprecipitation method. The earliest JDS immunocytochemistry papers involved localization of IGF-I receptors after treatment with bST (Glimm et al., 1992), effect of prolactin on induction of immunoglobulin receptors (Barrington et al., 1997), and localization of fibroblast growth factors during different development states (Plath et al., 1998). Such studies are important because they allow identification of proteins within specific populations of cells in mammary tissue or, in some cases, within cellular organelles. For example, the study by Malven and Keenan (1983) demonstrated the presence of immunoreactive prolactin within various subcellular fractions of bovine mammary cells. The study suggested that internalization of prolactin after receptor binding is linked to its transport via secretory vesicles into milk. A subsequent study (Akers and Kaplan, 1989) showed that disruption of microtubules, known to arrest cellular secretion, also interfered with transfer of prolactin from blood into milk. As suggested earlier, increased availability of specific antibodies to recognize targets on bovine cells (i.e., flow cytometry and analysis of circulating immune cells) or within bovine tissues has been key to discovery of multiple pathways and molecules that influence the bovine mammary gland. As a recent example, consider the fascinating and still unfolding story of how the classic neurotransmitter serotonin and its large family of

receptors act to modulate milk secretion (Hernandez et al., 2008; Weaver and Hernandez, 2016).

Ellis et al. (2012) discussed the utility and promise of imaging and computer advances that increasingly allow quantitative evaluation of the expression of receptors, transcription factors, and other important mammary proteins in populations of defined mammary cells within the mammary gland. Safayi et al. (2012) report the quantitative expression of markers for myoepithelial cells in mammary tissue from heifers during early development. Velayudhan et al. (2015) and Tucker et al. (2016) have reported not just the changes in the proportion of heifer mammary epithelial cells expressing estrogen and progesterone receptors but also the difference in expression of the receptors based on quantitative multispectral imaging using immunocytochemistry. This approach is essentially quantitative Western blot analysis applied to specific, identifiable populations of cells within the developing tissue. These examples simply confirm the importance of ongoing analytical and technical advances in our continued effort to understand mammary growth and function.

Cell and Tissue Culture

Incubations of bovine mammary tissues (slices or explants) or longer-term culture of isolated mammary acini or organoids (alveolar-like or duct-like isolated epithelial structures) and isolated epithelial cells (primary or immortalized) have a long history in the study of mammary cell proliferation, differentiation, and milk component biosynthesis. Kinsella (1968) authored an early JDS study demonstrating that mammary cells isolated from lactating cows could synthesis and secrete lipids based on incorporation of radiolabeled acetate into various lipid fractions. In a similar approach, Richmond and Hood (1973) showed incorporation of radiolabeled glucose into lactose by explants of mammary tissue from a lactating cow. Heald and Saacke (1972), although using mammary tissue from lactating rats, used tissue incubations or infusion of radiolabeled leucine along with timed fixation and autoradiography of sectioned tissue to track synthesis and secretion of milk proteins. Specifically, they showed progressive peak appearance of labeled proteins in the endoplasmic reticulum (3–5 min), to the Golgi (15–20 min), and into the alveolar lumen (40–60 min). In addition to demonstrating the utility of tissue culture, these examples illustrate the significance of availability of various radiolabeled substrates for measures of milk component biosynthesis.

Following initial characterization of isolation methods and culture procedures (Ebner et al., 1961), Larson (1965, 1969) reviewed the status of regulation of milk protein biosynthesis and especially the utility of results obtained by various culture techniques. Subsequently, Larson (1972) showed the stimulatory effect of methionine on milk protein synthesis, and Rao et al. (1975) demonstrated the effects of cell density on synthesis of lactose by bovine mammary cells. Progressing from cultures with rat mammary cells, Clark et al. (1978) reported studies that defined the limiting amino acids for milk protein synthesis by bovine mammary cells in culture. Park et al. (1979) subsequently described a method to isolate acini (alveoli) from lactating bovine mammary tissue and demonstrated specific synthesis of milk proteins.

Incubations of explants (Rivera, 1972) from pregnant animals or those treated with estrogen and progesterone were especially important in the delineation of minimal hormones needed to induce the secretory cell differentiation (structural and biochemical) necessary for milk synthesis and secretion. As reviewed by Heald (1974), rodent studies first characterized the effects of addition of insulin, hydrocortisone, and prolactin on the ultrastructure of mammary alveolar cells in mammary explants. Subsequent bovine studies characterized the dramatic differentiation of mammary cells just before and after parturition, as well as biochemical and structural responses of alveolar epithelial cells to addition of lactogenic hormones (Collier et al., 1977; Nickerson et al., 1978). Additional experiments have confirmed the importance of insulin and IGF-I in maintenance of mammary tissue, the importance of glucocorticoids in differentiation of alveolar secretory cells, and the importance of prolactin in induction of milk protein and lactose biosynthesis. For example, Goodman et al. (1983) demonstrated very clearly the dramatic positive effect of prolactin on induction of α-lactalbumin synthesis and secretion by bovine mammary explants as well as interplay between lactogenic hormones. That is, the presence of estrogen or hydrocortisone enhanced the inductive effect of prolactin, whereas progesterone had an inhibitory effect. These tissue responses closely mirrored the hormonal changes in mammogenic and lactogenic hormones around the time of parturition and correspondence with the onset of lactogenesis at calving. As noted earlier, explant cultures were also important tools in discovery of hormones and growth factors that stimulated proliferation of mammary cells.

CONTROL OF LACTOGENESIS AND GALACTOPOIESIS

As results from measurements of circulating concentrations of mammogenic and lactogenic hormones

became widely available in the late 1960s and 1970s, increasing attention was focused on hormonal regulation of the onset of lactation and hormones involved in the maintenance of lactation. Along with accurate hormone data, tools to evaluate milk component biosynthesis (Bauman et al., 2006) helped renew focus on understanding the onset of lactation. For example, incorporation of radiolabeled precursors into specific milk components (lactose, milk proteins, lipids; Bauman et al., 1973; Mellenberger et al., 1973), enzyme assays (Baldwin, 1966, 1969; Baldwin and Cheng, 1969), direct assays for milk components, and understanding of lactose synthesis (i.e., the significance of α-lactalbumin; Brew et al., 1968) prompted research efforts. In addition, application of higher resolution cytology and electron microscopy to characterize structural differentiation associated with onset of natural (Heald, 1974) or induced lactation (Collier et al., 1976; Croom et al., 1976) and milk removal (Akers et al., 1977; Akers and Heald, 1978) allowed researchers to piece together actions of hormones regulating differentiation of the alveolar epithelium and lactogenesis.

Just as surgical ablation and endocrine replacement helped identify key mammogenic and lactogenic hormones, selective pharmacology approaches allowed evaluations that were more specific. For example, the creation and availability of the dopamine agonist CB154 (2-Br-α-ergocryptine) allowed selective inhibition of prolactin secretion. Treatment with CB154 reduced prolactin in cows and was associated with reduced milk production after calving. However, studies by Akers et al. (1981a,b) showed that inhibition of prolactin secretion at calving impaired milk production in association with decreased biochemical and structural differentiation of the alveolar epithelium. Furthermore, infusion of prolactin along with treatment with CB154 restored milk production and full differentiation of the alveolar secretory cells. These data along with responses of mammary tissue and cells to prolactin confirmed the importance of prolactin for lactogenesis in cows. Interestingly, most subsequent studies (unlike in many other mammals) showed that neither inhibition nor administration of exogenous prolactin following parturition appreciably altered milk production in cows. However, recent studies using newly developed agents suggest that prolactin is likely galactopoietic in multiple dairy ruminants (Lacasse et al., 2016).

Debate surrounding regulation of lactogenesis focused on 2 hypotheses: that milk synthesis was initiated (1) because of a release from inhibition (i.e., removal of progesterone) or (2) by positive stimulation via increased secretion of cortisol and prolactin at the time of parturition. In other words, is lactogenesis pushed by positive agents or allowed to proceed by release from inhibition? The answer seems to be a bit of both. Certainly, changes in circulating concentrations of progesterone (i.e., dramatic decrease) within days and hours before parturition that coincide with increases in cortisol and prolactin just before and after parturition support this concept (Convey, 1974). Moreover, in mammary explants, prolactin-induced secretion of α-lactalbumin, synergism with added hydrocortisone, and antagonism of secretion with added progesterone support this idea (Goodman et al., 1983). Responses involving α-lactalbumin are especially telling, given that secretion of lactose is the primary driver of milk volume once lactation is established. In pregnant heifers, serum concentrations of α-lactalbumin become detectable in the last trimester of gestation, with modest increases until just before calving when concentrations markedly increase. This response pattern closely mirrors a 2-stage onset of lactogenesis, with a modest increase (stage I) in milk component biosynthesis in the last month before calving followed by a marked increase (stage II) just before and after calving (McFadden et al., 1987). Measurements of mammary tissue activity (incorporation of radiolabeled precursors into milk components and increased expression of milk protein genes) and increased structural differentiation support this pattern of development. In addition to changes in circulating hormones, there are corresponding changes in the expression of glucocorticoid, prolactin, and progesterone receptors.

Specific to the role of progesterone in inhibition of onset of lactation, there is also the curious situation of simultaneous pregnancy and lactation in cows and the apparent lack of inhibition of high progesterone concentrations on milk synthesis. This seems to reflect differences in expression of progesterone receptors in mammary tissue of pregnant lactating cows compared with mammary tissue of nonlactating cows late in the dry period or in the mammary tissue of late pregnant heifers (Capuco et al., 1982). Schams et al. (2003) provided gene expression data for bovine mammary tissue expression of estrogen and progesterone receptors and an immunocytochemical survey for staining across developmental stages. However, to my knowledge, no one has evaluated either the proportion of mammary cells expressing the progesterone receptor or the degree of expression in positive cells using modern quantitative immunocytochemical techniques for tissues in these 2 distinct conditions.

As described earlier, a wealth of information demonstrates the galactopoietic responses to GH, as well as the metabolic adjustments that allow the nutrient mobilization to support increased milk production

(Hart et al., 1979; Bauman and Currie, 1980; Bauman et al., 1985). Evidence also shows that cows selected for increased milk production correspondingly exhibit increased secretion of GH (Barnes et al., 1985; Kazmer et al., 1986).

Induced Lactation

The desire to induce lactation in nonpregnant animals, even humans, is not new. Consider the comments by Becker and McGilliard (1930): "Literature relative to milk secretion by virgin and non-fecund females, and males of several species have been reviewed extensively by Hammond (4), Hill (5), Marshall (6), Movoisin (7) and Velich (8)." Two short volumes (Fulkerson, 1979, 1981) provide summaries of work focused on the hormonal control of lactation across multiple species and, in particular, a comprehensive listing of induced lactation studies for multiple species. The first successful induction of lactation in a ruminant was in the goat (Frank and Rosenbloom, 1915) via injection of crude lipid extracts prepared from ovaries. The discovery and isolation of estrogen by Allen and Doisy (1923) led to production of synthetic estrogens and availability of these agents. Many of the early studies utilized long-term injections of estrogens and later progestins. These longer-term treatments mimicked a timeframe similar to that of gestation length (Turner et al., 1956).

Two reports (Smith and Schanbacher, 1973, 1974) ushered in renewed interest in hormonal induction of lactation that created a flurry of research activity. Their initial efforts aimed to stimulate colostrum-like secretions and immunoglobulin production in nonlactating cows. They observed that some of the steroid-treated animals began to "bag up" after a week of treatment. With continued collection of samples, the composition became less colostrum-like and more milk-like in appearance. This led to the development of a short-term protocol that successfully induced lactation in most treated cows. Subsequent modifications included treatment with glucocorticoids near the time of onset of milking, treatments to increase prolactin secretion during the "lactogenic" phase of induction, effects of season, response to exogenous bST, and so on in multiple dairy ruminants. Comparisons have also included induction of animals that failed to conceive as well as heifers before normal breeding age (Macrina et al., 2011, 2014). Induced lactation in young heifers provides a means to collect milk before a normal lactation for early testing of transgenic animals as possible mammary bioreactors (Ball et al., 2000; Kaiser et al., 2017). Variations on this short-term induced lactation scheme continue to be explored.

Photoperiod Effects on Mammary Development and Milk Production

A report in *Science* by Peters et al. (1978), which demonstrated that supplemental lighting stimulated growth and increased milk production in dairy cows, stimulated many papers evaluating the effects of altered photoperiods on mammary growth, secretion of mammary active hormones, and feeding behavior in dairy ruminants, followed by studies to discover the mechanisms involved in these responses. Subsequent reviews (Dahl et al., 2000; Collier et al., 2006) outlined the effects of photoperiod, its practical uses, and more broadly, the environmental effects on dairy cows.

Milking Interval, Milking Frequency, and Milk Ejection

Interest in the effects of milking on milk production, composition, and productivity has a long history in JDS. Ragsdale et al. (1924) was the first JDS report concerned with the effect of milking interval; that is, the effect of milk accumulation on milk secretion. Such studies confirmed the relevance of 12-h milking intervals for twice-daily milking. A quote from a *Hoard's Dairyman* article emphasizes ongoing discussion of increasing milk frequency from the usual twice-daily milking: "Practical dairymen (3) have also observed the advantages of frequent milking" (Riford, L. D., 1922, *It pays to milk 3 times a day*. LXIII, no. 19, 661). Interest in udder capacity and effects of milking interval and milk accumulation have evolved to consider effects of residual milk and internal pressure on the apparent rate of milk secretion (Tucker et al., 1961).

Milking frequency has been studied repeatedly (Pearson et al., 1979), and there is no doubt that increasing milking frequency from 2 to 3 or 4 times a day increases milk yields. However, economics and especially labor costs and availability of labor are key factors. In recent years, as herd sizes have increased, more frequent milking has also increased on many dairies. An especially interesting twist has evolved from experiments showing that increased milking or suckling in early lactation could produce carry-over effects such that the animals (or glands) that received extra milking continued to product more milk later in lactation when milking frequency returned to "normal" (Bar-Peled, 1995). This highly cited paper stimulated many studies focused on trying to understand the mechanisms responsible for the continued increase in milk production when cows revert to a normal milking frequency after a period of increased milking frequency in early lactation (Hale et al., 2003; Wall et al., 2006; Wall and McFadden, 2010; Murney et al., 2015a).

As reviewed (Petersen, 1942; Bruckmaier and Blum, 1998), discovery of the principles behind the milk ejection reflex and factors involved in milking and suckling led to development of the modern milking machine. The first reported demonstration of the effect of oxytocin (i.e., a solution prepared from the posterior pituitary) on milk let-down was by Ott and Scott (1910). Bruckmaier and colleagues have published extensively on the effects of milking procedures on release of oxytocin (Bruckmaier and Blum, 1998), proportions of cisternal versus alveolar milk, factors influencing milk ejection, effects of milk accumulation on transport of milk and serum components across the milk–blood barrier, and general effects on milk secretion. Many of these papers have appeared in the *Journal of Dairy Research* (e.g., Bruckmaier and Blum, 1996; Kaskous and Bruckmaier, 2011) but recent JDS papers illustrate these ongoing efforts (Weiss et al., 2004; Waters et al., 2015; Besier et al., 2017). Indeed, Dr. Bruckmaier's extensive bibliography (i.e., 65 papers published in JDS since 2010) suggests that milk secretion research for dairy ruminants is now largely a European affair. Specific to the United States, I think this reflects a lack of ongoing institutional research support for dairy research (direct research expenses, graduate student, and technical support) and minimal competitive federal grant funds to support lactation biology research in farm animals. In addition, even among the US land-grant universities, the traditional focus on farm animal research and the balance among tripartite missions (research, teaching, and extension) are increasingly disturbed. Trends suggest that an emphasis on short-term trials to increase profitability, seeking patent possibilities, supporting local new entrepreneurs, diverting faculty efforts toward human biomedical research, "big" science, and creating teams, centers, or destination areas will continue (Fribourg, 2005). It is also evident that most basic dairy animal research funding depends on highly competitive grants. This situation seems to minimize sustained efforts in broad topical areas in favor of efforts to make the latest grant proposal awash with the latest buzzwords or scientific fads. Pundits, not so tongue in cheek, frequently comment that American agriculture, including the dairy industry, is a victim of its own success. It remains to be seen how much mammary biology research, slanted toward dairy animals, can continue in the future.

It is certainly true that concerns about support for animal agriculture research and training of future scientists are not new. Consider the following quote from Thompson (1973), "Currently there is a clarion call from numerous federal sources for relevance in research. If we gear our research primarily to someone else's need to solve immediate ... problems or moneymaking schemes, our hopes of scholarly excellence ... better faculty, better students, .. may never be filled." More recently, Senger (2008) and Roberts et al. (2009) similarly lament the relative lack of support for farm animal research and training for future animal and dairy scientists in the United States. If the internationalization of JDS over the past decade is any indication, we can be grateful that the rest of the world apparently recognizes the ongoing relevance of dairy research in general and continued study of the development and function of the mammary gland specifically.

Mammary Stem Cells and the Bovine Mammary Gland

Clearly, the past decade or so has ushered in a wealth of information regarding the identification, characterization, isolation, and quantification of putative mammary stem cells (**MaSC**). It should not be a surprise that progress has been much more extensive in rodent models than in cattle. Furthermore, the gold standard for identification of MaSC has been the demonstration that even a single isolated MaSC (Kordon and Smith, 1998; Shackleton et al., 2006) can regenerate a complete mammary gland when transplanted into cleared (i.e., native epithelium removed) MFP in mice. Nonetheless, some progress has been made related to identification and counting of presumptive bovine MaSC and progenitor cells. Very few MaSC-related papers appear in JDS but consideration of this topic is nonetheless very relevant.

For background, true MaSC can undergo 2 types of cell division. With symmetric division, 2 daughter stem cells are formed and the stem cell population expands. With asymmetric division, there is self-renewal of the stem cell and production of a progenitor cell. These "common" progenitor cells subsequently generate daughter cells that are the progenitors for the ductal and luminal epithelial cells and the myoepithelial cells. This scheme, as tailored to the bovine, has been previously described (Capuco et al., 2012).

Regardless of the experimental hurdles (Capuco and Ellis, 2005; Capuco et al., 2012), several studies have sought to identify stem and progenitor cells in the bovine mammary gland. Ellis and Capuco (2002) quantified proportions of lightly stained, intermediate, and darkly stained epithelial cells in growing bovine mammary glands. The population (~10% of the total) of lightly stained epithelial cells in tissue sections accounted for about 50% of the proliferating cells. The conclusion was that these "pale" cells included a mixture of stem cells and progenitor cells. Capuco (2007) subsequently de-

scribed the identification and quantitation of putative bovine MaSC based on long-term labeling of DNA with 5-bromo-2-deoxyuridine (**BrdU**); that is, the presence of label-retaining epithelial cells (**LREC**) coupled with the absence of estrogen receptor (**ESR1**) expression. The number of heavily labeled cells correlated with expected differences in cell proliferation in regions of the developing udder based as well as proportions of MaSC based on murine studies. Choudhary et al. (2013) used laser capture microdissection and gene expression to evaluate the transcriptomes of LREC positioned in the mammary epithelium compared with LREC embedded within the epithelium. They also compared LREC with nonlabeled control cells within the ductal epithelium. They reported 592 genes differentially expressed between basal LREC and basally located control cells as well as 110 genes differentially expressed genes between LREC embedded within the epithelium and control epithelial cells also embedded within epithelium. These data support the idea that basally located ESR1-negative LREC are likely true bovine MaSC, whereas LREC positioned within the epithelial layer are more likely common progenitor cells. In companion studies, these researchers (Capuco et al., 2009) showed that intramammary infusions of xanthosine increased the population of presumptive MaSC or progenitor cells.

Others have used enzymatic digestion of mammary tissue and cell sorting with panels of antibodies to separate populations of epithelial cells to characterize MaSC and progenitor cells (Motyl et al., 2011; Rauner and Barash, 2012). In particular, researchers use expression of multiple members of the cluster of differentiation (CD) proteins to segregate populations of epithelial cells believed to represent authentic MaSC and various progenitor cells. These isolated cells are then tested in various cell cultures (e.g., formation of colonies, development of mammospheres, growth response, or appearance of duct-like or alveolar-like structures) following transplantation into the cleared fat pads of immunocompromised nude mice. The appearance of specific phenotypes has allowed the putative identifications of ductal, alveolar, and myoepithelial progenitor cells and bovine MaSC (Rauner and Barash, 2016) as well as estimation of populations over the lactation cycle (Perruchot et al., 2016). Others have estimated the effects of prepubertal nutrition (Daniels et al., 2009), ovariectomy (Ellis et al., 2012), or treatment with antiestrogens (Tucker et al., 2016) on populations of putative bovine MaSC via counting of LREC. Others have estimated possible populations of bovine MaSC based on counting of cells expressing HNF4A in water buffalo (Choudhary et al., 2016) or Musashi-I in mammary tissue from sheep (Colitti and Farinacci, 2009) across different stages of development. Specific to myoepithelial cells and presumptive myoepithelial progenitor cells, Ellis and colleagues (Ballagh et al., 2008; Safayi et al., 2012; Tucker et al., 2016) have shown that smooth muscle actin or common acute lymphoblastic leukemia antigen are excellent cytoplasmic markers and transformation-related protein 63 an excellent nuclear marker for basally located myoepithelial cells and their progenitors.

Despite the experimental advances, responses of presumptive bovine MaSC or progenitors in culture or following transplantation into mice does not really reproduce the bovine mammary gland and its function. Certainly, these tools and approaches are valuable but healthy skepticism is warranted. The recent report (Bruno et al., 2017) demonstrating that the extracellular matrix isolated from the mammary gland can induce embryonic or testicular cells to acquire a mammary phenotype structurally and physiologically illustrates the significance of tissue environment in regulation of mammary morphogenesis and function. As dairy lactation physiologists, our focus is correctly on the developing glandular tissue but this finding is an important reminder that the local environment of the epithelium is also a critical player in mammary development and function.

In summary, despite many years of study evaluating the effects of hormones and growth factors, diet and management, genetics, and other factors on regulation of ruminant peripubertal mammary development and associated expression of genes and proteins, we remain far from complete understanding. However, new and exciting imaging tools (Ellis et al., 2012) and the capacity to identify and study distinct cell populations within the growing mammary gland continue to provide opportunities and unexpected approaches to decipher the keys that control mammary development and ultimately function.

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APPENDIX

Table A1. Timeline of important discoveries and developments in mammary development and lactation

Date	Milestone	Reference
1910s–1920s	Mammary active hormones are discovered, including oxytocin, ovarian hormones, and anterior pituitary hormones.	Ott and Scott, 1910; Allen and Doisy, 1923; Stricker and Grueter, 1928 (reviewed in Tucker, 2000)
1930s	Evaluations of mammary growth and development are published.	Swett and Matthews, 1934; Turner, 1952 (review and summary)
1936	First large-scale report is published on the galactopoietic effect of growth hormone (pituitary extracts) in cows.	Asimov and Krouze, 1936
1940s	Study effects of milk frequency, milk removal, breeds, nutrition, thyroproteins, exogenous hormones, and milk ejection reflex are reported.	Lewis and Turner, 1941; Peterson, 1942
1953	DNA is first used to quantify mammary growth (in rat).	Kirkham and Turner, 1953
1960s	Cell and tissue culture techniques are developed for bovine.	Ebner et al., 1961
1961	DNA is first used to quantify mammary growth in bovine.	Williams and Turner, 1961a,b
1964	Restoration of milk production is demonstrated in hypophysectomized goat.	Cowie et al., 1964
1969	Isometric versus allometric mammary growth (DNA) is described in peripubertal heifers.	Sinha and Tucker, 1969
1960s–1970s	Radioisotopes are used to measure milk component biosynthesis and mammary tissue enzyme activity.	Baldwin, 1966; Larson, 1972; Mellenberger et al., 1973
1970s	Radioimmunoassays are developed for hormones and growth factors in bovine blood and milk.	Hansel and Snook, 1970; Koprowski and Tucker, 1971; Malven and McMurtry, 1974
1973	Shortened induced lactation scheme is reported for bovine.	Smith and Schanbacher, 1973, 1974
1976	Glucocorticoid receptors are measured in bovine mammary tissue.	Gorewit and Tucker, 1976a,b
1977	Lactogenic complex is demonstrated in explant cultures of bovine mammary tissue.	Collier et al., 1977; Nickerson et al., 1978
1978	Effects of an increased photoperiod on milk production are described in cows.	Peters et al., 1978 (reviewed by Dahl et al., 2000)
1980s	Steroid and prolactin receptors are measured in bovine tissue.	Capuco et al., 1982; Akers and Keys, 1984

Continued

Table A1 (Continued). Timeline of important discoveries and developments in mammary development and lactation

Date	Milestone	Reference
1982	Nutrition is shown to affect periparturient mammary development in bovine.	Sejrsen et al., 1982, 1983
1984	Lactogenic hormone receptors described in bovine mammary tissue.	Akers and Keys, 1984
1985	Galactopoietic effectiveness and safety of recombinant bST is demonstrated.	Bauman et al., 1985
1986	Growth hormone regulation of mammary growth is demonstrated in peripubertal heifers.	
1988	Early gene expression studies in bovine are conducted using Northern analysis and other techniques.	Choi et al., 1988
1990s	Initial measures of gene expression in bovine mammary gland are reported.	Schanbacher et al., 1993; Koff and Plaut, 1995
1992	Bovine somatotropin is recognized as an emerging animal technology.	Bauman, 1992
1994	US Food and Drug Administration approves recombinant bST for use in lactating cows.	Bauman et al., 1999
1990s–2000s	Transgenic animals and the mammary gland as bioreactor show fleeting promise.	Karatzas and Turner, 1997; Bremel et al., 2001; Donovan et al., 2001
1990s–2000s	Researchers recognize effects of altered photoperiods, milking frequency, and environment (e.g., heat stress) on mammary development and function.	Dahl et al., 2000; Collier et al., 2006
2003	Estrogen and progesterone receptors are localized in bovine mammary gland using immunocytochemistry.	Schams et al., 2003
2003–2010	Milking frequency in early lactation is shown to have carry-over effects on mammary gene expression.	Hale et al., 2003; Wall et al., 2006, Wall and McFadden, 2010
2007	Ovarian steroids are shown to regulate gene expression in bovine mammary gland.	Connor et al., 2007
2008	Evolving significance of serotonin and its receptors in local regulation of mammary function is recognized.	Hernandez et al., 2008 (reviewed in Weaver and Hernandez, 2016)
2008	The lactocrine hypothesis—mammary secretions are more than just neonatal nutrition—is proposed.	Bartol et al., 2008
2010–current	Bovine mammary "-omics" data (e.g., proteomics, transcriptomics, metabolomics) continue to rapidly expand.	Wang et al., 2015
2010–current	Putative bovine mammary stem cells are discovered and identified.	Capuco and Ellis, 2013; Rauner and Barash, 2016

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A 100-Year Review: Reproductive technologies in dairy science[1]

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ABSTRACT

Reproductive technology revolutionized dairy production during the past century. Artificial insemination was first successfully applied to cattle in the early 1900s. The next major developments involved semen extenders, invention of the electroejaculator, progeny testing, addition of antibiotics to semen during the 1930s and 1940s, and the major discovery of sperm cryopreservation with glycerol in 1949. The 1950s and 1960s were particularly productive with the development of protocols for the superovulation of cattle with both pregnant mare serum gonadotrophin/equine chorionic gonadotrophin and FSH, the first successful bovine embryo transfer, the discovery of sperm capacitation, the birth of rabbits after in vitro fertilization, and the development of insulated liquid nitrogen tanks. Improved semen extenders and the replacement of glass ampules with plastic semen straws followed. Some of the most noteworthy developments in the 1970s included the initial successes with in vitro culture of embryos, calves born after chromosomal sexing as embryos, embryo splitting resulting in the birth of twins, and development of computer-assisted semen analysis. The 1980s brought flow cytometric separation of X- and Y-bearing sperm, in vitro fertilization leading to the birth of live calves, clones produced by nuclear transfer from embryonic cells, and ovum pick-up via ultrasound-guided follicular aspiration. The 20th century ended with the birth of calves produced from AI with sexed semen, sheep and cattle clones produced by nuclear transfer from adult somatic cell nuclei, and the birth of transgenic cloned calves. The 21st century has seen the introduction of perhaps the most powerful biotechnology since the development of artificial insemination and cryopreservation. Quick, inexpensive genomic analysis via the use of single nucleotide polymorphism genotyping chips is revolutionizing the cattle breeding industry. Now, with the introduction of genome editing technology, the changes are becoming almost too rapid to fully digest.
Key words: artificial insemination, multiple ovulation and embryo transfer, in vitro embryo production, sexed semen

INTRODUCTION

Artificial insemination was the first reproductive technology applied to cattle, initially in Russia and Denmark during the early 1900s (Ivanoff, 1922; Perry, 1945). The primary driving force behind AI was its potential to increase the rate of genetic gain in livestock populations by widespread use of sires with elite genetic merit. Cooperative AI centers began in Denmark in 1936 and were replicated internationally (Perry, 1945). Not since the invention of the milking machine has a technology had such an effect. For farmers, transition away from natural service breeding required changes in herd reproductive management. In addition to genetic gains, AI breeding avoided the need to have bulls on each farm and contributed to improved safety for farm employees. Today, use of AI has grown to the extent that internationally approximately 130 million cattle are submitted for AI annually (Vishwanath, 2003).

Birth of the first calves from the use of frozen–thawed semen (Polge and Rowson, 1952) and embryos (Wilmut and Rowson, 1973) represented important milestones during the past century. Both developments were critical to the feasibility and growth of large-scale AI and embryo transfer (**ET**) operations globally because it became no longer essential to use only unfrozen (fresh) semen and embryos. Today, the vast majority of inseminations and transfers are performed with frozen–thawed semen and embryos, respectively (Vishwanath, 2003; Hasler, 2014).

Techniques for multiple ovulation and ET for cattle were developed in the 1940s and 1950s (Casida et al., 1943; Rowson, 1951; Willett et al., 1951; Dziuk et al., 1958); however, large-scale ET operations were not established in North America until the 1970s, in Europe until the 1980s, and in South America until the 1990s (Hasler, 2014). In vitro developments in oocyte maturation and sperm capacitation, fertilization, and embryo

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Table 1. Key events in our understanding of reproductive biology before the early 1900s[1]

Event	Year
de Graaf describes testis structure.	1668
de Graaf describes follicle structure.	1672
Van Leuwenhoek and Ham describe spermatozoa.	1678
Spallanzani reports AI of a bitch followed by birth of pups.	1784
Spallanzani reports that sperm survived chilling.	1803
Von Baer describes oocytes.	1827
Kölliker demonstrates that the testes produce sperm.	1841
Barry observes the fusion of sperm and oocyte.	1843
Leydig describes Leydig cells.	1851
Sertoli describes Sertoli cells.	1865
Waldeyer describes the ovary and oocyte formation.	1870
Von Ebner describes spermatogenesis.	1871
Dreisch demonstrates artificial embryo twinning in the sea urchin (an invertebrate).	1885
Heape performs the first mammalian embryo transfer.	1890
Spemann demonstrates artificial embryo twinning in the salamander (a vertebrate).	1902

[1]Data from Marshall (1910); Genetic Science Learning Center (2014).

culture during the 1970s and 1980s led to the birth of the first completely in vitro embryo produced calves in 1987 (Lu et al., 1987). The ovum pick-up (**OPU**) method for repeated oocyte recovery from live donor females was developed during the late 1980s by Pieterse et al. (1988). Protocols for in vitro embryo production (**IVP**) were further developed in the 1990s as an alternative to multiple ovulation and ET by combining OPU, in vitro fertilization (**IVF**), and ET (Looney et al., 1994). The practice of IVP has grown rapidly since the 2000s, with large-scale commercial operations established primarily in South America (Hasler, 2014).

Cloning techniques for production of identical sheep began in the 1970s, first by embryo splitting (Willadsen, 1979) and subsequently replaced by nuclear transfer (Willadsen, 1986; Prather et al., 1987). A far more powerful technology, however, involved what is referred to as somatic cell nuclear transfer (**SCNT**), allowing the cloning of an animal whose genetics and morphology were already known. Dolly the sheep was the first example of success with SCNT (Wilmut et al., 1997). The technique was also applied to the production of transgenic cattle (Cibelli et al., 1998) and has so far found its greatest use in production of transgenic and gene-edited animals for research or pharmaceutical use. Examples include development of cattle with mastitis resistance (Liu et al., 2014) and polled traits (Carlson et al., 2016). Nuclear transfer can be combined with genomic selection to further accelerate genetic gain by reducing the generation interval (Kasinathan et al., 2015).

Production of offspring of predetermined sex has been long sought after by livestock producers. Sorting of X- and Y-chromosome-bearing sperm by flow cytometry has been possible since the 1980s (Garner et al., 1983), but the initial procedures killed sperm. It was not until 1989 that the first offspring (rabbits) from sexed semen were born (Johnson et al., 1989), and it was 1993 before the first calf from sex-sorted semen was born (Cran et al., 1993). In recent years, the use of sexed semen has grown internationally to the extent that bovine semen is currently being sex sorted in approximately 15 countries.

It is a credit to the many scientists, farmers, veterinarians, and breeding organizations that have translated the basic science to on-farm and laboratory technologies. In this review, we highlight the scientific advances that contributed to the development of reproductive technologies in dairy science. To put the advances of the past century in perspective, key events in reproductive biology preceding the early 1900s are summarized in Table 1. The review begins with the development of AI and continues chronologically with each advancement (see Appendix Table A1).

DEVELOPMENTS IN AI

Before the development of AI, cows were bred by natural service and bulls were often shared among farms. It was in Denmark in 1936 that the first large-scale bovine AI organization was established before similar organizations were established in the United States and worldwide (Perry, 1945). Some producers and breeders initially opposed the use of AI because early procedures for collection, handling, and insemination were cumbersome (Polge, 2007; Wilmot, 2007). The benefits of AI over natural service, however, soon became obvious. Artificial insemination was critical to increasing the reproductive potential of sires with elite genetic merit. The reduction in disease transmission between animals and the opportunity to evaluate sperm production and characteristics provided additional importnant benefits (Ivanoff, 1922; Perry, 1945). Most important, AI enabled precise genetic evaluation of bulls via hundreds

of thousands of daughters. Its growth during the past 80 yr to approximately 130 million inseminations internationally per year (Vishwanath, 2003) was driven by some key advancements, primarily in the collection, extension, and preservation of sperm.

AI Techniques

Techniques for AI were developed initially for mares and were translated for cattle by teams in Russia and Denmark (Ivanoff, 1922; Perry, 1945). The earliest method for AI of livestock was to deposit semen in the vagina, as would be the case with natural service. The speculum method was developed next; this involved viewing the cervical os with a light source and shallowly inseminating the semen into the cervix. The concept of low sperm dose insemination was introduced with the development of the speculum method. Kozlova (1935) reported similar fertility rates after insemination of 0.2 mL of semen into the cervix compared with 4 mL of semen into the vagina (cited in Salisbury and VanDemark, 1951). Even though the vaginal deposition and speculum methods were both relatively easy to perform, neither were very efficient (Olds, 1978). Large numbers of sperm were required, low numbers of pregnancies were achieved, and the large effort to maintain good hygiene with either method was not conducive to widespread adoption of AI. The success of AI was transformed by the development of the rectovaginal method by Danish veterinarians around 1937, which remains the method of AI still practiced today. With this method, a gloved hand in the rectum holds the cervix and guides the insemination gun through the cervix. Its superiority compared with the speculum method was exemplified by data collated by Olds (1978). Across 9 studies, average nonreturn rates were 48.9% for the speculum method compared with 60.1% for the rectovaginal method. The other major benefit of the rectovaginal technique compared with the vaginal deposition method was that much fewer sperm were required. Adoption of the rectovaginal method was also greatly assisted by the development of the stainless steel insemination gun, shoulder-length plastic gloves, replacement of glass ampules with plastic semen straws (Cassou, IMV Technologies, L'Aigle, France), and disposable plastic sheaths. Together these developments made the procedure of AI easier to perform, improved animal hygiene and biosecurity, and culminated in greater fertility.

Semen Collection

Early efforts at semen collection were cumbersome, with large losses of sperm and a high risk of disease transfer between animals. A bull mounted a cow and ejaculated in the vagina. Semen was removed from the vagina in a sponge or bag that was already in situ with a spoon or a syringe (Ivanoff, 1922; Perry, 1945). Alternatively, semen was ejaculated from bulls after transrectal massage of the ampullae and accessory glands; however, contamination of the sample with urine and low concentrations of sperm were common problems (Case, 1925; Miller and Evans, 1934). The artificial vagina (**AV**) was a major advancement in the process of collecting clean semen samples. The AV was first developed for the collection of semen from dogs by Amantea in 1914 and was modified for use with bulls by researchers in Russia in the 1930s. Bulls were trained to mount teaser animals and to ejaculate into the AV. The AV has been a great resource for researchers also because it is now possible to collect clean samples that are not contaminated by vaginal secretions. Electroejaculators were invented in the 1930s (Gunn, 1936), and their use with bulls was first reported in 1954 (Dziuk et al., 1954). The technique is primarily used with bulls that are not suitable for semen collection by teaser mounting and AV.

As demand for AI sires began to overtake supply during the 1940s, methods to maximize semen collection received increased focus. It became clear that exposure of bulls to several positive stimuli was important. Inducing sexual excitement in bulls by allowing longer periods of time with the mount animal and some false mounting before ejaculation were demonstrated to increase the concentration and motility of sperm (Collins et al., 1951). It also became common practice for semen to be collected 2 to 3 times per day at 3- to 4-d intervals (Bratton and Foote, 1954; Hafs et al., 1959). This change had the major effect of increasing sperm production. For example, the number of motile sperm collected per week was 60% greater with no loss in fertility when semen collection was increased from once to twice per 8-d period (Bratton and Foote, 1954). At this rate, 30 billion sperm could be collected per week from Holstein bulls (enough to inseminate 3,000 cows with 10 million sperm each).

Semen Evaluation

One of the indirect benefits for producers from using AI versus natural service has been the knowledge that only high-quality semen is used to inseminate their livestock. Initial assessments of each ejaculate involved measurement of the volume and the sperm concentration to estimate the number of sperm collected (Salisbury et al., 1943) and a visual assessment of the proportion of sperm displaying progressive motility in a diluted sample at a magnification of 400× (Elliott,

1978). These assessments provide important information on the suitability of the ejaculate for processing, the reproductive performance of the sire, and the number of doses that can be produced and are required for accurate dilution and packaging of semen. Criteria for assessing sperm morphology were developed by Williams (1920), who also reported associations between abnormal sperm and fertility. Hence, sperm morphology is routinely assessed by commercial semen processing labs; ejaculates typically are required to have ≥65% morphologically normal sperm to be further processed.

Cell sorting became feasible in the 1960s with the development of flow cytometry (Fulwyler, 1965; Dittrich and Goehde, 1968). The ability to sort, count, and assess sperm cells gave semen processing centers a major impetus to automate aspects of their quality control procedures and was central to the establishment of sex-sorted sperm (to be discussed later). Sperm staining and flow cytometry have been combined to assess various aspects of sperm quality (Garner et al., 1986). Today this methodology is used to assess sperm membrane integrity, acrosome status, sperm energetics, and sperm DNA integrity.

The accuracy and reliability of sperm motility assessments were greatly advanced by the development of computer-assisted semen analysis (Liu and Warme, 1977). These systems were developed to provide objective measurement of sperm velocity and to determine the proportion of the sperm population with total and progressive motility. More recently, some units are capable of assessing sperm morphology. Even though it is possible to assess several sperm quality characteristics, fertility remains the definitive test.

Semen Processing and Preservation

The ability to preserve sperm viability until insemination was a major advance in the establishment of organized AI because of the difficult logistics created by the fragmented nature of farms in rural areas. The basic principle of preserving sperm viability is to slow down or inhibit its metabolic activity by cooling to low temperatures. Spallanzani demonstrated in an 1803 report that sperm could survive chilling. The next major demonstrations of the potential to preserve sperm viability were performed by Walton (1926) with pregnancy in rabbits in Edinburgh, Scotland, after insemination with semen collected 48 h previously in Cambridge, England, and by Edwards et al. (1938) with pregnancy in cows in the Netherlands after insemination with semen collected 57 h previously in England. The aforementioned studies demonstrated that storage of raw, undiluted semen at 5°C with ice was sufficient to reduce the metabolism of the sperm and extend their survival, typically for 2 to 4 d.

It became obvious to researchers early on that a single ejaculate contained sufficient sperm for thousands of inseminations, yet only 10 to 20 inseminations were possible with undiluted semen (Ivanoff, 1922). Initially semen was diluted with a medium containing glucose, phosphate buffer, and sodium sulfate. A major breakthrough reported in 1939 was the success of egg yolk phosphate buffer as a semen extender (Phillips, 1939). It was later demonstrated that boiled milk could replace egg yolk as a suitable semen extension medium with comparable fertility (Thacker and Almquist, 1953). Semen extenders were distinct from previous media because of their ability to extend the motility and fertility of sperm (Foote, 1978). Phillips (1939) demonstrated that high levels of sperm motility were maintained for more than 150 h after the addition of an equal-volume mixture of egg yolk and phosphate buffer solution to semen stored at 5 to 10°C; pregnancy was achieved even from extended semen stored up to 180 h. Subsequent replacement of phosphate buffer with citrate buffer achieved the same preservation ability but with the added benefit of dispersing fat globules from the egg yolk (Salisbury et al., 1941). This characteristic greatly improved the clarity of the extended semen and the ability to examine sperm microscopically (Salisbury et al., 1941). Fertility was greatest with 2-d-old semen compared with 1-d-old semen because of a decrease in the proportional number of abnormal sperm from the first to the second day (Salisbury and Flerchinger, 1967). In practice, semen was discarded 2 d after collection because fertility declined when older semen was used (Salisbury and Flerchinger, 1967).

During the 1940s, awareness of microorganisms in semen ejaculates and their implications for fertility increased (Gunsalus et al., 1941; Prince et al., 1949) as a consequence of the greater incidence of bacterial growth in semen extended with nutrient-rich egg yolk. The negative effect of some microorganisms on fertility was clearly demonstrated when the addition of antibiotics (penicillin and streptomycin) to egg yolk-extended semen reduced bacterial growth (Almquist et al., 1949) and increased 60- to 90-d nonreturn rates by 11% (Foote and Bratton, 1950). Extension of semen with antibiotics has been standard practice ever since. This in conjunction with improved sanitary procedures and disease screening made a substantial contribution to the eradication of venereal diseases in particular.

It seems appropriate here to reiterate that the aforementioned studies were performed at a time when it was still not possible to maintain the viability of bovine sperm after the freeze–thaw process. In the absence of a

cryoprotectant, the two mechanisms through which the freeze–thaw process damages sperm are the formation of internal ice crystals that affect sperm structure and an increase in osmotic pressure, and the interaction between the two (Pickett and Berndtson, 1978). Working with unfrozen (fresh) semen with a useful life span limited to 2 d required some innovative thinking to maximize the number of inseminations before the semen had to be discarded. In some regions, semen processing labs dispatched packaged semen daily to distant AI technicians by airplane (Underwood, 2012). Such programs became irrelevant with perhaps the most important breakthrough in the development of the AI industry. In 1949, the cryoprotective properties of glycerol on sperm after the freeze–thaw process were reported (Polge et al., 1949). Sperm of fowl frozen with glycerol at −79°C (the temperature of solid carbon dioxide) resumed normal motility after thawing (Polge et al., 1949). This was the first report that living cells could survive the freeze–thaw process. The same success was subsequently demonstrated with bovine sperm (Polge and Rowson, 1952). The suitability of glycerol is largely attributed to its ability to buffer electrolytes in sperm (Lovelock, 1953). The arrival of the first calves from the AI of frozen–thawed semen in Reading and Cambridge in England (Polge and Rowson, 1952) heralded a new era that revolutionized the AI industry. With the ability to successfully cryopreserve sperm, extenders suitable for frozen semen were developed. Studies reported in 1965 demonstrated that the motility of sperm was 18 percentage units greater in egg yolk-extended semen buffered with Tris compared with no Tris (Davis et al., 1963a,b). Egg yolk extenders using Tris and glycerol achieved extensive use in semen processing labs because of good results whether the semen was stored at 5°C, stored at ambient temperature, or frozen (Vishwanath and Shannon, 2000).

The main risk to sperm viability during the freeze–thaw process is the period when sperm are exposed to temperatures ranging from −15°C to −60°C (Mazur, 1984). Hence, methods for processing semen during freezing and thawing were developed that maximized postthaw sperm motility and fertility. First, the ejaculate is partially extended at 37°C to buffer the sperm and to provide antibiotic and thermal protection; then it is cooled to 5°C. Slow cooling over a period of a few hours was demonstrated to improve postthaw sperm motility and fertility (Graham et al., 1957; Martin, 1965), probably because it permits the sperm to adjust to low temperatures before cryopreservation. The final extension is then performed to achieve the desired sperm concentration per straw, typically 10 to 20 million sperm per frozen dose. In the original work of Polge and Rowson (1952), extended semen with glycerol was frozen to −79°C with solid carbon dioxide and alcohol. Unfortunately, solid carbon dioxide was cumbersome, and sperm were damaged by the formation of crystals. Storage of semen in liquid nitrogen was also difficult until glass vacuum containers were replaced by insulated liquid nitrogen tanks developed in 1954 by American Breeders Service (Deforest, WI) and Linde Air Products (Murray Hill, NJ). The freezing process was later modified so that semen was frozen with liquid nitrogen vapor to −196°C (Forgason et al., 1961) or with programmable freezers (Almquist and Wiggin, 1973). Liquid nitrogen was subsequently adopted as the preferred coolant for freezing semen because postthaw sperm viability was greater and it facilitated longer term, lower maintenance storage of frozen semen compared with solid carbon dioxide (Fowler et al., 1961). Similar to when freezing semen, the primary risk to sperm viability after thawing is when sperm are exposed to the temperature window of −15°C to −60°C (Mazur, 1984). Sperm survival is dependent on the effects of freezing and thawing, and sperm cooled rapidly should be thawed rapidly (Mazur, 1984). A thawing temperature of 35°C applied for 45 s is broadly practiced and increases sperm motility but not nonreturn rates when compared with semen thawed at other temperatures (Pickett and Berndtson, 1978; Vishwanath and Shannon, 2000).

Frozen semen brought about many advantages, including indefinite storage of sperm, ease of transport, a greater choice of sires, and, most influentially, the international trade of semen of sires with elite genetic merit. However, the freeze–thaw process does result in damage to about 50% of the sperm, and consequently fertility performance of frozen–thawed semen has consistently been inferior to that of liquid semen (Shannon and Vishwanath, 1995). Despite the limited viability of liquid semen, fresh-semen AI programs are available in New Zealand and Ireland, for example, and have the advantage of maximizing the number of inseminations per ejaculate because fewer sperm can be packaged without a reduction in fertility. Typical doses for fresh semen usage are 1 to 5 million sperm per straw compared with 10 to 20 million sperm per straw for frozen semen (Shannon and Vishwanath, 1995). The major developments on extenders for liquid semen began with development of the Illini variable temperature diluent by VanDemark and Sharma (1957). The distinguishing feature was that extended semen was saturated with CO_2, which had the effect of immobilizing and preserving sperm for about 3 d, but the results were variable. The Cornell University extender developed by Foote et al. (1960) was based on the same principle as the Illini variable temperature diluent but was described as "self-carbonating"—that is, CO_2 was released into

the extended semen by the action of citric acid on sodium bicarbonate (Vishwanath and Shannon, 2000). Nonreturn rates of 76% by 60 to 90 d were reported with liquid semen extended with the Cornell University extender and stored for up to 4 d at 5°C (Foote et al., 1960). Continuing advancements in technologies meant that it became possible to store liquid semen at ambient temperatures (18–24°C) for up to 4 d without loss of fertility following the development of the Caprogen extender in 1965 (Shannon, 1965; Shannon and Curson, 1984). Caprogen works on the basis that saturation of extended semen with N_2 gas reduces O_2 concentration in the semen (Shannon, 1965). These authors also demonstrated that the viability of sperm stored in the liquid state was greater when stored at the ambient New Zealand temperature compared with storage at 5°C (Shannon and Curson, 1984).

Sexed Semen

In a review of the history of sexed semen use in cattle, Garner and Seidel (2008) provided the following statement: "The most sought after reproductive biotechnology of all time, selection of sex at conception, has a long history of great optimism, along with many disappointments." The recorded historical attention to determination of sex goes back at least to the early Greeks, when Democritus (470–402 BC) suggested that the right testis produced males whereas the left testis produced females. Many unsuccessful studies and a large number of inoperative registered patents for the sex separation of sperm cells, based on a variety of different principles, have been produced in the past 50 yr. Currently, only the separation of stained X- and Y-chromosome-bearing sperm by flow cytometry cell sorting has proven successful.

Initial breakthroughs involving laminar flow cytometry at the Lawrence Livermore National Laboratory in Livermore, California, led to success determining the DNA content differences between X- and Y-sperm from cattle, sheep, pigs, and rabbits (Garner et al., 1983). Two critical advances facilitated this process: (1) the use of the DNA-specific live cell stain Hoechst 33342 (membrane-permeable fluorophore) that allowed sorting of living sperm and (2) modifications to cell flow sorting analysis (dual orthogonal fluorescence detection) that created sufficient signal-to-noise ratio to allow sperm differentiation based on the small (3.8%) difference in chromosomal DNA content between X- and Y-chromosome-bearing bovine sperm. Ultimately, a live stain (food coloring) was included in the process, which allowed sperm to be sorted simultaneously for both sex and viability. Critical to the development of sexed semen technology was the announcement by Johnson et al. (1989) at the USDA laboratories in Beltsville, Maryland, of the live birth of rabbits, with 94% female pups, from sexed sperm. The work led to a US patent covering the technical details of flow sorting sperm for sex (Johnson, 1992). The original sexing patent by Johnson has long expired, but more than 200 patents related to all aspects of the production, freezing, and use of sexed bovine sperm have been registered by XY Inc. (now XY LLC) and Sexing Technologies (Navasota, TX).

Live calves resulting from IVF of oocytes with sexed bull semen were first reported by Cran et al. (1993). Cogent, located in Chester, United Kingdom, was the first company to commercialize sexed semen for AI. Currently, Sexing Technologies/XY LLC has licensed approximately 40 semen processing facilities to produce sexed semen in 15 countries. It has been estimated that semen from approximately 1,800 sires was sorted globally in 2013; the majority of the sires were Holstein-Friesian and Jersey. In the United States, between 4.5 and 5 million straws of sexed semen were processed in 2016, of which more than 90% were from dairy sires. There are 2 limiting factors involving the current technology used to sex semen. First, the processing of sexed semen involves flow cytometers that are both expensive to purchase and expensive to operate. Depending on factors such as individual bull variation and the desired accuracy of sex selectivity, approximately 7 to 12 straws/h can be processed. Consequently, multiple flow cytometers are used simultaneously to speed up the processing of each ejaculate. This is despite the fact that in most cases only 2 million sexed sperm are packaged per straw compared with the 10 to 20 million unsexed sperm in conventional straws. The accuracy of selection of either X- or Y-chromosome-bearing sperm is 90%, and the accuracy is closely related to the speed of sorting. Second, the process of sexing sperm results in varying levels of damage to the cells. This is reflected in a decrease in conception rates and embryo production following AI in both dairy heifers and cows compared with unsexed frozen semen (DeJarnette et al., 2009; Kaimio et al., 2013; Mikkola and Taponen, 2017). In terms of offspring performance, there is evidence for (DeJarnette et al., 2009; Healy et al., 2013; Siqueira et al., 2017) and against (Tubman et al., 2004; DeJarnette et al., 2009) negative health consequences in calves generated from sex-sorted semen, and 1 study to date (Siqueira et al., 2017) has reported less milk production from cows generated from in vitro-produced sexed embryos compared with cows generated from timed AI, although potential mechanisms have not been determined.

DEVELOPMENTS IN EMBRYO TECHNOLOGIES

Superovulation and ET are seen as methods for rapid multiplication of animals. Although recovery and transfer of embryos from female cattle does not match the enormous potential genetic contribution of individual stud bulls, it does allow production of more calves per female than what is possible with once-yearly natural births. Credit for the first successful transfer of mammalian embryos is given to Walter Heape. In 1890, Heape transferred two 4-cell Angora rabbit embryos into an inseminated Belgian doe, which subsequently gave birth to 4 Belgian and 2 Angora young (Heape, 1891). A later paper described Heape's technique for handling rabbit embryos, which involved spearing them on the tip of a needle and transferring them to a recipient without an intermediary step of placing them in holding medium (Heape, 1897). In vitro production of embryos by combining OPU, IVF, and ET was developed as an alternative to multiple ovulation and ET. Embryo culture medium enabled embryo manipulation techniques to develop. It is now possible to freeze embryos, store them for future use or transport them internationally, bypass certain disease situations, initiate production of calves from reproductively immature heifers, and evaluate embryos (for genomic value or genetic diseases) before transfer.

Superovulation

The sequence of events leading to the transfer of bovine embryos usually starts with superovulation. The first superovulations in cattle were performed by Casida et al. (1943). Later, Tim Rowson and colleagues at the Agricultural Research Council Unit of Animal Reproduction in Cambridge, England, developed methods for inducing follicle stimulation and oocyte production by using pregnant mare serum gonadotrophin/equine chorionic gonadotrophin (Rowson, 1951). Because of its long half-life, only a single injection of equine chorionic gonadotrophin was required for inducing superovulation in cattle. The outcome was quite variable, however, with unovulated follicles and poor-quality embryos frequently recovered.

Methods for superovulation with FSH were reported by Dziuk et al. (1958), and when ET programs changed to porcine FSH, injected twice daily over a period of 4 d, more consistent superovulatory results were achieved (Elsden et al., 1978). For many years, in commercial ET programs, FSH injections were initiated on any day starting between d 8 to 13 of the estrous cycle (Hasler et al., 1983). This required knowing the day of the previous estrus. Estrous synchronization protocols made initiating superovulation easier and more convenient. More recently, superovulation with 1 or 2 injections of FSH using hyaluronic acid as a diluent was shown to be comparable with multiple injections with saline as the diluent (Tríbulo et al., 2011, 2012).

A striking characteristic of superovulation is the variability in response among animals to gonadotrophin treatment. Using similar superovulation protocols, responses within breeds of cattle ranged from 0 to more than 100 oocytes recovered, of which 0 to more than 60 were good-quality embryos. The average number of embryos collected from cattle has changed only modestly, if at all, during the past 25 yr. The American Embryo Transfer Association (**AETA**) reported that beef donors produced on average slightly more embryos (7.1) than dairy donors (6.2) during 1998 to 2010. These averages are practically unchanged from the average of 6.2 reported by Looney (1986) for more than 2,000 beef donors and the average of 6.4 reported by Hasler et al. (1983) for more than 600 Holstein donors, each superovulated for the first time.

Embryo Collection

Bovine embryo collection was first performed surgically. The procedure was invasive, expensive, time consuming, and hard work for the people involved. It required sophisticated surgical facilities and could not be performed on farm. The large udder of dairy cows and lactating beef cows made it very difficult to perform either embryo recoveries or transfers. In addition, repeated surgical recoveries on individual animals often revealed the development of serious adhesions involving ovaries and fimbriae.

Nonsurgical techniques are easier on both ET practitioners and animals, simple, and relatively low in cost. The initial techniques were developed by Rowson and Dowling (1949) in Cambridge. That team designed a piece of apparatus for the extraction of embryos from the living cow; it was a catheter with an inflatable cuff that could be passed through the cervix to flush the uterine horns. Developments in the nonsurgical embryo recovery techniques were also reported by Dziuk et al. (1958) at the University of Minnesota and by Sugie (1965, 1968) in Japan. Developments on using Foley catheters were reported by Drost et al. (1976), Elsden et al. (1976), and Rowe et al. (1976), all published simultaneously in *Theriogenology*. Thereafter, a rapid increase in the number of commercial ET operations ensued (Hasler, 2014).

Nonsurgical recovery of bovine embryos generally involves using a relatively large volume (500–2,000 mL) of flush medium that is introduced into and out of the uterus. In the early days of the industry, this medium was often collected in a 1-L graduated cylinder and the

embryos were allowed to settle to the bottom for 30 min or more. Most of the medium was then siphoned off and discarded, and 100 mL was searched for embryos. In the mid-1980s, the first of several embryo filter models was made commercially available. The filters could be connected in line to the outflow tubing of the nonsurgical flush equipment, and the embryos were then trapped in a plastic cup by a stainless steel or nylon screen that allowed the flush medium to pass through. This was convenient and shortened the time spent searching for ova and embryos at the end of the flush.

In the early days of the industry, before the availability of commercial medium products, most ET practitioners made their own media. Modified Dulbecco's PBS with 1% heat-treated newborn calf serum was typically used for flushing and 10% newborn calf serum was used for holding embryos (Hasler, 2014). Today, several commercial companies manufacture and sell media specifically for flushing, holding, transferring, and cryopreserving bovine embryos. In most cases these media no longer contain serum, but BSA is a common component. Synthetic media, containing no components of biological origin and requiring no refrigeration, have also proven efficacious (Hasler, 2010).

Bovine embryos are normally collected 6 to 8 d after estrus, although d 7 is preferred when cryopreservation is anticipated. Embryos usually range in development on those days from late morulae to expanded blastocysts. Microscopic evaluation includes determination of the stage of development, particularly in reference to the day of collection, organization of the embryo, morphological appearance of the blastomeres, degree of fragmentation, and other signs of degeneration. Descriptive definitions of embryo quality were originally provided by Elsden et al. (1978) and are still cited as the basis for embryo quality evaluation. The quality evaluation of bovine embryos is now internationally recognized and based on morphology and is thoroughly described by Robertson and Nelson (2009).

ET

Although Umbaugh (1949) reported 4 pregnancies in Texas resulting from the transfer of cattle embryos in 1949, all the recipients aborted before term, possibly due to brucellosis. It was 1951 before the first ET calf was born following the surgical transfer of a slaughterhouse-derived 5-d embryo (Willett et al., 1951). Bovine embryos were almost exclusively transferred surgically via midline incision during the 1970s, and as with surgical embryo recovery, this approach required specialized surgical facilities. A relatively simple surgical flank transfer procedure, developed by R. D. Baker at McGill University in Montréal, Québec, Canada, was quickly applied to on-farm transfers. Later, this proved highly successful in large-scale ET programs in the United States, with pregnancy rates exceeding 70% (Coleman et al., 1987; Hasler et al., 1987). Even the flank transfers, however, were not ideal because they included clipping and surgically prepping the paralumbar region, injecting a local anesthetic, surgically opening the flank, exteriorizing the uterine horn under aseptic condition, transferring the embryo through a puncture of the uterine horn, and then suturing the incision. An interesting feature of this transfer method was that most veterinarians learned it quickly and achieved high conception rates without having much previous experience. Subsequently, successful nonsurgical transfer actually required a great deal more experience—specifically, extensive experience in transrectal palpation (prior AI experience was and is also advantageous)—than did the now-discontinued surgical approach (Hasler, 2014).

There was no evident loss of pregnancy rate with the transition from surgical to nonsurgical transfer of embryos in a commercial ET program (Hasler, 2001). Development of nonsurgical ET in cattle benefitted from the development of timed AI protocols. The latter permitted the donors and the desired number of recipients of embryos to have their estrous cycle synchronized—important for a successful ET program. This reduced the size of the recipient herd needed. If a group of donor cows within a herd was synchronized, they could all be efficiently inseminated at one time and embryos could be collected later at one time. In one study (Hasler et al., 1987), the pregnancy rate was slightly but significantly greater in recipients induced into to estrus with prostaglandin compared with those exhibiting natural estrus. Estrous asynchrony affected fresh and frozen–thawed embryo pregnancy rates in a similar fashion, with no evident effect when synchrony was within 24 h (Hasler, 2001). One early problem with nonsurgical ET was that no specific equipment was used. This changed in 1984 and 1985 when IMV Technologies introduced equipment specific for nonsurgical ET (Hasler, 2014). Thereafter, pregnancy rates were similar between transfer methods when fresh embryos were used. Lower pregnancy rates still existed, however, when frozen embryos were transferred nonsurgically (Hasler, 2001). Table 2 shows the annual total number of flushes for selected years between 2000 and 2015 in the United States.

Embryo Cryopreservation

Despite successful cryopreservation of sperm first reported in 1949 (Polge et al., 1949), small somatic cell cryopreservation resulted in 100% death with embryos. Embryo cells contain far more water than do sperm

Table 2. Selected yearly total number of bovine uterine flushes and ovum pick-up (OPU) recoveries from dairy cattle in the United States[1]

Year	No. of flushes	Dairy donors as % of total flushes	No. of OPU recoveries	Dairy donors as % of total OPU
2000	37,680	39	2,099	29
2003	34,896	36	2,781	61
2009	37,127	39	4,885	47
2011	41,151	39	8,689	63
2013	44,685	36	22,046	58
2015	41,921	30	32,636	53

[1]Source: American Embryo Transfer Association.

cells, and effective dehydration of these cells to prevent ice damage is critical to survival. Success with mammalian embryos was not reported until 1972 (Whittingham et al., 1972) using mouse embryos. Success with bovine embryo cryopreservation was first reported in 1973 (Wilmut and Rowson, 1973). As with sperm cryopreservation, an optimal cooling rate applies also to embryo cryopreservation. The optimum cooling rate depends on the amount of water in the cells and the ease with which it can leave the cells. The expectation was that the optimum cooling rate would have to be very slow (<1°C/min) because the cells of early embryos are very large (Wilmut and Rowson, 1973). Systematic comparisons were made of the effect of cooling rate, warming rate, and the use of different cryoprotective agents. The main principle that made success possible was to cool the embryo from around −5 to −7°C to around −30 to −35°C at a rate of 0.5°C/min. This cooling rate gave water sufficient time to exit cells osmotically so that damaging intracellular ice crystals would not form. The next refinement was to plunge the cooled (−30 to −35°C) embryos into liquid nitrogen before the cells became too dehydrated. A third essential requirement was to remove cryoprotectant from cells postthawing in ways that minimized osmotic swelling (Seidel, 2015). The potential value of embryo cryopreservation for international exchange of valuable genetic material was demonstrated when frozen cow embryos were exported from England to New Zealand in 1976 (Polge, 2000). The early history of embryo cryopreservation was reviewed by Maurer (1978).

Today, cryopreservation of embryos is a very large component of ET programs. In fact, some 70% of cattle embryos in the United States are frozen rather than transferred immediately after collection (AETA, personal communication). Embryos can be collected at one time and place and used as needed with the exact number of recipients required at another time and place. Dimethyl sulfoxide was the cryoprotectant most often used for freezing embryos until it was replaced by glycerol in the early 1980s. The industry quickly and widely accepted the development of a direct transfer procedure that used ethylene glycol as the cryoprotectant (Voelkel and Hu, 1992). Direct transfer allowed the straws to be thawed and placed in the ET gun. Before this, the straw was thawed and the embryo was rehydrated with sucrose and reloaded into straws for transfer into the recipient. The uptake of direct transfer with ethylene glycol was rapid and had a positive effect on the ET industry. The thawing and loading process was much quicker, and there was no longer a need after thawing for an embryologist with a microscope to search for and locate embryos for rehydration after thawing.

With the transition from fresh to frozen–thawed ET, there was a loss in pregnancy rate of 10 to 13 percentage points (Hasler, 2001). Stage of embryo development was also shown to affect pregnancy rates following transfer of frozen–thawed embryos (Hasler, 2012). This study, based on almost 73,000 transfers, reported the following pregnancy rates relative to embryo stage: stage 4 (late morula) = 53.7%, stage 5 (early blastocyst) = 55.3%, stage 6 (blastocyst) = 52.1%, and stage 7 (expanded blastocyst) = 43.8%. Stages 6 and 7 resulted in significantly lower pregnancy rates than did stages 4 and 5. This is an example of how large sample sizes can be used to determine significant differences but also how small but significant differences in pregnancy rates may translate into lost opportunities for success.

In Vitro Embryo Culture

The abbreviation IVF is often used to reference all the steps comprising the IVP of embryos, a series of steps usually including in vitro maturation, IVF, and in vitro culture. The necessity of sperm capacitation for fertilization was first demonstrated by Chang (1951) in the rabbit and by Austin (1951) in the rabbit and rat. The rabbit was the first mammal to deliver live offspring produced by IVF (Chang, 1959). However, IVF procedures for rabbits and mice initially were not successful in cattle. Successful IVF in cattle was first reported in 1977 by Iritani and Niwa (1977) using sperm that had been capacitated in the oviduct or uterus of cows in estrus or in the uterus of rabbits. By

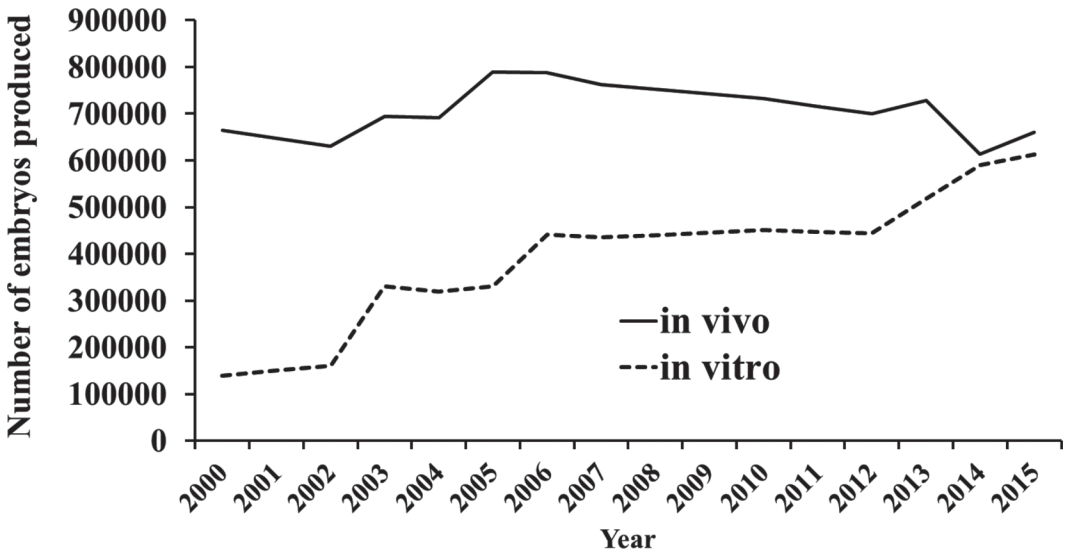

Figure 1. Comparison of the numbers of flushed, in vivo-derived bovine embryos and ovum pick-up–in vitro embryo production embryos produced internationally since 2000. Source: International Embryo Technology Society.

the end of the decade it had been demonstrated that oocytes matured in vitro could be fertilized in the cow oviduct and produce calves (Newcomb et al., 1978). The first birth of a live calf from IVF occurred in 1981 (Brackett et al., 1982) at the University of Pennsylvania. Subsequent births were reported following transfer of in vitro maturation–IVF bovine embryos cultured in the oviduct of a sheep for 5 d (Critser et al., 1986). Two calves were born following transfer of embryos resulting from IVF with sperm capacitated using calcium ionophore and the resulting zygotes cultured in rabbit oviduct (Hanada et al., 1986). The first calves produced entirely from in vitro maturation, IVF, and in vitro culture were born at University College Dublin, Ireland, in 1987 (Lu et al., 1987). Throughout the 1980s, bovine in vitro techniques remained primarily research technology with little use by the ET industry.

Introduction of real-time transrectal ultrasound led to the development of transrectal ultrasound-guided aspiration of follicles (Pieterse et al., 1988), often referred to as OPU, and has become the predominant technique for oocyte collection from living cattle. During the 1980s, developments in OPU and IVP set the stage for their uptake by commercial ET programs (Hasler, 2014). Two additional noteworthy findings during this period were the discovery that IVF of bovine oocytes proceeds more efficiently at 39°C (Lenz et al., 1983) and the development of in vitro sperm capacitation with heparin (Parrish et al., 1988). The annual total number of OPU collections in the United States grew from 2,000 collections in 2000 to 32,000 collections in 2015 (Table 2). Over the same period, in vitro embryo production has also increased internationally to the extent that a similar number of embryos are now produced by in vivo and in vitro methods (Figure 1).

The genomic testing of cattle is now significantly affecting IVP programs. There is increased demand for producing embryos from not only young heifers but also calves that are too young for effective superovulation and traditional flushing protocols. Small ultrasound OPU probes are available, and the commercial practice of producing IVP embryos from younger females is growing. Recently, Landry et al. (2016) showed that Holstein calves aged 5 to 7 mo produced more ova following OPU compared with a control group of cycling heifers aged 16 to 18 mo. Although the percentage of the ova that developed into blastocysts was greater in the control group, the actual numbers of blastocysts (in the range of 6–8) were not different between the calves and the control group.

Embryo Splitting

Embryo splitting was a technique elegantly demonstrated very early, without the help of modern equipment, by Hans Spermann in Germany (Spemann, 1902). With the objective of creating identical offspring by artificial twinning, Spemann tightened a strand of baby hair around a 2-cell salamander embryo until the cells separated. Each cell subsequently developed into an adult.

Modern mammalian splitting started in the Cambridge laboratory of Steen Willadsen (Willadsen, 1979), who removed single blastomeres by pipette from

incised 2-cell stage sheep embryos and transferred them into evacuated oocytes. The homozygous embryos were then cultured in oviducts, collected, and transferred in pairs to new recipients. Five sets of twins were born, each twin a clone. Key to the success of this procedure was that once the blastomere was transferred to the evacuated oocytes, the new embryo was embedded in agar; this step sealed the damaged zona and enabled the embryo to develop in the female reproductive tract (Willadsen, 1979). This approach was also translated to bovines with the production of twin calves (Willadsen et al., 1981). Identical twins—and in some cases quadruplets—were created in sheep, cattle, pigs, and horses. Such genetically identical animals could be regarded as clones derived from single embryos. Willadsen et al. (1981) also demonstrated that mammalian embryo splitting could be just as well accomplished using micromanipulation on postcompaction morulae and blastocysts recovered from the uterus compared with the cleavage-stage embryos originally used. This discovery was followed up by Ozil et al. (1982), Lambeth et al. (1983), and Williams et al. (1984), all of whom demonstrated that it was no longer necessary to put the embryo halves into agar chips in ewe oviducts. The half embryos were, however, transferred with one in the original zona and the other half placed into a zona obtained by removing either unfertilized or degenerate embryos from zonas. This required a double micromanipulation setup and the use of a microsuction syringe system.

Later, it became clear that conception rates with zona-free demi embryos were comparable with those with zona-clad demis. Production of zona-free embryos was faster and required less equipment. In this protocol, the embryo adheres to the bottom of a Petri dish in surfactant-free medium and the embryo is split from above with a microblade attached to a single micromanipulator or hand held. The resulting demi embryos are then transferred zona free. Initial enthusiasm for embryo splitting technology among dairy cattle breeders diminished rather quickly when it became difficult to justify the costs associated with increasing the number of bull calves. A peak in reported commercial embryo splitting occurred around 1986, when the Holstein Association reported the registration of 158 female calves in the United States. This would have represented approximately 300 embryos, half of them males, and an almost 50% conception rate for the female demis. In breeds with distinctive hair color patterns such as Holsteins, although genetically identical, calves produced by splitting may have obvious differences in color patterns because the epidermal melanocytes are not completely under genetic control during fetal development (see Figure 2). In 2011, AETA reported that a total of only 36 dairy embryos of all breeds were split. In addition to the problem of increasing the number of dairy bull calves, the decreased demand for splitting was due to the inconvenience of on-farm splitting, the availability of sex-selected semen, and the increased emphasis on freezing most embryos from most donors.

Embryo Biopsy and Embryo Sexing

Today, with the availability of sex-selected semen, embryo splitting is used much less often to produce identical half-embryos. The technique has however been adopted for the purpose of embryo biopsy, with just a few cells removed from the edge of the embryo with a microblade. The biopsy can then be used for embryo sex determination or more comprehensive genetic testing.

The original technique for sexing embryos involved chromosomal karyotyping, and the first calf sexed as an embryo by this technique was born in 1975 (Hare et al., 1976). This technique was tedious and expensive and necessitated the use of d-14 elongated embryos so that a sufficient number of trophoblast cells were available for karyotyping. The development of PCR assays using Y-chromosomal-specific probes made it possible to sex embryos with just a very small biopsy (Bondioli et al., 1989; Herr and Reed, 1991). A plethora of articles dealing with sexing embryos using PCR and electrophoresis were published throughout the 1990s. A unique PCR procedure that did not necessitate gel electrophoresis was also developed and used conveniently in the field (Bredbacka et al., 1995). Few commercial ET practitioners adopted the technology, however. There are undoubtedly more published papers dealing with sexing of bovine embryos than there are practitioners currently offering the service.

Cloning

Following from the production of identical offspring by embryo splitting, the concept of cloning by nuclear transfer—a method previously demonstrated in amphibians by Briggs and King (1952) and Gurdon et al. (1958)—was extended to mammals. The first mammals cloned by nuclear transfer were sheep (Willadsen, 1986), and the birth of live cloned calves from nuclear transfer was first reported by Prather et al. (1987). As discussed by Willadsen (1989), the main difference between embryo splitting and nuclear transfer is that an electrical charge is applied after transfer of the blastomere to the evacuated oocyte. This has the effect of fusing the nucleus and oocyte and reprogramming the nucleus of the embryo to the developmental stage of a just-fertilized oocyte. Splitting embryos at the 8-cell

Figure 2. Embryo donor dam and identical twin Holstein calves produced from an embryo split by micromanipulation.

stage or later and transfering blastomeres individually has not been very successful because, in the absence of reprogramming, cellular mass by the time of blastulation is insufficient for the embryos to remain viable; hence, splitting is limited to the production of 4 embryos. With reprogramming of the nucleus, as occurs with nuclear transfer, cellular mass at blastulation is sufficient for the embryos to remain viable; therefore, there is potential for unlimited production of identical offspring.

Before 1996, mammalian cloning had been demonstrated only with donor nuclei from embryonic cells—blastomeres and inner cell mass cells (Sims and First, 1994). The field of cloning was further advanced when it was demonstrated that a blastocyst-derived cell culture (Campbell et al., 1996) and an adult mammary epithelial cell culture (Wilmut et al., 1997) provided suitable donor nuclei for cloning sheep, the latter leading to the birth of Dolly, the first mammal cloned from somatic cells. Cloning by utilization of somatic cells is referred to as SCNT. Birth of live calves from SCNT was first reported by Cibelli et al. (1998). Commercial services providing livestock cloning services were established shortly after these initial reports. For commercial breeding organizations, nuclear transfer has been used for the production of animals that are transgenic (Cibelli et al., 1998), gene edited (Liu et al., 2014), or genotyped for genomic evaluation (Kasinathan et al., 2015). For commercial dairy producers, the technology is not widely used, although some dairy cows and sires of elite genetic merit have been cloned. The main limitations to the use of cloning are society's reluctance to accept produce from cloned livestock and their offspring into the food chain (despite regulatory approval from food standards agencies internationally), the high costs of the procedure ($15,000–$20,000 each), and the low success rate (only about 10% of embryos transferred lead to healthy, productive animals). An important limiting factor to the widespread use of SCNT to date is the high rate of losses throughout pregnancy. A comparative ET study between IVP embryos and cloned embryos derived from embryonic, fetal, and adult cells was reported by Heyman et al. (2002). The study demonstrated significant differences in pregnancy rate at 70 d (49.0% vs. 37.3% vs. 22.5% vs. 14.3% for IVP embryos and embryonic, fetal, and adult cell clones,

respectively) and at calving (49.0% vs. 34.3% vs. 15.0% vs. 6.8% for IVP embryos and embryonic, fetal, and adult cell clones, respectively). Altered gene expression and epigenetic status and the development of hydrops and cotyledonary hyperplasia in SCNT-derived embryos compared with IVP embryos are the main factors implicated in the excessive pregnancy losses (Dean et al., 2001; Lee et al., 2004; Everts et al., 2008).

In 2001, when it became apparent that animal cloning may become a commercial venture to help improve the quality of herds, the US Food and Drug Administration (**FDA**) requested livestock producers and researchers to keep food from animal clones or their offspring out of the food supply. Since then, the FDA has conducted an intensive evaluation that included examining the safety of food from these animals and the risk to animal health. Based on a final risk assessment, a report written by FDA scientists and issued in January 2008 concluded that meat and milk from cow, pig, and goat clones and the offspring of any animal clones are as safe as the food we eat every day (US FDA, 2014). Similar conclusions have been reached by studies in other countries (Panarace et al., 2007; Watanabe and Nagai, 2011).

APPLICATION OF REPRODUCTIVE TECHNOLOGIES TO GENETIC IMPROVEMENT

With the arrival of AI, breeding programs were developed to maximize genetic gain in traits of economic importance, primarily milk production, and more recently functional, health, and fertility traits. Incorporation of AI into progeny testing schemes was essential to the incredible improvement in milk production per cow during the past century. The establishment of cooperatives from the 1930s onward provided the infrastructure for the collection and distribution of semen from sires of elite genetic merit. Until the past decade, the identification of such sires was based solely on progeny testing schemes, first envisaged by Lush (1935). With this approach, sire genetic evaluations were based on the performance records of their daughters. Elite sires were then selected for mating with females to generate the next generation of offspring.

Genomic selection, now implemented globally, has revolutionized animal breeding programs (García-Ruiz et al., 2016). The framework for genomic selection was first outlined by Meuwissen et al. (2001), but sequencing of the bovine genome (Elsik et al., 2009) and development of SNP genotyping chips (Matukumalli et al., 2009) such as the Illumina SNP chip (BovineSNP50, Illumina Inc., San Diego, CA) were critical to its establishment. The greatest effect of genomic selection to date has been the reduction of the generation interval and the increase in the accuracy of genetic selection. In the future, the generation interval could be further reduced and the accuracy of selection may be increased by incorporating sexed semen, genomic selection, genome editing, and gene drives into juvenile in vitro embryo production and ET or SCNT programs as outlined by Kasinathan et al. (2015), Carlson et al. (2016), and Gonen et al. (2017), respectively.

Production of genome-modified cattle is proceeding on a modest scale; however, a widespread effect on the genetic improvement of cattle populations has not yet occurred. To date, cattle have been generated to produce milk containing pharmaceutically valuable human proteins (Bauman et al., 2006; Robl et al., 2007), with mastitis resistance (Liu et al., 2014), and with polled traits (Carlson et al., 2016). The first actual example of making mammalian transgenic embryos was reported by Jaenisch and Mintz (1974). They microinjected viral DNA into the blastocoel cavities of mouse embryos, surgically transferred the embryos, and demonstrated that the viral DNA had probably been integrated into the genome of mice. Much later, cloned embryos produced by culturing enucleated oocytes each injected with a single blastomere or from embryonic stem cells, fetal cells, or adult somatic cells (Campbell et al., 1996; Wilmut et al., 1997; Cibelli et al., 1998) all led to significant progress in the production of transgenic animals. A good deal of money was spent and great expectations were promoted relative to producing better cattle, including enhanced milk production, carcass quality, and traits such as polledness, with modest success. Several companies, including Advanced Cell Technology, Infigen, Pharming, Gala Design, and Genetic Savings and Clone were started to pursue these goals, and most of them are no longer in business. Recently, several research teams announced the development of the CRISPR-Cas9 system to target and cleave any chosen DNA sequence in vitro (Cain, 2013). Potential applications in livestock of this extremely powerful genome modification technology were recently reviewed (Josa et al., 2017). A great deal of attention in the public press has focused on applications of this technology for food animal production. The number of genome modifications possible may soon be limitless. Their role in food animal production and biomedicine, however, cannot be evaluated or realized in the absence of regulatory approval.

FUTURE DIRECTIONS

Here, we propose some areas on which to focus research and discussion in the future.

- Additional methods of extended sperm storage, such as lyophilization
- Additional methodology for sex separating sperm cells and improving the fertility of sex-separated sperm
- The role of seminal plasma and microbiome on uterine environment and embryo development
- Mechanisms by which reproductive technology affects offspring health and performance
- Better communication and understanding between the public, especially animal rights organizations, and the animal sciences
- The wise and practical application of powerful new technologies, such as CRISPR/Cas9, to animal science

In addition to this listing, we call attention to a recent paper by Seidel (2016) dealing with future research that is needed in the field of assisted reproduction with gametes and embryos.

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APPENDIX

Table A1. Selected key advancements in the field of reproductive technology since the early 1900s

Year	Milestone	Reference
1935	Progeny test estimates sire genetic merit based on progeny performance.	Lush, 1935
1938	Bovine artificial vagina prevents contamination of ejaculate.	Milovanov, 1938
1938	Rectovaginal method improves the effectiveness of AI.	Sorensen, 1938
1939	Phosphate-buffered egg yolk extender maintains viable sperm for 180 h.	Phillips, 1939
1950	Addition of antibiotics to semen extender improves fertility.	Foote and Bratton, 1950
1951	Protocol to superovulate cattle with pregnant mare serum gonadotrophin is developed.	Rowson, 1951
1951	The first calf is born after surgical embryo transfer.	Willett et al., 1951

Continued

Table A1 (Continued). Selected key advancements in the field of reproductive technology since the early 1900s

Year	Milestone	Reference
1951	Researchers discover that sperm capacitation is a prerequisite for fertilization.	Austin, 1951; Chang, 1951
1952	The first calf is born after insemination with frozen–thawed semen.	Polge and Rowson, 1952
1954	Manufacture of liquid nitrogen storage tanks begins.	American Breeders Service, Linde Air Products
1954	Electroejaculator to stimulate ejaculation in bulls is developed.	Dziuk et al., 1954
1958	Protocol to superovulate cattle with FSH is developed.	Dziuk et al., 1958
1959	Live rabbits are born after in vitro fertilization.	Chang, 1959
1964	Plastic Cassou straws replace glass ampules for the storage of frozen semen.	Cassou (IMV Technologies, L'Aigle, France)
1973	Calf is born following transfer of frozen–thawed embryo.	Wilmut and Rowson, 1973
1982	Live calves are born after in vitro fertilization.	Brackett et al., 1982
1983	X- and Y-chromosome-bearing sperm are separated by flow cytometry.	Garner et al., 1983
1986	First mammal clone is born from nuclear transfer-derived sheep embryo.	Willadsen, 1986
1987	First calf is born from completely in vitro produced embryo.	Lu et al., 1987
1988	In vitro capacitation of sperm is induced by incubation with heparin.	Parrish et al., 1988
1988	Ovum pick-up method is developed for repeated oocyte collection from live donors.	Pieterse et al., 1988
1992	Ethylene glycol enables the direct transfer of frozen–thawed embryos.	Voelkel and Hu, 1992
1993	Calves are born following separation of X- and Y-chromosome-bearing sperm.	Cran et al., 1993
1996	Dolly the sheep is born. The first mammal is cloned from an adult somatic cell.	Wilmut et al., 1997
1998	Live transgenic cloned calves are born.	Cibelli et al., 1998
2009	Bovine genome sequence heralds a new era of genomics and genomic selection.	Bovine Genome Sequencing and Analysis Consortium et al., 2009

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A 100-Year Review: Practical female reproductive management[1,2]

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ABSTRACT

Basic knowledge of mechanisms controlling reproductive processes in mammals was limited in the early 20th century. Discoveries of physiologic processes and mechanisms made early in the last century laid the foundation to develop technologies and programs used today to manage and control reproduction in dairy cattle. Beyond advances made in understanding of gonadotropic support and control of ovarian and uterine functions in basic reproductive biology, advancements made in artificial insemination (AI) and genetics facilitated rapid genetic progress of economically important traits in dairy cattle. Technologies associated with management have each contributed to the evolution of reproductive management, including (1) hormones to induce estrus and ovulation to facilitate AI programs; (2) pregnancy diagnosis via ultrasonography or by measuring conceptus-derived pregnancy-associated glycoproteins; (3) estrus-detection aids first devised for monitoring only physical activity but that now also quantitate feeding, resting, and rumination times, and ear temperature; (4) sex-sorted semen; (5) computers and computerized record software packages; (6) handheld devices for tracking cow location and retrieving cow records; and (7) genomics for increasing genetic progress of reproductive and other economically important traits. Because of genetic progress in milk yield and component traits, the dairy population in the United States has been stable since the mid 1990s, with approximately 9 to 9.5 million cows. Therefore, many of these technologies and changes in management have been developed in the face of increasing herd size (4-fold since 1990), and changes from pastoral or dry-lot dairies to increased housing of cows in confinement buildings with freestalls and feed-line lockups. Management of groups of "like" cows has become equally important as management of the one. Management teams, including owner-managers, herdsmen, AI representatives, milkers, and numerous consultants dealing with health, feeding, and facilities, became essential to develop working protocols, monitor training and day-to-day chores, and evaluate current trends and revenues. Good management teams inspect and follow through with what is routinely expected of workers. As herd size will undoubtedly increase in the future, practical reproductive management must evolve to adapt to the new technologies that may find more herds being milked robotically and applying technologies not yet conceived or introduced.
Key words: dairy cow, management, reproduction, technology

INTRODUCTION

Management of reproduction in dairy cattle requires a mixture of science, technology, and the art of animal husbandry. Reproduction is a complex science, so much so that William Hansel, formerly of Cornell University, always told his students, "it is not a wonder that reproduction sometimes fails, but rather a miracle that so many pregnancies terminate successfully" (Bearden and Fuquay, 1997). It is also a wonder that so many pregnancies occur, given the innumerable ways in which the human can interfere in attempts to control the complex physiologic pathways and mechanisms that lead to and support pregnancy and eventual parturition.

Several factors during the last 100 yr have influenced and altered how dairy cows are managed to promote reproductive efficiency. As the total number of dairy cows decreased with increased milk production per cow (Figure 1), herd size increased and nearly all dairy farms qualified as grade A milk producers, requiring upgrading of facilities and equipment. As a perspective from the 1920 US Census, 19.6 million dairy cows produced milk on 4.5 million dairy farms, representing an average of 4.4 cows per farm, and annual yield per cow was 1,385 L. In 2016, 9.3 million cows (223 cows per farm) produced milk on 41,809 dairy farms, and average production per cow was approximately 10,024 L (USDA, 2017). By 1950, average herd size was only

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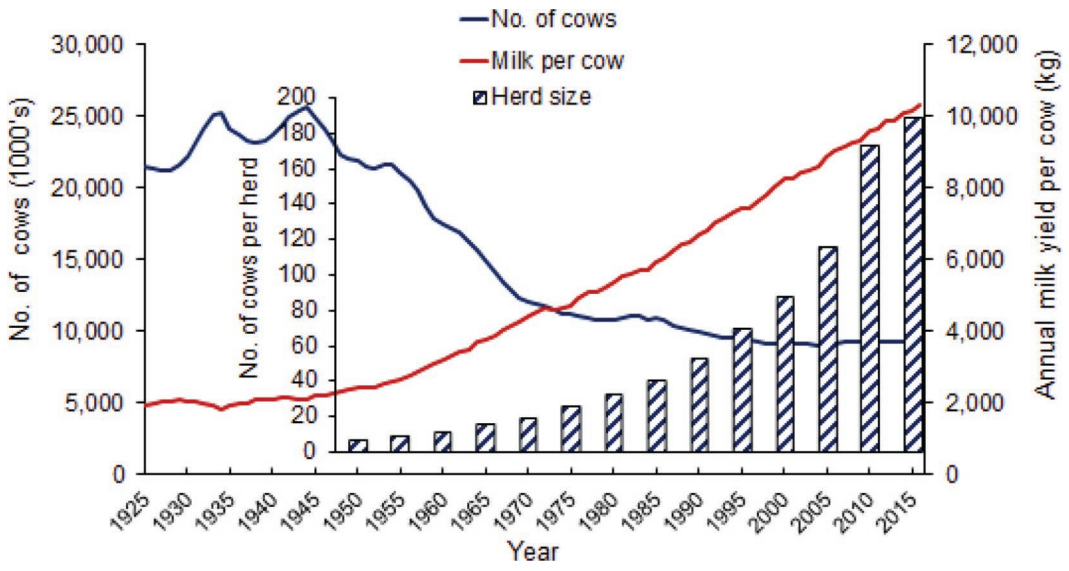

Figure 1. A historical summary of milk yield per cow, herd size, and numbers of dairy cows in the United States. Artificial insemination became commonplace in the 1950s with the advent of frozen semen and its adoption by dairy producers. Genetic progress in milk yield per cow is evident by the increasing yields after the 1960s when daughter–dam comparisons were replaced by a more sophisticated animal model that included genetic contributions of all female relatives of dairy sires in addition to their progeny. Source: NMPF (2016). Color version available online.

6.5 dairy cows per farm, but since then, it has increased exponentially (Figure 1). The numbers of herds with larger numbers of cows have also clearly changed. The number of cows per US dairy operation and its distribution by herd size reveals the exponential growth in large dairy herds beginning in the 1960s (Figure 2). Furthermore, proportion of total US milk produced annually by larger herds has increased dramatically since 1980. In 2012, 63% of milk was produced by herds of 500 or more cows (NMPF, 2016). With 49,300 dairy herds in 2012, 63% of US milk was produced by only 35.7% of the dairy farms.

Average number of cows managed or volume of milk sold per dairy worker has increased with time and that is particularly true in larger than in smaller dairy herds (NDFS, 2015). Moreover, the labor force has changed culturally from predominantly immigrant Americanized Europeans to predominantly Hispanics, with the need for bilingual managers with a working knowledge of Spanish. Currently, Hispanic employees (of which approximately 63% are Mexican) represent approximately 75% of the hired labor force on dairy farms in California (Bello et al., 2012).

During the early half of the past century, most dairy cows grazed pastures and may have been housed in tiestall or stanchion barns during winter months. Housing types on US dairies then changed by growth of operations housed in freestall and dry-lot systems (NAHMS, 2007). The NAHMS (2007) survey was conducted in 17 of the United States' major dairy states, representing 79.5% of US dairy operations and 82.5% of US dairy cows. Estimates in the NAHMS report refer to calendar year 2006. Further changes in the 1960s included greater usage of confinement housing, in which cows were housed almost exclusively on concrete after first calving. In these larger herds, adoption of feedline head lockups to confine cows individually after the morning milking introduced opportunities to manage much more efficiently reproduction and health care for large numbers of cows. Insemination, pregnancy diagnosis, health monitoring, and assessment of paint or tail chalk rubs as a means of detecting prior standing activity became much easier. Some managers without feedline lockups took advantage of return-alley sorting gates to segregate cows into palpation rails for various reasons or into a sorting pen with feedline lockups.

Key technologies and discoveries (Appendix Table A1) have led to changes in reproductive management and clearly have become important drivers for change in day-to-day management of dairy operations, particularly in larger herds. Breeding strategies have moved from predominantly natural service to an overwhelming prevalence of AI and ovulation-synchronization programs before timed AI (Caraviello et al., 2006). In the AI industry, changes in frozen semen packaging and sperm dose and use of sex-sorted semen facilitated genetic progress by making available more progeny of genetically proven sires (Foote, 1996). Detection of estrus by means of measuring correlated traits such as increased physical activity or body temperature became

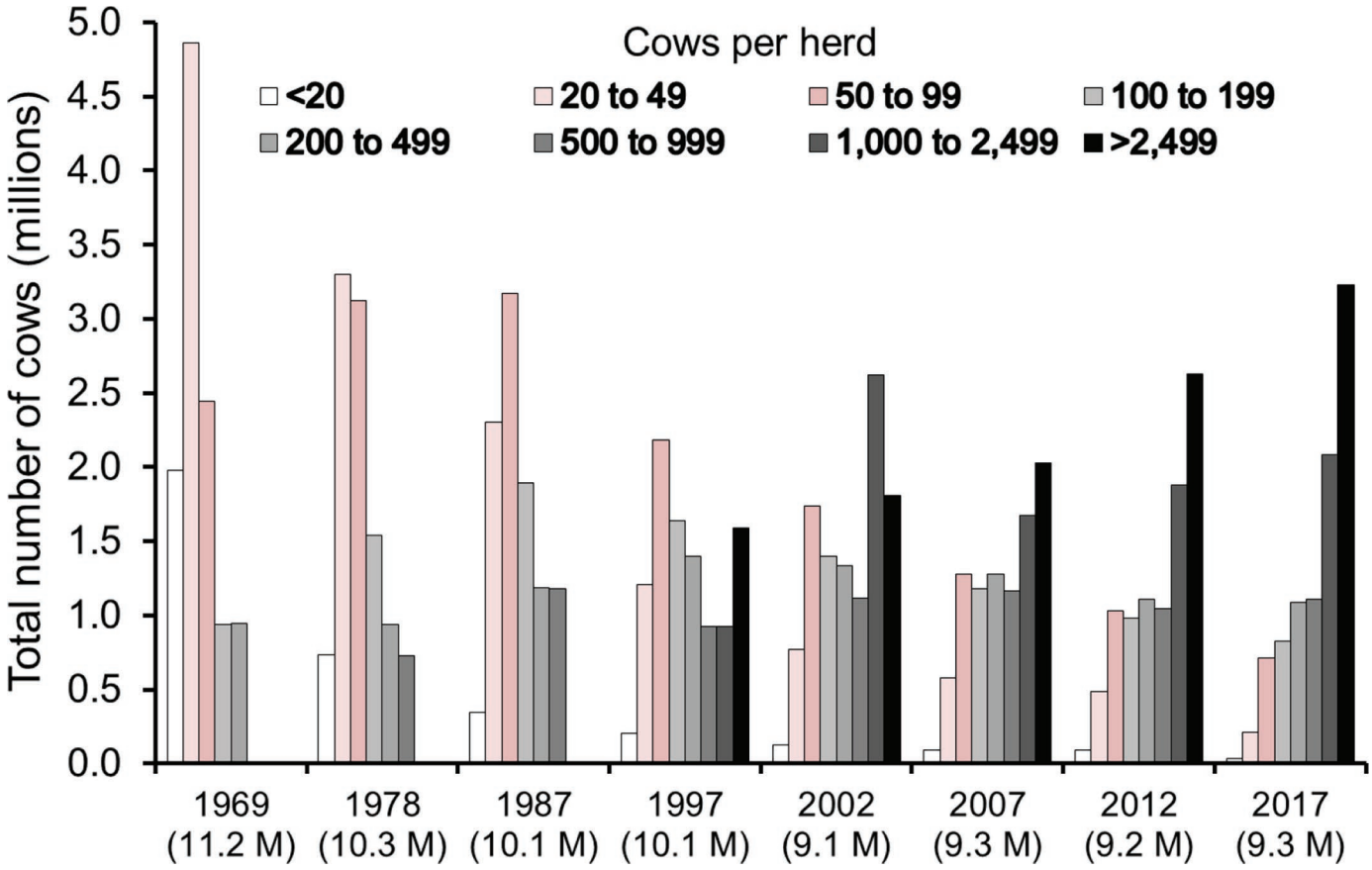

Figure 2. Change in the number (millions; M) of dairy cows per herd during the past 48 yr encompassing the period when freestall barns and larger parlors were built and much of the technology used today began to emerge. Data for 2017 represent a linear extrapolation from previous values. Source: USDA Census of Agriculture (http://agcensus.mannlib.cornell.edu/AgCensus/homepage.do; accessed March 24, 2017). Color version available online.

available in addition to single-use heat-mount detectors and permanent rump-mounted, pressure-sensitive electronic detectors that recorded mounts received by the cow in estrus (Roelofs et al., 2010). Pregnancy diagnosis occurred by means of transrectal palpation, transrectal ultrasonography, or by chemical tests as early as 28 d post-AI (NAHMS, 2007). Introduction of other assisted-reproductive techniques included embryo transfer, in vitro maturation of oocytes collected from ovaries of cows at slaughter, in vitro fertilization of those oocytes, followed by in vitro culture and transfer to recipient surrogate cows for gestation (Rodriguez-Martinez, 2012). Applying recombinant DNA–derived bovine somatotropin enhanced milk yields in addition to improved pregnancy rates of dairy cows in some studies.

The focus of this review will be to describe the history and impact of science and technology on reproductive management of dairy cattle during the 100-yr history of the *Journal of Dairy Science*. Readers are referred to previous reviews during the last 100 yr, which provide some insights to earlier assessments of technologies and reproductive management (Willett, 1956; Gomes and Erb, 1965; Foote, 1979, 1996, 2002; Britt et al., 1981; Moore and Thatcher, 2006).

HISTORICAL DEVELOPMENTS IN REPRODUCTIVE MANAGEMENT

At the dawn of the 20th century, there was limited knowledge of mechanisms that controlled reproduction in mammals. Discoveries made early in the century laid the foundation to develop technologies used today to manage and manipulate reproduction in dairy cattle. In this section, we briefly describe discoveries and advances that moved management of reproduction from breeding cows by natural service bulls of unknown genetic potential to more precise management that has emerged in the 21st century. A timeline illustrates the technologies and significant discoveries that were part of the past 100 yr in reproduction and its management (Appendix Table A1).

Reproductive Records

Williams (1919) recognized the need for common ways of comparing reproductive efficiency among herds. He proposed a simple approach by assigning 100% to each month that a heifer or cow remained in the herd with a calving interval of 12 mo or less, and 0% for each month beyond a 12-mo calving interval. His "Reproductive Months" comprised all months from when a heifer reached 15 mo of age until she was culled from the herd. Spielman and Jones (1939) subsequently utilized Williams' method to measure reproductive efficiency of different cow families and breeds from a 25-yr collection of records in the dairy herd at Oregon State Agricultural College. They reported different reproductive efficiencies among cow families (54.5 to 87.2%) and among breeds (67.6 to 81.3%). This scoring method represented the first attempt to measure reproductive performance using a single index.

Growth of AI associations in the United States beginning in the 1940s led to a systematic collection of reproductive data to compare fertility of bulls, AI technicians, and herds. Nonreturn rates (60 or 90 d) were often used to estimate conception risk in lieu of actual pregnancy diagnosis. A related measure, services per conception, became a common variable for measuring herd fertility (Davis and Brost, 1953). None of these measures accounted for cows that were culled without being serviced, so they lacked a true assessment of the entire herd's reproductive performance.

Mead (1938) recognized this weakness and devised a method for tracking a herd's progress in time. His method used parallel hand-drawn lines on a large sheet of paper in which each cow's reproductive event (e.g., calving, estrus, pyometra, abortion, repeat service) was represented symbolically on one line. Each sheet could accommodate approximately 70 cows during a period of 5 yr and established a systematic way of examining a herd's reproductive performance over time. This method was especially useful in visually depicting how a condition such as a *Trichomoniasis* infection affected herd performance.

Days open became a broadly used criterion in the 1960s because it included all cows eligible for breeding. Days open were also related to parity and economic returns from milk yield, leading Louca and Legates (1968) to recommend 13-mo calving intervals for primiparous cows and 12-mo calving intervals for multiparous cows. A reproductive index (Herd Reproductive Status; HRS) was developed in the 1960s and became part of some DHI record systems (Johnson et al., 1964). The HRS index included both average days open as well as the proportion of problem cows (those not pregnant >100 d).

These systems gradually lost their meaningfulness when synchronized breeding entered the picture—first with synchronized estrus and later with timed AI programs. Because almost all cows were inseminated by appointment on a weekly basis, a shift occurred to use voluntary waiting period (**VWP**) as the starting point for measuring reproductive performance at the herd level. By using VWP, managers had some leeway in deciding when to initiate first postpartum services without penalizing them for delaying first AI. A new status code "Do Not Breed" or DNB became vogue and allowed managers to identify cows that they planned to cull, thus excluding those cows from the breeding program and herd reproductive measures.

Traditional historical measures of reproductive efficiency such as days open, services per conception, and calving intervals have lesser value because they lack time sensitivity to current fertility trends in the herd. Risks for AI submission, estrus detection, and conception proved to have greater value but the combination of the latter in what is defined as the 21-d pregnancy rate has now become commonplace. By definition, a 21-d pregnancy rate is the proportion of breeding-eligible cows in the herd becoming pregnant every 21 d beginning at the VWP. Its usefulness in assessing current insemination success was documented by Ferguson and Skidmore (2013) as a means to compare herds and monitor intra-herd progress in pregnancy risk.

The economic value of improving 21-d pregnancy rates has been estimated by various economic models (Cabrera, 2017). The annual per-cow value of improving the 21-d pregnancy rate from its current rate can be determined. For example, increasing a current 21-d pregnancy rate from 13 to 17% could result in a predicted annual increase in income of $97 per cow per year. Increasing it from 18 to 24% results in a predicted increase of $100 per cow per year. It is usually easier to increase 21-d pregnancy rates from lesser to greater rates <20 than endeavoring to increase >25. Furthermore, the increased economic returns increase at a decreasing rate when improvements are made above a 21-d pregnancy rate of 25%.

Artificial Insemination

Artificial insemination of cattle on a broad scale was pioneered by E. I. Ivanoff in the late 19th and early 20th centuries in Russia. By 1938, an estimated 1.2 million cattle in Russia had been inseminated under his programs (Perry et al., 1945). Danish leaders adopted Russian techniques and launched their first artificial breeding association in 1936, and by 1939, the Danes had established 21 associations across Denmark. In the United States, Cooperative Artificial Breeding Associa-

tion No. 1 Inc. was launched in New Jersey in May 1938. By 1943, more than 14,000 cows had been inseminated in 1,600 herds served by 6 artificial breeding associations in New Jersey. In addition, approximately 100 breeding association cooperatives existed in the United States, with the greatest numbers in Wisconsin and New York (Perry et al., 1945).

Artificial insemination of cattle grew quickly and provided an incentive to understand more about time of ovulation in cattle and optimal timing for insemination. Brewster and Cole (1941) utilized repeated palpation of ovaries of heifers and cows during and after estrus to determine that ovulation occurred approximately 14 h after the end of estrus; however, duration of estrus was not measured accurately in that and many studies. Most believed that events at the end of estrus stimulated the follicle to ovulate. Nevertheless, these early estimates are within the range estimated by modern-day ultrasonographic estimates of follicle disappearance (Dransfield et al., 1998).

At first, chilled fresh semen was stored in stoppered or screw-top vials that may have had sufficient sperm for servicing more than one cow. When frozen semen became available, it was packaged initially in glass ampoules and later in 0.25- or 0.5-mL plastic straws that could be more easily stored in liquid nitrogen containers specifically manufactured to store frozen semen on the farm. Today, different suppliers of bovine semen for AI use both sizes of straws for packaging their frozen semen. Meta-analyses applied to fixed- or random-effect models of 15 different studies indicated that the average odds of having a greater pregnancy outcome were 3 to 4% greater with the 0.25-mL straw than the 0.5-mL straw (Stevenson et al., 2009). Based on these odds ratios, the expected proportion of difference in pregnancy outcome translated into a difference of only 0.74%.

As quality of semen extenders improved, studies focused on the optimal timing of insemination relative to onset of estrus and what types of daily service logistics were optimal for an AI technician to inseminate cows in a designated area. The question was whether technicians should visit farms in the morning and afternoon to inseminate cows detected in estrus at different times or whether once-daily visits were sufficient. Most AI organizations were using the a.m.-p.m. rule of thumb, in which cows first detected in estrus in the a.m. were inseminated later in the p.m. and those in first detected in the p.m. were inseminated the next a.m. (Trimberger and Davis, 1943). Inseminating cows during the a.m. and p.m. limited the number of herds that could be serviced by one AI technician. Field trials with chilled, extended semen (Foote, 1979) and frozen semen (Nebel et al., 1994) demonstrated that once-a-day service was optimal and that any cow detected in estrus after a previous daily visit should be inseminated when the technician arrived at the farm the next day. The number of doses of bovine semen produced worldwide during the last 50 yr exceeds 250 million (Rodriguez-Martinez, 2012).

The increase in milk yield per cow in the United States during the last century has been driven by genetic selection for milk yield and milk components (Figure 1). The first national sire evaluations were calculated in 1936. The USDA's Animal Improvement Programs Laboratory (Beltsville, MD) was originally tasked to conduct research, discover, test, and implement improved genetic evaluation techniques for economically important traits of dairy cattle. Data were collected and research was directed at genetic improvement of yield traits (e.g., milk, fat, lactose, and protein) and non-milk yield traits that affect health and profitability (longevity, udder conformation, fertility, and disease resistance) of dairy cows. Today, the Council on Dairy Cattle Breeding (Bowie, MD) utilizes records from herds participating in DHIA that are processed at 4 national processing centers (AgriTech Analytics, Visalia, CA; AgSource Cooperative Services/CRI, Verona, WI; DHI Computing Service Inc., Provo UT; and Dairy Records Management Systems, Raleigh, NC). These records provide information on progeny daughters of bulls from which sire summaries are calculated and published regularly.

Despite advantages of using AI, not all cows and heifers are bred by AI. A survey conducted in 103 Alta Genetics (Watertown, WI) progeny-test herds (averaging 613 cows) located in 18 states (Caraviello et al., 2006) found that 56% of the herds used AI for all breeding services. In addition, a national survey sponsored by the National Association of Animal Breeders (NAAB; Madison, WI) revealed that, depending on herd size, 55 to 63% of dairy heifers were serviced using AI. A more recent NAAB survey showed that 62 to 68% of dairy heifers received at least 1 AI service (Fricke, 2004). As recently as 2007, more than 72.5% of pregnancies achieved in dairy operations resulted from AI, whereas 26.8% occurred after natural mating, with relatively little variation among herds of different size (Table 1; NAHMS, 2007).

Today, about half of all dairy operations (51.5%) have bulls used for breeding dairy cows (NAHMS, 2014). The NAHMS (2014) study was conducted in 17 of the United States' major dairy states, representing 76.7% of US dairy operations and 80.3% of US dairy cows at the time of the study interview. Estimates provided here refer to calendar year 2013. Beef bulls were used for breeding dairy cattle on 5.5% of dairy operations, whereas dairy bulls were used on 48.1% of

operations. A smaller percentage of small operations (30 to 99 cows; 48.2%) used bulls compared with large operations (>500 cows; 62.8%). Natural service was the second most common practice used at first service for most heifers and cows (33.2 and 21.7% of operations, respectively).

Sex-sorted semen has been available in the US market since 2003. Altering the sex ratio in favor of heifer calves has led to a greater abundance of replacement heifers in dairy operations, facilitating, if desired, internal growth of the herd. Sorting sperm based on their DNA content was successfully done in 1987 by flow cytometry, allowing relatively pure populations of X- and Y-chromosome-bearing sperm to be collected (Moore and Thatcher, 2006). Disadvantages of applying sex-sorted semen are its cost and reduced fertility. The early technology for sorting sperm was relatively slow (150 to 200 straws of sexed semen per machine per day), and commercial straws have smaller doses (e.g., 2 million sperm per straw) compared with conventional semen, which is packaged in doses of 10 million or more sperm per straw. A big improvement during the past 10 yr has increased speed and efficiency of each sorting unit, resulting in a doubling of output. Packaging 2 million sperm per AI dose under ideal conditions now produces approximately 14 straws of sex-sorted semen of each sex per hour or 672 straws per day (Seidel, 2014).

Pregnancy outcomes with sex-sorted sperm are generally 70 to 85% of what can be achieved with conventional semen in well-managed herds (DeJarnette et al., 2009). Because of the cost of producing sex-sorted semen and poorer fertility associated with its application, most of it is used in heifers because they are the most fertile females on a dairy operation. For dairy operations with pregnancies produced by AI during 2006, sex-sorted semen was used to inseminate 11.4% of heifers and 3.5% of cows (NAHMS, 2007).

Table 1. Average percentage of pregnancies conceived during 2006 according to breeding method and size of US dairy operation (NAHMS, 2007)

	Breeding method (%)		
Herd size	Natural service[1]	AI[2]	Embryo transfer[3]
<100 cows	29.1	70.3	0.6
100 to 499 cows	22.0	77.0	1.0
≥500 cows	19.7	79.9	0.4
All herds	26.8	72.5	0.7

[1]Bull bred.

[2]After detected estrus or timed AI.

[3]Superovulated or in vitro-derived embryos.

Discovery and Assay of Reproductive Hormones

Pituitary and gonadal reproductive hormones were discovered in the third and fourth decades of the 20th century. Estrogens were isolated and characterized from ovarian follicular fluid (Allen and Doisy, 1923), progesterone from corpora lutea (Allen, 1930), and LH and FSH from pituitary glands (Fevold et al., 1931).

Bioassays were used to estimate relative amounts of hormones in urine and tissues during the first 7 decades of the 20th century. The earliest bioassays estimated estrogenic hormones in urine of cattle during pregnancy (Hisaw and Meyer, 1929; Nibler and Turner, 1929). For example, Nibler and Turner (1929) stimulated cows to urinate at planned times throughout gestation and then extracted estrogenic activity by mixing olive oil with the urine. The oil was then separated from the aqueous fraction and used in a rat uterine bioassay. Estrogenic activity estimated by rat units (relative increase in weight of the uterus) increased 19-fold from the beginning to the end of gestation in cows.

Casida (1938) provided an insightful overview of actions of crudely purified gonadotropic hormones in laboratory animals and cattle. Bioassays of gonadotropins often used responses observed in immature female rats or male chicks. For example, Nalbandov and Casida (1940) removed pituitaries from cattle at different stages of pregnancy, minced pituitary pieces in acetone, dried the material, and created a powder with mortar and pestle. Approximately 25 mg of powder was loaded into gelatin capsules and then inserted (i.m. or s.c.) into rats at approximately 31 d of age. Ovaries of rats were removed 4 to 5 d later and weighed. Heavier weights reflected greater gonadotropic activity. For male chicks, dried powder was injected as an aqueous solution daily for 7 d beginning at 14 d of age, and testes were removed and weighed when chicks were 21 d of age.

Hormone assays were not very sensitive until the late 1960s, often requiring several milliliters or liters of urine or blood to assess a single sample. Various types of chromatography were used subsequently to separate active hormonal agents, which were then measured using spectrophotometric procedures (Gomes and Erb, 1965). In the late 1960s and early 1970s, protein binding assays and radioimmunoassays were developed and these increased sensitivity up to a thousand-fold (Midgley et al., 1969; Niswender et al., 1969). Immunoassays were sufficiently sensitive, requiring assay of only microliters of biological fluid (e.g., tissue extracts, blood, or milk). Increased assay sensitivity facilitated frequent and multiple sampling of animals to detect secretory patterns over minutes, hours, or days. It became possible to detect pulsatile secretion of hormones surrounding key reproductive events such as parturition, estrus, ovulation, or luteolysis.

Eradication of Brucellosis

No historical report summarizing reproductive management of dairy cattle would be complete without mentioning efforts made to eradicate brucellosis (Ragan, 2002). Brucellosis (Bang's disease) caused by *Brucella abortus* was by far the most significant reproductive disease of cattle in the United States during the first half of the previous century. It affected dairy herds by increasing abortions and reducing fertility. Furthermore, it was a threat to human health through transmission to humans who consumed unpasteurized milk. Steps were underway in some states in the 1930s to eradicate the disease, but the problem was nationwide. The US Congress appropriated funds in 1954 to launch a national eradication program. Testing spread to all states by 1957 and 124,000 infected herds were soon identified. Testing revealed from one-third to one-half of herds were infected based on data from cattle slaughtered for meat production.

One of the authors (JHB) recalls veterinary visits to his family's dairy farm when all cows were blood tested and cows that were "positive" were branded on the jaw with a "B" and those that had a "suspect" test were branded with an "S." If a cow received such a brand, its owner was entitled to an indemnity and branded cows had to be slaughtered. It took almost half a century to eradicate brucellosis in cattle herds, and small pockets of the disease remained in bison and deer in various regions of the United States. Brucellosis is still prevalent at modest to high rates in countries around the world.

Control of Estrous Cycles

Progesterone and Progestins. Progesterone was first used to manipulate estrous cycles of cattle during the late 1940s and early 1950s (Ulberg et al., 1951). These early studies showed that progesterone (50 mg) administered daily in a vegetable oil base was effective in preventing estrus and ovulation. Estrus typically occurred 4 to 5 d after progesterone withdrawal, but fertility at that estrus was compromised (Trimberger and Hansel, 1955). To avoid having to administer daily injections, Nellor and Cole (1956) used microcrystalline progesterone suspended in a starch solution administered in a single injection to synchronize estrus. Their approach was successful but the precision of synchronization was poorer than that observed following daily injections.

Orally active progestins were first fed to cattle to synchronize estrus in the 1960s (Patterson et al., 1989). For example, field trials evaluated effectiveness of 2 progestins [6-chloro-Δ^6-dehydro-17-acetoxyprogesterone (CAP) and 6-methyl-17-acetoxyprogesterone (MAP)] provided in liquid or pelleted forms to control time of estrus in cows (Hansel, 1961). Progestins were typically fed for 14 to 18 d and cows exhibited estrus 4 to 6 d after withdrawal of the progestin from feed (Boyd, 1970). Synthetic progestins such as 17α-acetoxy-9α-fluoro-11β-hydroxyprogesterone (cronolone) also were incorporated into vaginal sponges for use in cattle (Carrick and Shelton, 1967).

Although progestins were reasonably effective in synchronizing time of estrus, fertility was consistently less than that observed in contemporary controls. Furthermore, concerns were expressed about synthetic progestins appearing in milk of dairy cows. One synthetic progestin, melengestrol acetate (**MGA**), was found to be very effective in preventing expression of estrus in feedlot heifers while also improving weight gain and feed efficiency (Lauderdale, 2009). This product continues to be used for this purpose and is used in combination with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to synchronize estrus in dairy heifers and beef cattle.

Grouping Cows for Reproductive Management. Once progestin products for synchronizing estrus became available, the concept of grouping or clustering cows (i.e., weekly or biweekly) according to calving dates for group management was introduced (Britt et al., 1972; Folman et al., 1984). The scheme utilized in the former report for dairy cows (Figure 3) used MGA to synchronize first inseminations and the next potential insemination, whereas in the latter report a progesterone-impregnated intravaginal insert and $PGF_{2\alpha}$ analog were used. These grouping schemes are prototypes for what is done today to cluster cows for timed AI.

Intervention Technologies

In the late 1960s, discovery of hypothalamic peptides that control secretory function of the anterior pituitary

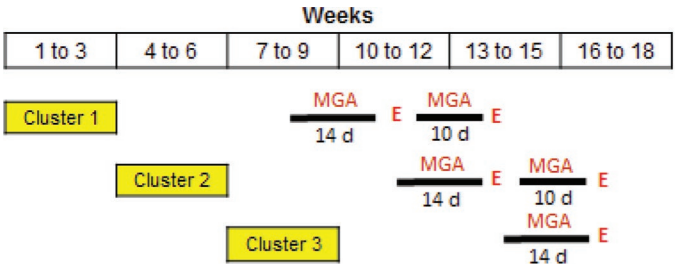

Figure 3. Proposed clustering of dairy cows after calving to facilitate synchronization of estrous cycles by feeding melengestrol acetate (MGA: 0.5 mg/cow per day) first for 14 d and thereafter for 10 d to cows not inseminated initially at estrus (E) after the first 14-d feeding (Britt et al., 1972). Clustering accommodated synchrony of all breedings to just a few days per week. Color version available online.

led to sequencing and synthesis of gonadotropin-releasing hormone (GnRH). At about the same time, highly sensitive and precise radioimmunoassays were developed to detect nanomolar concentrations of biological substances in blood and tissue. These discoveries led to a shared Nobel Prize in Physiology or Medicine by Roger Guillemin, Andrew Schally, and Rosalyn Sussman Yalow in 1977 (Yalow, 1978).

Several newly discovered hormones and delivery technologies were introduced for control of estrous cycles and ovulation in domestic animals in the early 1970s. Many early reports of these technologies were presented at a 1974 conference on Control of Sexual Cycles in Domestic Animals (Anon, 1975). Among technologies that were highlighted at this conference were applications of (1) $PGF_{2\alpha}$ and its analogs as luteolytic agents; (2) GnRH and its analogs to induce ovulation; (3) intravaginal progesterone-impregnated silicone spirals for administering progesterone to synchronize estrus; and (4) timed AI to inseminate cows after synchronized estrus or ovulation.

$PGF_{2\alpha}$. The finding that $PGF_{2\alpha}$ could decrease progesterone in ovaries of pseudo pregnant rats and terminate pseudo pregnancy led to its discovery as a luteolytic hormone (Pharriss and Wyngarden, 1969). Several preliminary reports in 1972, and later reviewed by Lauderdale (2009), documented luteolytic actions of $PGF_{2\alpha}$ in cattle. Potent analogs were developed subsequently and use of luteolytic prostaglandins expanded to application in multiple species.

GnRH. Discovery of the amino acid sequence of LH-releasing hormone soon led to its synthesis (Amoss et al., 1971; Matsuo et al., 1971a,b). Thereafter, several reports documented the effectiveness of GnRH for inducing secretion of LH in heifers (Zolman et al., 1973), normal cows, and cows with ovarian follicular cysts (Kittok et al., 1973), and for inducing LH release and ovulation in lactating dairy cows (Britt et al., 1974). Studies demonstrated that injection of GnRH induced release of LH and FSH (Britt et al., 1981), and later that it was effective for inducing ovulation of bovine ovarian follicles $\geq$10 mm in diameter during approximately 64% of the estrous cycle (Moore and Thatcher, 2006).

Progesterone Inserts. A progesterone-impregnated silicone-coated flat stainless steel insert, twisted into a coil so that it could be easily inserted into the vagina, later marketed as the progesterone-releasing intravaginal device (**PRID**), released progesterone at concentrations sufficient to suppress estrus for more than 3 wk. When tested in cows, the PRID successfully synchronized estrus (Mauer et al., 1975; Roche, 1975). Another intravaginally placed progesterone-impregnated insert known as the controlled internal drug release or **CIDR** insert was introduced in the 1990s in New Zealand (Macmillan and Peterson, 1993) and is now used worldwide in combination with $PGF_{2\alpha}$, GnRH, or both.

Timed AI. With hormonal products available to synchronize estrus or ovulation, scientists sought to inseminate cows at preplanned times without regard to detection of estrus. The first 2 reports of success with timed AI applied similar approaches. In the first, Mauer et al. (1975) inserted PRIDs into 396 nonlactating cows and heifers for 21 d, injected GnRH 28 to 30 h after PRID removal, and inseminated cows with frozen-thawed semen 18 to 24 h after GnRH. Conception risk did not differ between cows inseminated by appointment and those inseminated based on detected estrus. Roche (1975) shortened the duration of PRID treatment in 367 Friesian dairy cows to 12 d, injected 5 mg of estradiol benzoate and 50 mg of progesterone at the time of PRID insertion, and injected GnRH 30 h after PRID removal. Cows were inseminated 48 h after PRID removal or 18 h after GnRH. Fertility was reported to be normal.

CURRENT TECHNOLOGIES AND MANAGEMENT

Estrus-Detection Systems

Detecting Estrus. Artificial insemination is the most viable option to produce pregnancies in dairy cows; however, implementation of a successful AI program on dairy farms requires accurate and efficient detection of estrus, even if timed AI is used for first or later services. Several significant physiological changes have been reported to occur during the periestrual period (Lewis and Newman, 1984; Roelofs et al., 2010) that enable detection of estrual behavior and other correlated traits in cows. Some of these changes include physical activity; vaginal cytology and pH; electrical resistance of vaginal mucus and genital tissues; body temperatures; pulse and heart rates; blood flow; rumination time; pheromones, blood metabolites, and hormones; milk yield; and feed intake.

Cows are monitored traditionally for visual signs of estrus such as "standing to be mounted" by a herdmate. To be effective in identifying when to inseminate, visual observation of cows should occur for at least 30 min twice daily in the morning and late afternoon or early evening. Challenges associated with visual detection of estrus were increased by changes in housing and footing surfaces that occurred in the early 1970s. Approximately 76% of large dairy herds (>500 cows) in the United States house dairy cows in confinement freestall

barns with concrete flooring. An estimated 32% of those freestall operations also have turnout dry lots. Only 17% of all large operations have cows only in dry lots (NAHMS, 2014). One of the most important factors limiting expression of sexual behavior in lactating Holstein cows is the surface on which they are observed for estrus. Cows are likely to express estrous behavior more often and for longer durations when detected for estrus on earthen surfaces rather than on dry grooved concrete surfaces (Britt et al., 1986).

Estrus-Detection Aids. To reduce labor and improve efficiencies associated with detection of estrus, procedures and methods were developed to detect estrus in absence of visual observations. The state of the art for early estrus-detection aids was reviewed thoroughly by Foote (1975). Described were pressure-sensitive devices affixed to the tail head of cattle that were triggered (color indicators) when a cow stood to be mounted and a marking harness worn by a sexually aggressive or testosterone-treated cow or heifer (Kiser et al., 1977) that applied a colored ink stripe to the back of estrual females when mounted by the marking animal. The newest version of tail-head-applied patches is made like a lottery or scratch-off ticket that reveals a new color once the outer gray film of the patch is rubbed off by the mounting female.

Mounting pressure-sensitive sensors to the rump of the cow that are activated by a mounting herdmate were developed in the mid 1990s (Stevenson et al., 1996). The accuracy and efficiency of these systems are quite high because they are associated with specific sexual behavior and are functional 24 h/d (Roelofs et al., 2010). The downside to these systems is the labor associated with applying the sensor patches and the maintenance to keep sensor patches affixed to cows until pregnancy occurs.

Today, technologically simple products such as paint or chalk applied to the tail head remain the most common forms of estrus-detection aids in US dairy operations, second only to visual observation (Figure 4). When painted or chalked cows are mounted from the rear, some of the chalk or paint is rubbed off, indicating that the rubbed cow possibly stood in estrus when mounted by a herdmate. Validation of accurate rub marks by concentrations of progesterone in milk or subsequent pregnancy after insemination showed that accuracy of chalk- or paint-rub marks as estrus-detection methods varied widely from 33 to 90% (Stevenson and Phatak, 1999). It seems from many studies that 5 to 30% of cows or heifers inseminated are not really in estrus (Roelofs et al., 2010).

Automated Estrus Detection. Automation of estrus detection by novel systems in dairy cows is well documented (Firk et al., 2002). Before 2006, automated estrus-detection aids were limited to pressure-sensitive rump-mounted devices and pedometers (Figure 4; NAHMS, 2007). Technologies developed to take advantage of physiological correlates of estrus include

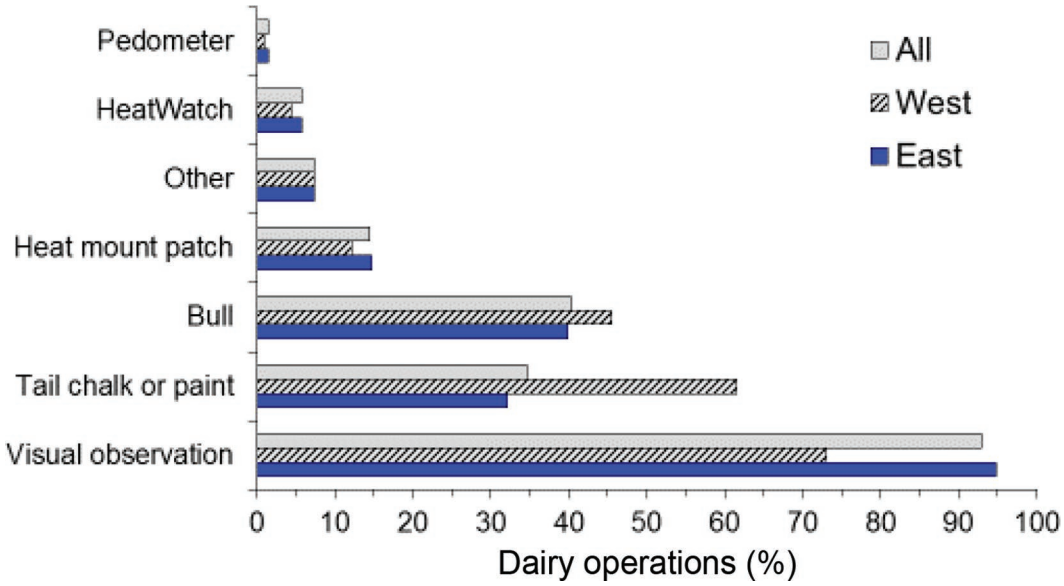

Figure 4. Percentage of US dairy operations by region using various estrus detection methods during 2006 (Source: NAHMS, 2007). HeatWatch is produced by CowChips LLC (Manalapan, NJ). Color version available online.

pedometry or activity monitors, pressure-sensitive, rump-mounted radiotelemetric devices, temperature sensors, and milking inline chemical sensors.

Early in the 21st century, neck-mounted activity tags containing a microprocessor and a 3-dimensional accelerometer became available for detection of estrus. An accelerometer allows accurate measurement of cow movement. Activity tags monitor specific estrus-related movement and its intensity, resulting in detection accuracies up to 90% (Roelofs et al., 2010). By 2010, the bestselling system in the world with approximately 1 million estrus-detection tags sold demonstrated that dairy farmers were willing to invest in technologies that provide a real solution to detection of estrus. Since the NAHMS survey in 2007 (Figure 4), at least a dozen companies are now offering activity monitor technology for use in dairy operations and will likely have a larger impact in dairy operations seeking for low-cost methods of identifying cows in estrus (Nebel, 2015). Some of the newest activity monitors in the market are equipped to monitor ear temperature, resting or lying time, rumination or feeding time. Some have global positioning sensor (GPS) capabilities to help locate individual cows in large pens. When integrated with daily milk production data, managers have the tools to monitor health and activity associated with estrus. Regardless of methods applied, visual detection of estrus has been the gold standard management practice upon which a successful AI program is built.

These activity systems are effective management tools in AI programs because their use can increase estrus-detection rates. Greater estrus-detection rates increase AI submission rates and more potential pregnancies. Several within-herd studies demonstrated that using activity monitors to detect estrus before AI compared with timed AI programs applied to control cows resulted in similar pregnancy risk (Neves et al., 2012; Stevenson et al., 2014; Denis-Robichaud et al., 2016)

Despite the increase in estrus-detection rates, 2 recent studies indicated that only 70 to 75% of dairy cows validated by progesterone concentration and transrectal ultrasonography of ovarian structures to have had luteolysis were detected in estrus by activity monitors (Valenza et al., 2012; Sauls et al., 2017). Furthermore, more than 50% of the cows not detected by the activity monitors ovulated in the absence of detected estrus.

Video systems have also been investigated to capture activity in freestall barns (Bruyère et al., 2012), but the cost and practicality of such systems require further study. Devices have been patented that claim to detect ovulation by changes in body temperature via an implanted temperature sensor or an implanted vaginal telemetry system that apparently detects tissue impedance, temperature, and activity of the animal. Radio-telemetered monitoring of vaginal or ear skin temperature was accomplished with greater than 80% accuracy of detection and only a few false positives (Roelofs et al., 2010).

AI Breeding Programs: Development of Timed AI

Timed AI programs were used (NAHMS, 2007) to manage reproduction in at least some of the heifers and cows by 58.2% of operations (survey of 17 of major US dairy states representing 79.5% of US dairy operations and 82.5% of US dairy cows) with a greater percentage of using timed AI for cows (57.6%) than for heifers (25.4%). More than 60% operations had used timed AI for 5 yr or more. Regarding reasons for using timed AI, the greatest percentage of operations (48.8%) used timed AI occasionally during the previous 12 mo to catch up on nonpregnant cows, and the reason that timed AI was used by the second greatest percentage of operations was to control all first and subsequent services (27.7%). In another 103-herd survey, the majority of large US dairy operations (>500 cows; Caraviello et al., 2006) used timed AI programs almost exclusively to inseminate their cows. More than 85% of those large herds surveyed were using timed AI at first services after calving and 77% for resynchronizing repeat services.

Hormone-based timed AI breeding programs have been commonplace in the dairy industry since the late 1990s. Although the discovery in the early 1970s that $PGF_{2\alpha}$ is the natural uterine luteolysin in cattle, its use on dairy operations was originally limited to inducing estrus for first AI services (Britt et al., 1981) or inducing estrus in cows diagnosed not pregnant but having a palpable corpus luteum (**CL**; Plunkett et al., 1984). Injections of $PGF_{2\alpha}$ successfully regressed the CL, with the majority of estrus activity occurring on d 2 through 5 after treatment with $PGF_{2\alpha}$. Early in its application, 2 injections of $PGF_{2\alpha}$ were given 11 to 14 d apart to allow cows to be inseminated at detected estrus after the first injection and follow through with the second injection for all noninseminated cows. Waiting 11 to 14 d before reinjecting noninseminated cows allowed for some of the remaining cows to have a $PGF_{2\alpha}$-sensitive CL (not responsive to the first injection) or for the remaining cows to develop a new $PGF_{2\alpha}$-sensitive CL. The latter group of cows was most likely those that responded (CL regression) to the first $PGF_{2\alpha}$ injection but were not detected in estrus. These simple programs are still common in southwest and western dry-lot dairies in which rates of detected estrus average greater than 70%.

Variation in the interval to estrus after $PGF_{2\alpha}$ was not understood until application of transrectal ultra-

sonography to monitor growth patterns of individual follicles during the mid-1980s (see Thatcher, 2017). It was discovered that follicles emerge together every 8 to 10 d (a follicle wave) with cows having 2 or 3 follicular waves per estrous cycle. Based on understanding of normal follicular wave dynamics of the estrous cycle, brought about by using transrectal ultrasonography, it became clear that synchronizing follicle growth must be coupled with induced regression of the CL to better synchronize subsequent ovulation.

As early as 1983, it was known that giving GnRH 3 d after $PGF_{2\alpha}$ would enhance synchronized ovulation and subsequent pregnancy risk (Lucy and Stevenson, 1986), but it was not recognized until the 1990s that GnRH administered before $PGF_{2\alpha}$ to dairy heifers and cows could synchronize follicular development before lysing the CL with $PGF_{2\alpha}$ (Twagiramungu et al., 1995), thus resulting in various successful timed AI programs used on dairy operations (Wiltbank and Pursley, 2014).

Timed AI Programs Before First Service

Ovsynch. A history of the most commonly applied timed AI program (Ovsynch) in dairy cows is available elsewhere (Wiltbank and Pursley, 2014). Among versions of Ovsynch, the most commonly used is the 7-d Ovsynch scheme (Figure 5A, 5B, and 5C). Variations of this scheme include a 5-d program with doses of $PGF_{2\alpha}$ given on d 5 and 6 and timed AI on d 8 (0 to 16 h after the second GnRH injection; Figure 5D). Insight is provided in detail in recent reviews (Bisinotto et al., 2014; Stevenson, 2016).

Presynch $PGF_{2\alpha}$ Programs. Early studies indicated that pregnancy outcomes at first AI might be

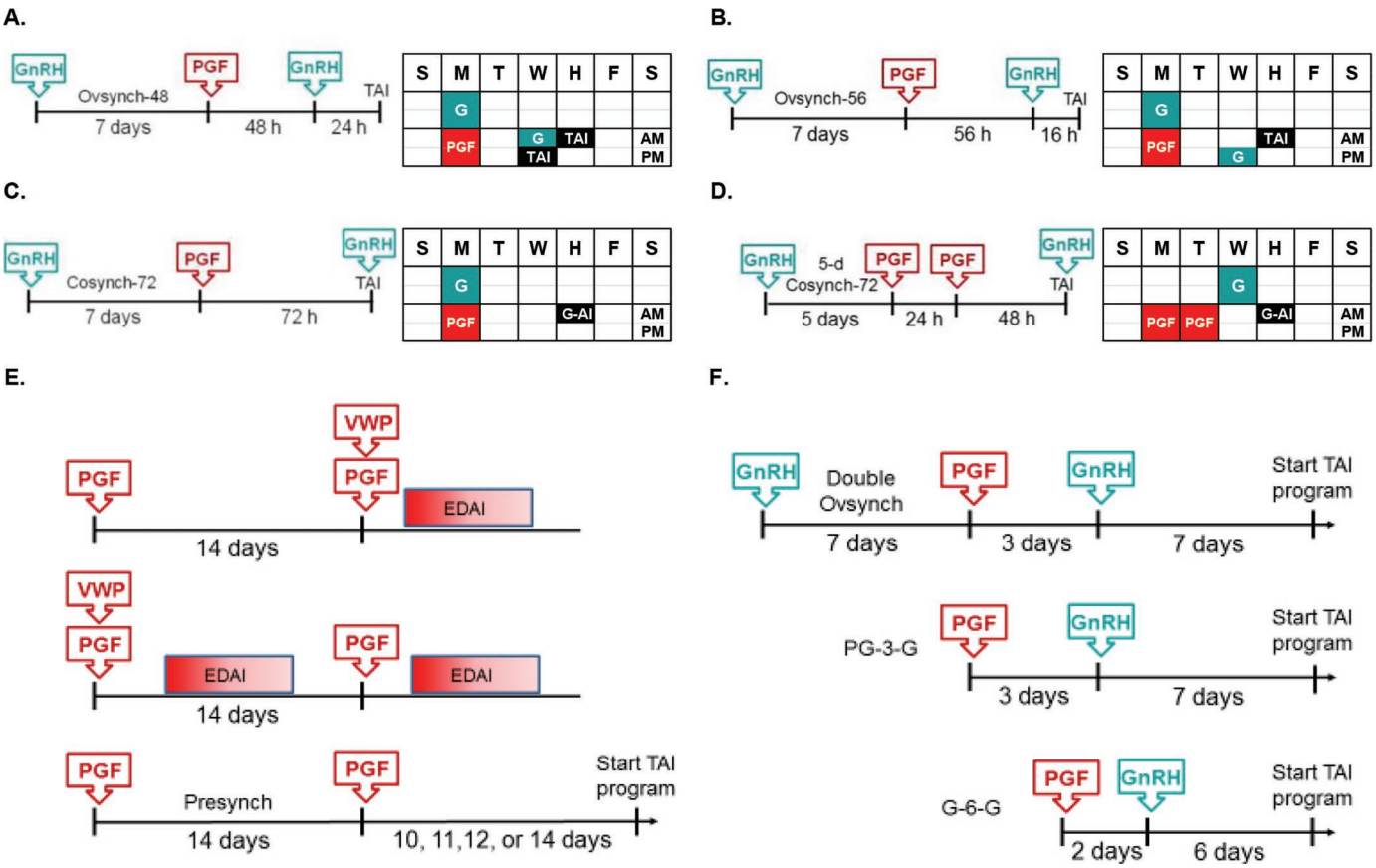

Figure 5. The basic ovulation-synchronization timed AI (TAI) program is known as Ovsynch. It consists of a GnRH injection (G; 100 μg) to induce an LH surge to cause ovulation followed in 7 d with $PGF_{2\alpha}$ (PGF) to lyse the corpora lutea (CL) before a second dose of GnRH is administered at 48 h (A; Ovsynch-48), 56 h (B; Ovsynch-56), or 72 h (C; Cosynch-72) after $PGF_{2\alpha}$. An alternative short version is a 5-d program (D; 5-d Cosynch-72) in which 2 doses of PGF are necessary to lyse any new CL that formed after GnRH. Progesterone supplementation can be applied via an intravaginal progesterone-releasing controlled internal drug release (CIDR) insert during the interim between GnRH and PGF. For first services after calving, cows can be treated with either 1 or 2 PGF injections administered 14 d apart and then inseminated after detected estrus (E; EDAI) or presynchronize estrous cycles by 2 $PGF_{2\alpha}$ injections before any timed AI programs (A, B, C, or D) at first services. Other presynchronization treatments before first services can include GnRH and $PGF_{2\alpha}$ (F) before any timed AI programs (A, B, C, or D) at first services. VWP = voluntary waiting period. Color version available online.

improved when cows were at specific stages of an estrous cycle at initiation of the timed AI program. Cows beginning the timed AI program on d 5 through 12 of their estrous cycles had greater ovulatory responses to the first GnRH injection and greater fertility than cows at other stages of the cycle (Vasconcelos et al., 1999). Thus, 2 research groups tested whether estrous cycles could be staged to meet this ideal by applying 2 injections of $PGF_{2\alpha}$ administered 14 d apart and then initiating the timed AI program 12 d later (named Presynch-12; Figure 5E). Their so-called Presynch treatment before Ovsynch resulted in greater pregnancy outcomes after first services compared with applying only Ovsynch to cows at random stages of their estrous cycles (Moreira et al., 2001; El-Zarkouny et al., 2004). A Presynch-14 program also was superior to Ovsynch alone for timed AI pregnancy outcome (Navanukraw et al., 2004).

Other permutations of the original Presynch have been applied (e.g., Presynch-14, Presynch-11, and Presynch-10) in which the 2 injections of $PGF_{2\alpha}$ were consistently administered 14 d apart, but the interval between the last injection to the onset of the timed AI program was 14, 11, or 10 d, respectively (Figure 5E). The interval between the second Presynch $PGF_{2\alpha}$ injection and the onset of Ovsynch determines the stage of the cycle at the onset of Ovsynch with decreasing days from the second Presynch $PGF_{2\alpha}$ injection resulting in earlier stages of the cycle at the onset of Ovsynch when a dominant follicle is capable of ovulating after GnRH-induced LH release (Vasconcelos et al., 1999).

In nearly all published studies, these Presynch $PGF_{2\alpha}$ systems have produced improved pregnancy outcomes compared with outcomes in cows submitted to the timed AI program at random stages of the estrous cycle without Presynch (Stevenson, 2016). Overall, lactating dairy cows exposed to Presynch $PGF_{2\alpha}$ programs have 42% greater odds of pregnancy compared with cows receiving only the timed AI program (Bisinotto et al., 2014). Furthermore, improved pregnancy outcomes were reported in cows treated with Presynch-11 than Presynch-14 before a timed AI program (Stevenson, 2016), probably because more cows were at the ideal stage of the cycle after Presynch-11 treatment (d 6 to 9 vs. d 9 to 12, respectively). Further reducing the interval between Presynch and Ovsynch, however, may reduce pregnancy outcomes. An additional benefit of Presynch $PGF_{2\alpha}$ programs is the flexibility of choosing to inseminate cows detected in estrus after $PGF_{2\alpha}$ rather than waiting to inseminate after the Ovsynch program (Figure 5E; Stevenson, 2016).

Presynch GnRH Programs. A major limitation to Presynch $PGF_{2\alpha}$ programs is their inability to improve fertility in anovular cows—those which have not initiated estrous cycles after calving—which may represent up to 41% of dairy cows at the end of the VWP (Bisinotto et al., 2014). Newer presynchronization systems that include GnRH administration in addition to injections of $PGF_{2\alpha}$ increase the odds for pregnancy by 1.65 times (Bisinotto et al., 2014) and have resulted in 3 different schemes: GnRH-7 d-$PGF_{2\alpha}$-3 d-GnRH (Double Ovsynch); $PGF_{2\alpha}$-3 d-GnRH (PG-3-G); and $PGF_{2\alpha}$-2 d-GnRH (G-6-G) (Figure 5F). In some cases, Double Ovsynch and PG-3-G may improve pregnancy outcomes compared with the 2 standard Presynch $PGF_{2\alpha}$ variants (Presynch-12, Souza et al., 2008; and Presynch-10, Stevenson and Pulley, 2012). The PG-3-G program produced better timed AI pregnancy outcomes during summer than Presynch-10 (Stevenson and Pulley, 2012). These Presynch-GnRH programs cause a larger proportion of cows to be at the most ideal stage of the cycle to initiate Ovsynch (d 6 to 8). Based on a meta-analysis considering 27 herds and 9,813 AI, cows with 100% timed AI after completing a $PGF_{2\alpha}$ Presynch-Ovsynch program at first service had more pregnancies compared with cows that were inseminated after detected estrus or received timed AI for cows showing no signs of estrus (Borchardt et al., 2016). In a second meta-analysis, comparing presynchronization programs with or without GnRH before timed AI, benefit was detected for pregnancy outcomes in primiparous cows presynchronized with a Presynch-GnRH program at first service, but this benefit was not observed in multiparous cows (Borchardt et al., 2017).

Improved pregnancy outcomes of Presynch GnRH programs may be realized in herds where more cows are anovulatory because more total cows ovulate in response to both GnRH injections of Ovsynch. Thus, Presynch options that include GnRH administration increase opportunities for anovulatory cows to ovulate either before or in response to the timed AI program. In addition, cows that lack a CL at the first GnRH administration of the timed AI program, which includes anovular or cycling cows treated during proestrus, estrus, and metestrus, have reduced concentrations of progesterone compared with cows with a CL. These groups of cows may represent approximately 30 to 40% of cows subjected to timed AI and are those that have the poorest pregnancy outcomes. In fact, cows without a CL treated experimentally with 2 CIDR inserts at the beginning of Ovsynch had similar pregnancy risk as contemporary cows starting Ovsynch in diestrus with a CL (see Bisinotto et al., 2014).

Presynch Five-Day Programs. Ovulation in response to the first GnRH injection of Ovsynch improves synchronization of the estrous cycle and reduces the period of follicular dominance; both factors may be associated with improved pregnancy outcomes (Vasconcelos

et al., 1999; Santos et al., 2010). One limitation to the 5-d program is the inability of a single dose of $PGF_{2\alpha}$ to induce complete CL regression in all cows (particularly a "new" CL that resulted from GnRH-induced ovulation). Therefore, 2 doses of $PGF_{2\alpha}$ are required (one on d 5 and another on d 6; Figure 5D) to optimize luteal regression by either $PGF_{2\alpha}$ or by one of its analogs. Recent studies included the addition of a second dose of $PGF_{2\alpha}$ in the 7-d program ($PGF_{2\alpha}$ on d 7 and 8) compared with a 5-d program ($PGF_{2\alpha}$ on d 5 and 6) and reported inadequate luteolysis in cows receiving only 1 $PGF_{2\alpha}$ on d 7 (Santos et al., 2016). Furthermore, addition of a second dose of $PGF_{2\alpha}$ in a multi-herd 7-d Ovsynch study increased luteolysis from 83 to 97%, but only increased pregnancy risk by 3 percentage points (Wiltbank et al., 2015).

Dose of GnRH. Increasing doses of GnRH in 7-d Ovsynch programs have increased LH responses to GnRH injections of Ovsynch (Giordano et al., 2012a), but did not increase the percentage of cows ovulating or the percentage of inseminated cows that became pregnant (Giordano et al., 2013).

Timing of GnRH. For 7-d programs, optimal timing of the second (or breeding) injection of GnRH to produce the greatest pregnancy risk is approximately 56 h after $PGF_{2\alpha}$ (Ovsynch-56; Figure 5B), with AI occurring approximately 16 h later (at 72 h) compared with timed AI at either 48 or 72 h, concurrent with the second GnRH injection (Figure 5A or 5C). In contrast, for cows subjected to the 5-d Ovsynch program, which results in smaller ovulatory follicles and reduced concentrations of estradiol in the plasma around the time of AI compared with the 7-d Ovsynch program, the duration of proestrus should be extended to 72 h, so the second GnRH injection and timed AI should occur at 72 after $PGF_{2\alpha}$ (Stevenson, 2016).

Programs Including Progesterone. Progesterone treatment with the CIDR insert has been used in some timed AI programs since its US market availability in 2003. The CIDR is either applied intravaginally for 5 or 7 d with an injection of $PGF_{2\alpha}$ upon insert removal or in combination with Ovsynch (inserted when the first GnRH is administered and removed 5 or 7 d later at the time of $PGF_{2\alpha}$ injection). About one-third of dairy operations (32.4%) used CIDR inserts during 2006 to treat anestrous females (65.7%) or cows with ovarian cysts (43.5%) or to synchronize estrus (34.3%) in females (NAHMS, 2007).

A review of the literature including a meta-analysis indicated that use of a single CIDR insert administered during the period between the first GnRH injection and the $PGF_{2\alpha}$ of the timed AI program increased the percentage of pregnant cows at d 60 after AI compared with untreated controls, and the benefit from progesterone supplementation was similar for cows with and without a CL (Bisinotto et al., 2015). In general, pregnancy risk in that summary was greater for cows treated with progesterone than for controls. Nevertheless, pregnancy risk for cows without CL treated with a single insert was 10.5 percentage points less than that of untreated cows that had a CL at the initiation of the timed AI program.

Cows benefitting the most from supplemental progesterone were those that were anovular or not in diestrus at the onset of the timed AI program. Incorporating 1 or 2 progesterone inserts as part of a timed AI program increased fertility in cows that lacked a CL at the first GnRH injection, but did not restore fertility to the same level as those cows starting the timed AI program in diestrus (reviewed by Stevenson, 2016).

Resynchronization Programs

Not-Pregnant Diagnosis. Increased efficiency of reproduction occurs when pregnancy status in previously inseminated cows is determined as soon as possible. When $PGF_{2\alpha}$ was introduced in 1979, a common practice of treating nonpregnant cows was to inject $PGF_{2\alpha}$ when a palpable CL was found upon not-pregnant diagnosis (**NPD**). Cows were either inseminated upon detected estrus or by appointment at 72 and 96 h after $PGF_{2\alpha}$ (Plunkett et al., 1984). When inseminations only occurred after detected estrus, failing to follow up on noninseminated cows resulted in nonpregnant cows remaining uninseminated for variable periods until they were detected in estrus.

Once timed AI programs such as Ovsynch became in vogue, studies were conducted using timed AI programs initiated in nonpregnant cows starting either a 5- or 7-d resynchronization (Resynch)-Ovsynch program on the day of NPD. When using transrectal ultrasonography to diagnose pregnancy on a weekly basis, it is common to evaluate cows that were inseminated 30 to 36 d before the NPD. If the NPD falls on a Monday, then most cows that received a timed AI on Thursday would be at d 32 of pregnancy. If transrectal palpation were used for pregnancy diagnosis, then a common interval for days since last AI would fall between d 37 and 43, with most cows receiving a timed AI on d 39.

Presynchronization Before Resynch. In some herds, cows diagnosed not pregnant are started on a timed AI program on the day of NPD. In other herds, to reinseminate cows sooner after a NPD, the first GnRH injection of the timed AI is administered to all cows eligible for the next pregnancy diagnosis (unknown pregnancy status) either 5 or 7 d before NPD depending on which timed AI program is used (i.e., 5 vs. 7 d). Applying a presynchronization treatment

such as GnRH or human chorionic gonadotropin (hCG) to induce ovulation and initiate a new follicular wave in cows of unknown pregnancy status has been tested for its profertility effects. Simple Resynch-Ovsynch programs initiated at d 32 or 39 after previous AI were compared with treatments that included a presynchronization GnRH or hCG injection administered 7 d before the NPD and the initiation of Ovsynch. At both initiation times, the pre-GnRH or pre-hCG injection increased pregnancy risk by 4 to 5 percentage units (Dewey et al., 2010; Giordano et al., 2012b,c; Bruno et al., 2014).

Addition of progesterone to such treatments in nonpregnant cows in designed studies in 7-d Resynch-Ovsynch programs initiated at the NPD failed to increase pregnancy risk of cows, whereas when tested in a 5-d Ovsynch program initiated at d 32, progesterone increased pregnancy outcome (Bisinotto et al., 2010). As previously noted, the greatest pregnancy advantage accrues from applying progesterone to cows without a CL rather than those with a CL.

In the early Resynch studies, detection of estrus was not applied in those designs, thus all cows received only a timed AI. In herds applying regular estrus detection, a large proportion of cows may be detected in estrus subsequent to the NPD. Therefore, in more recent studies, cows at NPD either received $PGF_{2\alpha}$ 7 or 11 d before initiating a Resynch-Ovsynch or a GnRH injection was administered as a presynchronization treatment 7 d before NPD as a control. In 2 such studies (Silva et al., 2007; Bruno et al., 2013), fewer cows were detected in estrus and inseminated before the timed AI program was initiated when the presynchronization GnRH injection was applied compared with the control Resynch + $PGF_{2\alpha}$ that included AI of cows after detected estrus. Although overall resulting pregnancy risks did not differ, cows detected in estrus and inseminated became pregnant sooner after the NPD than timed AI cows (Chebel et al., 2013). In general, when applying Resynch programs to dairy cows at NPD, employing presynchronization $PGF_{2\alpha}$ facilitates estrus expression, whereas using a pre-GnRH injection suppresses estrus expression.

Replacement Heifer AI-Breeding Programs

When heifers are inseminated after detected estrus without any hormone synchronization, it is common for conception risk to exceed 60%. Even when inseminations are performed after $PGF_{2\alpha}$ injections (and the majority of heifers express estrus on d 2 through 5 after injection) and inseminations are made after detected estrus, conception risk is commonly greater than 60%. These are traditional and proven programs for heifers, but they require time and skilled labor for detection of estrus. In large groups of heifers, such as found in heifer grower operations, inseminations are often based on both estrus and timed AI programs, followed by cleanup bulls. Sex-sorted semen is often used when inseminating replacement heifers, limiting conception risk to approximately 70 to 80% of what is achieved with conventional semen (DeJarnette et al., 2009).

Seven-Day Programs. When applying timed AI in heifers, pregnancy risk will be slightly reduced to the mid-50% range compared with insemination following detected estrus. The benefit of not needing estrus detection expertise, however, makes timed AI of heifers attractive for many heifer developers. A 7-d timed AI program in heifers includes a progesterone insert (e.g., CIDR) used for 7 d and $PGF_{2\alpha}$ injected upon its removal, in addition to a GnRH injection at CIDR insertion and a second GnRH injection between the $PGF_{2\alpha}$ injection and timed AI. Similar conception is expected for the 7-d CIDR programs when heifers are inseminated after detected estrus up until 72 h after $PGF_{2\alpha}$ and then the remaining heifers are injected with GnRH and inseminated at 72 h in the absence of estrus.

Five-Day Programs. The most consistent results for timed AI in heifers are found using the 5-d timed AI programs (Figure 6). A series of recent studies conducted in a large number of dairy heifers examined some variations of the 5-d timed AI program. In study 1 (Figure 6), ovulation in dairy heifers was synchronized and injections of $PGF_{2\alpha}$ were administered on d 5 (one when the CIDR insert was removed and another 12 h later). Pregnancy risk did not differ in 2 separate experiments, but ranged from 53 to 59% (Rabaglino et al., 2010a).

In study 2 (Figure 6), a comparison was made between using 1 or 2 $PGF_{2\alpha}$ injections at the time of CIDR insert removal. No differences in pregnancy risk were detected in 2 experiments (Rabaglino et al., 2010b).

In study 3 (Figure 6), only 1 $PGF_{2\alpha}$ injection was employed and the researchers addressed the need for the GnRH-1 injection. Results from study 3 showed no advantage in using the GnRH-1 injection when 1 $PGF_{2\alpha}$ injection was administered because pregnancy risks ranged from 48 to 52% (Lima et al., 2011).

In study 4 (Figure 6), no GnRH-1 injection was used with only 1 $PGF_{2\alpha}$ injection and the proper timing of GnRH-2 was tested. Results showed that the inconvenience of handling the heifers an extra time to administer GnRH-2 at 56 h (16 h before the timed AI) was not necessary to maximize pregnancy risk. In contrast, for those heifers that did not show estrus during the 72 h after $PGF_{2\alpha}$ and CIDR removal, extending the time to the GnRH injection to 72 h (coincident with the timed

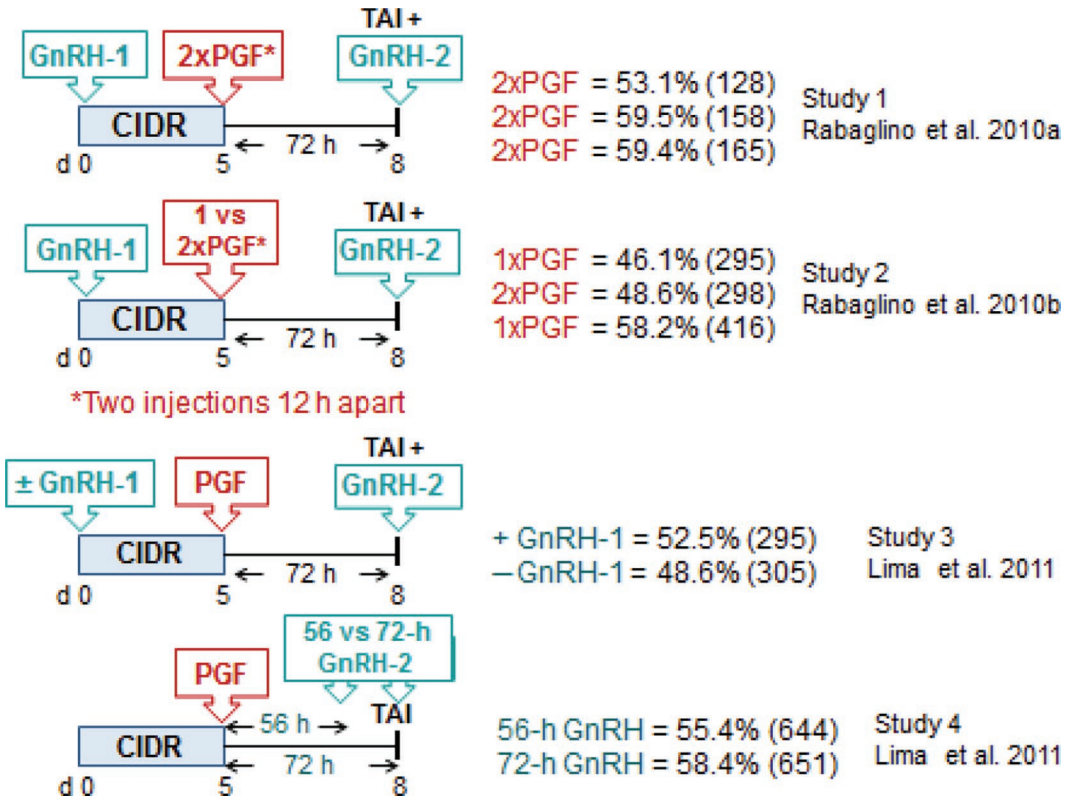

Figure 6. Variations in 5-d timed AI programs for replacement heifers are discussed in the text. CIDR = controlled internal drug release progesterone insert; PGF = $PGF_{2\alpha}$; TAI = timed AI. Color version available online.

AI) improved their pregnancy outcome from 44.7 to 53% (Lima et al., 2011).

One further study demonstrated that when GnRH-1 was administered at the time of CIDR insertion in a 5-d program, administering 2 $PGF_{2\alpha}$ injections (24 h apart; one with CIDR insert removal and one 24 h later) was advantageous to improve pregnancy risk compared with not using GnRH-1 and using only one $PGF_{2\alpha}$ injection (Figure 6).

In summary, synchronization of ovulation in dairy heifers by using a progesterone insert with administrations of GnRH and $PGF_{2\alpha}$ in 5-d programs can be successful. The results indicated that the 5-d program is effective and the GnRH-1 injection is not necessary when using only 1 $PGF_{2\alpha}$ injection. Extending the timing of the GnRH-2 to 72 h (coincident with the timed AI) benefitted heifers that did not show estrus during the 72 h after CIDR insert removal.

Pregnancy Diagnosis

Pregnancy exams are used on dairy operations to identify pregnancy status of inseminated or mated females. Because the majority of US dairies operate a continuous 365-d breeding season and cows calve year-round, it is critical to determine pregnancy status and identify nonpregnant cows so that they can be managed and reinseminated in a timely manner to reduce interinsemination and calving intervals. Additional benefits of pregnancy exams include detection of uterine or ovarian abnormalities, diagnosis of twins, and estimation of conception and calving dates for females with unobserved natural service by bulls.

In the 1800s, transrectal palpation of the uterus and its contents was first identified as a method of pregnancy diagnosis. Traditional diagnosis of pregnancy involves palpation of the conceptus (detection of fluid-membrane slip, or amniotic vesicle, or both) between 35 and 40 d after last insemination or mating. About two-thirds of all dairy operations (67%) performed pregnancy exams monthly or more frequently during 2006 (NAHMS, 2007). The majority of large (>500 cows) operations (75%) performed pregnancy exams weekly or every 2 wk, whereas 50% of smaller operations (<100 cows) performed exams on a monthly basis. Transrectal palpation is the method used most routinely to determine pregnancy status (85.7%), with the residual using transrectal ultrasonography (27.4%) and the blood test for assessing concentrations of pregnancy-associated glycoproteins (4.1%). Of dairy operations surveyed in

2014 (NAHMS, 2014), 85.7% used a veterinarian as a reproductive consultant.

A recent review (Fricke et al., 2016) highlighted current methodologies and implementation of pregnancy diagnosis in dairy cattle. Transrectal ultrasonography is becoming more accepted because portable, battery-powered units are available. Evidence of a viable embryo can be detected as early as 20 d in heifers, but accuracy and efficiency are best in routine herd exams for cows that are at least 28 to 30 d since last insemination.

Use of ultrasonography requires skill and training. Common errors include a false-positive diagnosis of uterine intraluminal fluid that is associated with estrus and not with the presence of a conceptus. On-farm ultrasonography is also diagnostic in assessing follicular structures (differentiating between follicular and luteal cysts), functional CL, twins, embryonic loss, and fetal sexing (Fricke, 2002). A major advantage of applying ultrasound is earlier and more accurate detection of pregnancy, which has great effect on reproductive management of dairy herd.

Pregnancy-specific proteins are produced by the conceptus during early pregnancy, and one class of these proteins is called pregnancy-associated glycoproteins (**PAG**; Ricci et al., 2015). Concentrations of PAGs are detectable approximately 25 d after AI, reach a peak near d 32, decrease to nadir by d 60, and then increase to greater concentrations throughout the remainder of gestation (Figure 7). Because of the described pattern in plasma and milk PAG profiles, the optimal time to conduct a first pregnancy diagnosis is approximately 32 d after AI, coinciding with an early peak in PAG concentrations (Ricci et al., 2015). Furthermore, because of the occurrence of pregnancy loss, all pregnant cows should be retested 74 d after AI or later, when plasma and milk PAG concentrations in pregnant cows have rebounded from their nadir. Although application of blood tests to identify PAGs was done on a minority of dairy operations in a recent survey (NAHMS, 2007), its use has increased greatly since then. At least 3 companies in North America are marketing the blood tests, which are routinely performed in veterinary clinics or other labs with a 24- to 36-h turnaround. One company also assays PAG milk samples. Incorporating these tests in reproductive programs usually involves collecting a blood or milk sample at 28 to 32 d after last insemination. The blood sample can be collected when a resynchronization program is initiated (first GnRH injection of the Ovsynch program is administered) so the program can be continued 7 d later when the not-pregnant status of the female is confirmed. Alternately, Resynch-Ovsynch is simply initiated after the blood or milk test results become available. These tests are relatively inexpensive, as accurate as transrectal ultrasonography diagnoses made by skilled technicians, and do not require expensive equipment or skilled training. Many of the DHI labs offer the PAG test as a service to dairy producers.

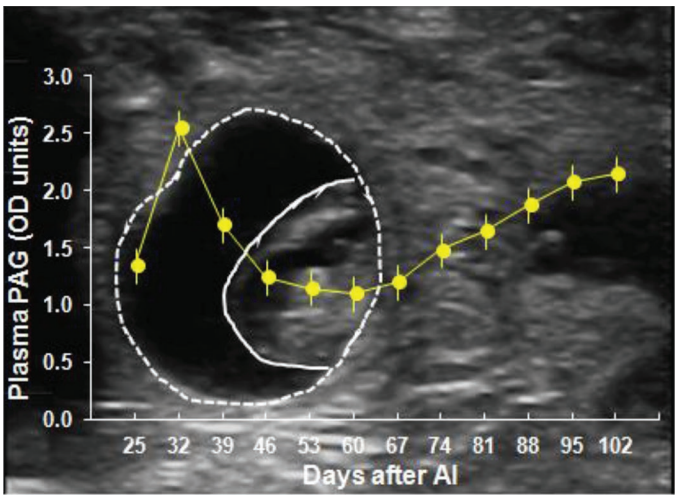

Figure 7. Plasma concentrations of pregnancy-associated glycoprotein (PAG) from 25 to 102 d of pregnancy are superimposed on an ultrasonogram of a 30-d bovine conceptus (solid line) suspended in a cross-section of a uterine horn (dashed line). The PAG data are from Ricci et al. (2015). OD = optical density. Color version available online.

Because pregnancy is diagnosed by ultrasonography or blood or milk tests before some natural spontaneous embryo losses occur, later verification of a viable pregnancy is essential. Losses of embryos identified as viable on d 28 can be up to 10 to 15% during the next 4 to 6 wk (Santos et al., 2004). If pregnancy was first detected at 35 to 45 d after last AI, some of these losses would never have been detected if the first diagnosis occurred after 45 d. For these reasons, it is recommended that early pregnancy diagnoses be verified later, perhaps by 80 to 100 d after AI, mid gestation, and again before terminating the lactation to begin the dry period.

Recombinant Bovine Somatotropin

One of the first potential biotechnology products for animal production was bST. Discovered more than 70 yr ago, bST was reported to increase milk yield by 15% in lactating dairy cows regardless of stage of lactation (Bauman, 1992). It was later synthesized and produced for commercial use through recombinant DNA technology similar to procedures used to produce human insulin.

Recombinant DNA derived–bovine somatotropin (**rbST**) also increases milk production by 15%. By 1978, research in the technology of bST involved hundreds of scientists publishing more than 1,000 studies involving more than 20,000 dairy cows. Treating cows with long-acting rbST (once every 14 d) not only increases milk

production on commercial dairy farms today but it was also observed, in a few studies, to have positive effects on pregnancy outcomes of well-managed cows (Moreira et al., 2001). First treatment with bST at first services after calving following a timed AI program (Ovsynch) or upon detected estrus resulted in increased pregnancy rates and reduced early embryonic death between d 31 and 45 after AI (Moore and Thatcher, 2006). The mechanism of bST action includes improved fertilization rate, accelerated embryo development, improved embryo quality, and increased conceptus growth at the time pregnancy is recognized approximately 15 to 16 d after AI (Ribeiro et al., 2014). Use of rbST has waxed and waned since it first became publicly available in the United States in 1994 (Caraviello et al., 2006), in part because of misunderstandings by consumers about the science and safety of such products and reluctance of dairy processors to market milk products coming from dairy operations using rbST. Today, most cooperatives and processors have restricted its use within their supply chain. The percentage of operations and the percentage of cows for which rbST was used increased as herd size increased. Overall, 9.7% of operations used rbST, and 14.7% of all cows received rbST during the most recent lactation (NAHMS, 2014).

Management Software Applications

Dairy record program software has been available for many years through the dairy record processing centers associated with the DHIA. The first DHI organizations were established in 1905 to provide a standardized method of collecting data from individual cows. Approximately 41% of US dairy operations participate in DHI testing programs, representing 47% of the 9.3 million dairy cows (NMPF, 2016). In the early days of DHI testing, summaries were prepared by hand, typed on forms, and sent to participating herds. Later, when computers came on line, print-outs were mailed to dairy producers within a few days of the monthly herd test summarizing milk yield, and various milk components (e.g., fat, protein, SCC, lactose, milk urea nitrogen) that were analyzed at DHI-affiliated laboratories. Daily yield of milk, milk component percentages, SCC, and other information such as calving and breeding dates, calf identification, pregnancy diagnoses, feed components and costs, culling information, and so on, are collated and entered into the software to produce the monthly printed reports.

In 2013, 47.5% of dairy operations had access to the internet for dairy information. Internet use generally increased as herd size increased, with 31.6% of very small operations accessing the internet for dairy information compared with 89.7% of large operations (NAHMS, 2014). Almost 100% of US dairies use on-farm computers that allow the same information to be collected, entered, and used in daily management routines in addition to measured milk and milk component data that occurs periodically. Each of the dairy record processing centers have their own unique software that shares most of the same output data in different formats for dairy producers. In addition, an independent software package used by many larger dairies is DairyComp 305 (Valley Agricultural Software, Tulare, CA). Another advantage of computerized records is to share that information with herd consultants and veterinarians who assist in closely monitoring client herd reproductive, production, and health issues. Consultants can easily obtain this information by obtaining a backup of each dairy's computer records or, with permission of the dairy producer, obtain a download from one of the dairy record processing centers.

Not only are computers commonplace but many dairy managers use handheld devices (including smartphones) that communicate with the farm computer to reveal needed information about cows as they work in cow pens. With the advent of electronic identification chips in radiofrequency identification (RFID) ear tags, cows can be identified by Bluetooth wands to retrieve various information about individual daily treatments. Milking equipment companies are now investing in in-line milk monitoring equipment that can measure metabolites as well as progesterone concentrations in the milk of cows during the milking process and produce elegant profiles of milk progesterone to assist managers in determining reproductive status (cycling or pregnancy status) of individual cows (Østergaard et al., 2005). Use of computers, computer-generated reports, and access to electronic data at the fingertips of dairy managers has not only influenced production but also increased efficiency of labor and management to provide better animal care. Future software upgrades may incorporate artificial intelligence that will interpret data and recommend possible actions and solutions.

Technologies of Emerging Application

During the last 25 yr, much has been learned about the molecular make-up of living organisms. The term "genomics" refers to the study of nucleic acids (e.g., DNA and RNA) within cells. In 2003, the USDA, in collaboration with the National Institutes of Health, announced the Bovine Genome Sequencing Project (Moore and Thatcher, 2006). The entire bovine genome has now been sequenced to reveal approximately 3 billion nucleotides with approximately 1% coding for functional genes, of which 40% or more of the genes have unknown functions. This technology is now being

applied in addition to traditional progeny testing to rank potential young sires that express desirable genes coding for important economic traits for breeding and future selection purposes. With release of genomic evaluations in 2009, information about a cow's or bull's genetic potential that previously would have taken years to obtain by progeny testing was revealed at a young age, reducing the generation interval from 5 to 2 yr, and thus increasing the rate of genetic progress.

With greater rates of genetic progress, it is important that proper emphasis of reproductive traits be considered. Part of the Holstein Total Performance Index includes a fertility index. The new fertility index combines several reproductive components into one overall index, including ability to conceive as a maiden heifer (heifer conception rate and daughter pregnancy rate), ability to conceive as a lactating cow (cow conception rate), and a cow's overall ability to reinitiate postpartum estrous cycles, express estrus, conceive, and maintain a pregnancy. Emphasis on these traits, including longevity, which itself is heavily dependent on reproductive efficiency of the cow, must continue. Therefore, future prospects are real for improving reproductive performance of dairy cows through genetic selection based on early genomic tests.

Although milk yield is often implicated as the cause of impaired fertility (Bello et al., 2012), the effect of inadequate body condition may be greater (Carvalho et al., 2014; Zachut and Moallem, 2017) because it has significant effects on the probability of conception, rate of embryo loss, and proportion of anestrous cows (Weigel, 2006). Selection for or against certain traits could lead to improved fertility of dairy cattle. For example, because of the high energy demand for rapidly increasing milk production and limited feed intake in the transition period around parturition, dairy cows require considerable metabolic adaptations. Some cows may be genetically less suited to cope with these metabolic needs than others, leading to adverse follow-up effects on longevity. In a recent study (Ha et al., 2017), functional lifetime of dairy cows was linked to the metabolic challenges in the beginning of the first lactation (e.g., sum of milk yield or the accumulated fat-to-protein ratio of the first 3 test-days (<120 DIM). Significant sire variance indicated a genetic component for metabolic adaptability and suggested that the ability to cope with metabolic stress in the transition period has a genetic component, which may be used to select metabolically more robust dairy cows.

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APPENDIX

Table A1. Timeline chronology of significant events and technologies developed to assist in reproductive management of dairy cattle; dates approximate development, introduction, patent issue, first use, or publication

Date	Milestone	Reference
1905	The Dairy Herd Improvement Association (DHIA) is organized.	
1914	First artificial vagina is developed.	
1918	Desirable age at first calving is described.	
1930	Gonadotropin extracts are first described.	Casida, 1938
1933	Progesterone is isolated and characterized.	Gomes and Erb, 1965
1937	Rectovaginal cervical fixation method of AI is developed.	Perry et al., 1945
1941	Time of ovulation is determined.	Brewster and Cole, 1941
1941	Semen diluents for AI are introduced.	

Continued

Table A1 (Continued). Timeline chronology of significant events and technologies developed to assist in reproductive management of dairy cattle; dates approximate development, introduction, patent issue, first use, or publication

Date	Milestone	Reference
1949	Frozen semen is developed and introduced to the AI industry.	Bratton et al., 1955
1951	"Frosty" is born (first calf to be born after AI with frozen-thawed semen).	
1952	Glycerol is added to semen diluents to protect sperm from freeze-thaw damage.	
1954	Frozen semen is first shipped in liquid nitrogen.	
1955	Progestins are used to synchronize estrus.	Hansel, 1961
1969	Prostaglandin $F_{2\alpha}$ is discovered to be luteolytic.	
1971	GnRH is discovered.	
1975	Progesterone-releasing intravaginal device (PRID) is developed.	
1977	First research report of pedometers used for detecting estrus is published.	
1979	Prostaglandin $F_{2\alpha}$ is approved for use in dairy cattle.	
1980	GnRH is approved for use in cystic cows.	
1982	Transrectal ultrasonography is first used to describe ovarian structures and diagnose pregnancy.	
1983	First patent is issued for a pedometer to detect estrus.	
1992	Blood test for pregnancy diagnosis is introduced as pregnancy-specific protein B.	
1993	HeatWatch pressure-sensitive estrus-detection sensors are introduced.	
1995	Use of transrectal ultrasonography for pregnancy diagnosis.	
1995	Timed AI programs are introduced with Ovsynch.	Pursley et al., 1997
1999	Presynch-Ovsynch is described for AI at first service.	Moreira et al., 2001
2000	Resynchronization programs are developed for open cows after a not-pregnant diagnosis.	
2005	Sex-sorted semen is introduced for sale by the AI industry.	
2005	Accelerometers are introduced in physical activity monitors for detection of estrus.	
2011	First eartag-based health and activity monitor is introduced.	
2014	Pregnancy-associated glycoproteins (PAG) can be used to diagnose pregnancy in milk.	

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A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle[1]

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ABSTRACT

Over the past 100 yr, the range of traits considered for genetic selection in dairy cattle populations has progressed to meet the demands of both industry and society. At the turn of the 20th century, dairy farmers were interested in increasing milk production; however, a systematic strategy for selection was not available. Organized milk performance recording took shape, followed quickly by conformation scoring. Methodological advances in both genetic theory and statistics around the middle of the century, together with technological innovations in computing, paved the way for powerful multitrait analyses. As more sophisticated analytical techniques for traits were developed and incorporated into selection programs, production began to increase rapidly, and the wheels of genetic progress began to turn. By the end of the century, the focus of selection had moved away from being purely production oriented toward a more balanced breeding goal. This shift occurred partly due to increasing health and fertility issues and partly due to societal pressure and welfare concerns. Traits encompassing longevity, fertility, calving, health, and workability have now been integrated into selection indices. Current research focuses on fitness, health, welfare, milk quality, and environmental sustainability, underlying the concentrated emphasis on a more comprehensive breeding goal. In the future, on-farm sensors, data loggers, precision measurement techniques, and other technological aids will provide even more data for use in selection, and the difficulty will lie not in measuring phenotypes but rather in choosing which traits to select for.

Key words: selection goal, production trait, functional trait, novel trait

INTRODUCTION

Genetic selection for important traits has helped transform and advance the dairy cattle industry. Specific traits considered for selection in dairy cattle populations have evolved with time as a response to changes to the needs of producers, consumers, and society with the aid of advances in technology and trait recording programs.

As outlined by Shook (1989), a potential trait must meet several criteria before it can be considered for selection in dairy cattle populations. First, either it should have an economic value as a marketable commodity or its improvement should reduce production costs. Second, the trait must have sufficiently large genetic variation and heritability. Third, the trait should be clearly defined, measurable at a low cost, and consistently recorded. Finally, an indicator trait may be favored if it has a high genetic correlation with the economically important trait, reduces recording costs, has a higher heritability, or can be measured earlier in life.

The economic value of traits has historically been the driver for genetic selection. From the 1930s to the 1970s, the focus of selection was solely on increasing milk production. Despite some early concern over selecting exclusively for yield, which was expected to cause a corollary decline in overall fitness, the industry strove to achieve maximum genetic change in the most financially lucrative area, which was production. The need to identify and select for additional traits emerged mainly from the recognition of the correlated genetic decline in other important traits. Many countries have shifted toward more balanced selection objectives by including more weight on previously undervalued nonyield traits (Miglior et al., 2005).

The second criterion concerns genetic variation and heritability of a trait, which are central to the rate of genetic progress possible within a selection program. Traits vary in the amount of phenotypic and genetic variation observed, and they may be more or less heritable. Traits may also be contingent on one another,

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with correlations either positive or negative and genetic and phenotypic correlations either strong or weak. Such correlations may be exploited by the use of indicator traits, which may be favored if they are more readily available than a trait of interest.

A major boon for the progression of genetic selection has been the recording and access to clearly defined, accurate, and cost-effective phenotypes. The continuous increase in data collected throughout the production chain has brought forth many opportunities and a large number of traits with genetic evaluations for consideration. However, this has also resulted in a large number of potential traits to be considered for inclusion in selection programs and ultimately balanced appropriately. Careless selection, changing selection goals, or having many different objectives can reduce selection pressure (Meadows, 1968) and can have an undesirable permanent effect on the population.

The conception, development, and application of multitrait index selection has played a pivotal role in successful and progressive selection in many countries. By weighting each trait according to its independent effect on net profit and using genetic and phenotypic parameters to weight traits measured in individuals and relatives, the correlation of index with genetic variation in net profit can be maximized (Hazel, 1943; Hazel et al., 1994). Traits considered in selection vary between countries because of differences in milk and component prices, costs of inputs and services, production environments, and availability of phenotypes. These factors can frequently change, and modifications need to be considered and researched continually. The identification of traits that are presently important for genetic selection and those that will be essential in the future is a vital aspect of selection research.

Milk recording began in North America in 1905 and thus provided the foundation for selection on milk production. Cattle shows at county fairs made conformation traits very popular as well. Technological advances, in particular the advent of AI in the late 1930s, created a division between producers who wanted cows to produce milk and those who wanted good-looking cows that produced milk. Artificial insemination organizations aimed their bull selection programs toward both types of producers. Gradually, producers recognized that fat and protein yields and longevity were also important to keeping the costs of production within reason. Behavior and health traits were incorporated soon afterward, demonstrating an increased awareness of the economic importance of these traits but also representing increasing societal concerns with intense production systems.

The rapid developments in genomic information, automated data recording technologies, and modern analytical techniques over the past decade are setting the stage for a new era in dairy cattle breeding. Here we review the development of phenotypes used in dairy cattle selection over the past century (see Appendix Table A1).

PRODUCTION

A century ago, selection in dairy cattle focused on high milk and fat production. In the late 1800s and into the 1900s, many breed associations, in addition to their standard herd register, worked to promote better dairy cows by recording cow merit through milk recording for the inclusion of cattle in advanced registries (Becker and McGilliard, 1929). These registries went by various names, including Advanced Registry for Ayrshire, Register of Production for Brown Swiss, Advanced Register for Guernsey and Holstein, and Register of Merit for Jersey. The Babcock test, invented and made public in 1890 by S. M. Babcock, provided an accurate and easy method for the determination of milk fat content in milk testing. The testing of a large number of purebred cows through milk testing programs and the organization of records into published volumes of the Advanced Register and Register of Merit provided the foundation for locating high-producing blood lines and the study of the inheritance of milk and fat production (Fohrman, 1926). Meade (1921) evaluated the performance of Guernsey sires and their transmitting ability and concluded that the best method for selection may be to consider the percentage production of all advanced registry daughters based on standardized requirements according to age. The male line received the greatest attention in selection because the sire's heredity was most accurately indicated by his daughters' production, more so than the dam based on her own production record (Graves, 1925). A recognized fault of production records in an advanced registry for selection purposes was that records included only daughters that were put on test and met advanced registry standards. Later, some breed organizations initiated a further herd test or herd-improvement registry where dairy producers were required to test and report production of all cows in their herd. Using the descendants of 2 cows disparate for their fat tests, Burrington and White (1925) demonstrated that the difference in test could be maintained over generations. Copeland (1927) expressed that dairy cattle breeders had thus far concerned themselves chiefly with increasing milk yield traits and concluded that more improvement in total fat production could be accomplished by selecting for high fat percentage along with milk yield.

Traits also considered by breeders to aid in the selection of animals to improve production were body

conformation traits. The thought was based on the view that conformation of the cow shows her probable production and that the conformation of the sire will be transmitted to his daughters, implying their probable production (Gowen, 1926). Gowen (1920) attempted to study conformation and its relation to milk-producing capacity through the calculation of phenotypic correlations. He confirmed the existence of the relationship between body conformation and production that was common belief among producers, although he concluded that conformation was a poor guide for milk production. Body weight and other measurements indicating size and shape were related to milk production (but not fat percentage) and were inferior to production records of ancestors for breeding for milk production (Gowen, 1926). Consideration of conformation traits remained in the forefront for breeders because an easily measured indicator for production was in demand. The true value of conformation in breeding for production was unclear, though, and Copeland (1941) reported only a slight relationship. Tyler and Hyatt (1948) found an intraherd relationship of 0.19 between conformation and average fat record and stated that selecting within a herd for conformation would not substantially improve fat production. Harvey and Lush (1952) found a genetic correlation of 0.18 between conformation and fat production from daughter–dam pairs. O'Bleness et al. (1960) studied the genetic correlations between production traits and individual conformation traits, the strongest of which were fat with pin bone width (0.39–0.40), rear udder shape (−0.54), and fore teat length (0.42) and with milk, dairy character (0.95–0.98). However, with an index, selection on the basis of milk production alone would be almost as effective as including conformation traits as well.

More measures of producing ability related to the lactation curve and persistency began to appear. The 2 main factors in total yearly milk and fat production were the yield during the maximum month and the persistency of production or the rate of decline (Turner, 1926). Turner (1926) suggested inheritance of fat production during the month of maximum production and endorsed consideration of persistency in selecting breeding animals. Copeland (1937) studied Herd Improvement Registry records of the American Jersey Cattle Club, discovered that some cows maintained their production longer than other cows under similar conditions, and ruled that lactation persistency was an inherited trait.

Gaines and Overman (1938) discussed the American Dairy Cattle Club's step toward requiring estimates of milk protein yield on the advice of the club's geneticist that protein was the most biologically valuable milk component. However, at the time no practical field test for protein existed. The importance of the nonfat component of milk, or SNF, increased due to the awareness of the nutritional value of SNF and its effect on milk processing. Data presented by Richardson and Folger (1950) suggested that SNF contents were inherited and that its relationship with fat was nonlinear. Around the 1950s, changes to the milk pricing structure were proposed to better reflect the importance of, and compensate producers for, both fat and SNF contents in milk. With an economic value projected for SNF, dairy cattle breeders would seek to improve contents in their cows. The heritability presented by Johnson (1957) for SNF was 0.34 for both Holstein and Jersey breeds. This study also reported very strong genetic correlations between different milk components, deducing that selection for one would equate to selecting for the others but with less pressure. Nonfat components were expensive and difficult to obtain for large numbers of cows, thus limiting the number of observations for this trait. Legates (1960) and Laben (1963) reviewed the many factors affecting SNF. Selection at the time emphasized total milk yield per cow, which returned the greatest value for milk, but farmers were encouraged to test for SNF to help build knowledge of its variation and possible future economic gains (Laben, 1963). The cost of testing milk for protein was an added expense that could potentially be avoided, as protein still increased by selecting for correlated fat and milk yields (Van Vleck, 1978).

Infrared methods for the analysis of milk samples for fat, protein, and lactose content delivered more rapid and less expensive measures than those previously available (Biggs, 1967). Protein testing became much more commonplace in the 1970s using this technology and became standard in milk testing. Milk processors began paying premiums for protein, and the number of cows with a higher genetic propensity to produce protein increased rapidly as protein was added into national selection indices (Shook, 2006). A shift toward increased emphasis on protein yield in selection indices occurred in many countries.

Although total protein content in milk was the primary consideration for selection, genetic variability and milk protein variants garnered additional attention. Aschaffenburg and Drewry (1955) first reported a genetic polymorphism of β-LG producing 2 different forms. Genetic variation was next reported in α-LA (Blumberg and Tombs, 1958) and in the CN (Aschaffenburg, 1961). The effects of the many discovered milk protein variants were considered important to achieving specific requirements for the dairy industry, including cheese manufacturing. Furthermore, several of the different protein variants have been associated with milk production and with fat and protein yield and percent-

ages (Ng-Kwai-Hang et al., 1984, 1986; Aleandri et al., 1990; Bovenhuis et al., 1992; Tsiaras et al., 2005). However, many contradictions in their effects exist in the literature, and it is not clear whether the protein genes or linked genes produce the effect (Bovenhuis et al., 1992).

The shape of the lactation curve became a trait of interest for selection once again because of issues of cow health from the stress of high peaks in production and for use in an index to improve total yield. Aspects of the lactation curve considered by Shanks et al. (1981) included the lactation curve, persistency, week of peak yield, and peak yield. Generally low heritabilities were found, except for peak yield, which had heritabilities ranging from 0.16 to 0.23. Ferris et al. (1985) reported low heritabilities with large standard errors for lactation shape measures, and indices formulated to flatten the lactation curve did so at the expense of milk yield. Jamrozik et al. (1997) applied random regression models to test-day yields to generate EBV for partial-lactation yields and persistency for animals with even single test-day records. Genetic evaluations are now performed in several countries for lactation persistency.

Much success has been achieved in the improvement of production traits as a result of genetic selection. The dominant role production traits held in selection programs for many decades has been diminishing as selection goals become broader. Selection for production traits needs to be examined in tandem with relevant nonyield traits.

CONFORMATION

Conformation, or type, of an animal has been of interest to dairy producers since the beginning of the selection process. The archetypes for conformation and beauty in dairy cattle have been passed down through time and conveyed from past breeders (Copeland, 1941). Producers strongly considered conformation traits for breed standards of perfection for registration and in the show ring as well as to garner top prices in public sales. The aesthetic aspect of the animal was the main reason for selection, but conformation traits were increasingly used to select dairy cows for other characteristics, such as higher production and longevity.

Several breed associations started classification programs to appraise conformation of all animals in the registry based on a scorecard or scale of points. In 1929, Holstein cattle in the United States were classified for 4 major categories: general appearance, dairy character, body capacity, and mammary system. Soon after, in 1932, the American Jersey Cattle Club established a similar classification program. The data collected through the classification programs confirmed an existing relationship between conformation and producing ability, highlighting the fact that both conformation and production should be considered in selection programs (Copeland, 1938).

The stability of conformation classification and the repeatability of the measurement were questioned in early studies (Johnson and Lush, 1942; Hyatt and Tyler, 1948). For example, Johnson and Lush (1942) reported high variation between evaluators and low repeatability for conformation traits ranging from 0.34 to 0.55. The heritability estimates reported for conformation traits were moderate to low. Tyler and Hyatt (1948) reported a heritability estimate for conformation of approximately 0.30. O'Bleness et al. (1960) reported heritability estimates for 27 different conformation traits ranging from 0.00 to 0.33.

In an effort to establish a more objective way to measure conformation traits, studies were conducted using data recorded from a conformation appraisal program initiated in 1953 in New York. The studies showed effects of appraiser (Van Vleck and Albrectsen, 1965), age, and lactation stage (Norman and Van Vleck, 1972) on conformation trait measures. Using data from the same appraisal program, Van Vleck (1964b) estimated heritabilities for conformation traits higher than those estimated previously and much closer to those reported in more recent studies (e.g., Rupp and Boichard, 1999).

In 1967, the Holstein-Friesian Association of America introduced a descriptive classification program that included an assigned code value for 12 conformation traits in addition to the 4 scorecard traits already recorded since 1929. These measures were also recorded on unregistered cows. The implementation of this system provided a large amount of data, allowing a more precise evaluation of conformation traits. Because of their negative correlation with milk production, conformation traits should be included in selection objectives to maintain cow appearance (Grantham et al., 1974).

Due to increased attention on linear conformation appraisal in the 1980s, a substantial change occurred in the methods used for estimating genetic values for conformation traits. The aim was to score conformation traits using a wider range of numerical scores (i.e., a 50-point basis). This measurement method presented several advantages, the major one being that it allowed analyses on a continuous scale and with mixed-model evaluation, as described by Thompson et al. (1983).

The predictive ability of conformation traits for additional traits of interest, other than production or longevity, was considered in several studies. Udder conformation traits showed varying but usually positive correlations with milking ability (Blake and McDaniel, 1979) and favorable correlations with udder health (Monardes et al., 1990; Rogers et al., 1991). As well,

mostly favorable correlations have been found between conformation and fertility (Dadati et al., 1985, 1986). The relationship between conformation and calving ease was negative when considering the conformation of the calf and positive when considering the conformation of the dam (Cue et al., 1990).

LONGEVITY

Longevity in dairy cattle has many different definitions and encompasses traits referring to the length of time a cow remains in the productive herd or its ability to remain in the herd. Measures of longevity have included age at disposal or last calving, number of lactations, and survival to a fixed age or lactation number. Cow longevity is a fundamental component of profitability in dairy production and, apart from production traits, has the greatest economic value (Allaire and Gibson, 1992). Longevity reduces the costs of replacements and maximizes the profitable period following the recovery of initial breeding and rearing costs. In addition, improving longevity could aid in breed development and genetic improvement because it would allow for more voluntary culling and greater selection intensity if fewer replacements were required.

The main goal in selecting for longevity is to decrease the premature disposal of cows or involuntary culling. By reducing the involuntary culling rate, dairy producers can consequently increase the voluntary culling rate and keep only the most productive animals. Reasons and strategies for culling are vast, may differ between years, and vary greatly between producers because they depend on the situation of the herd and involve a great degree of subjectivity and personal preference. Cows may be removed for voluntary reasons such as herd reductions, old age, level of production, body or conformation, management or workability, and sale for beef. Involuntary culling may occur for various reasons including reproductive performance, general health or illness, injuries, and accidents. Therefore, selection for longevity incorporates the improvement of many different components.

Automatic selection for increased longevity is presumed because cows remaining in the herd longer would produce more progeny and thereby contribute more to the succeeding generations (Parker et al., 1960). Because of the aforementioned importance of the trait to producers, further deliberate and direct selection for longevity was attractive and warranted. Measures of longevity were more easily recorded and accessible than records for fitness traits. Asdell (1951) examined DHIA herd culling records and stated that work needed to be done to develop longer living cows and reduce the loss of aging cows to sterility and udder troubles, which were on the rise. When studying the occurrence of cystic ovaries in a herd, Casida and Chapman (1951) found that there was a significant daughter–dam correlation for time spent in the herd. Wilcox et al. (1957) estimated in a single herd a heritability of 0.37 for longevity measured as number of parturitions. In a herd that had experienced no deliberate selection for conformation or production, Parker et al. (1960) found a near-zero heritability for longevity in terms of age at last calving. In general, the heritability of longevity in dairy cows is low (White and Nichols, 1965; Miller et al., 1967; Hargrove et al., 1969; Schaeffer and Burnside, 1975; Ducrocq et al., 1988; VanRaden and Klaaskate, 1993). Variation in reported heritabilities could be attributable to single or small numbers of herds used in early studies and differences in culling reasons between the populations.

To qualify a direct record for longevity, the cow or daughters of a sire must have reached the end of their productive life, which means the cow is no longer available and the generation interval is increased in evaluated sires. To overcome this and the low heritability, early measures for indirect selection for longevity were investigated. Parker et al. (1960) found a significant correlation between first-lactation fat production and longevity recorded as age at last calving. Gaalaas and Plowman (1963) found a tendency for better producing young cows to stay in the herd longer using an intrasire regression of age at last calving on production. The propensity for high first-lactation producers to complete more lactations was substantiated by Van Vleck (1964a), White and Nichols (1965), and Hargrove et al. (1969), indicating that selecting young sires on daughter first-lactation production records would indirectly improve longevity. This conclusion was in contrast to the belief of many producers, who thought that many young high-producing cows leave the herd early and do not live up to this high production later in life (Van Vleck, 1964a).

The physical characteristics of a dairy cow were assumed to be related to its longevity. Conformation traits were widely available for classified cattle, known early in life (usually first lactation), and heritable, making them attractive indicators of longevity. Specht et al. (1967) reported a correlation of 0.20 between overall first classification score and longevity of Holstein-Friesian cows. They found similar correlations between individual conformation traits and longevity. Using the daughters of AI sire Holsteins, Van Vleck et al. (1969) examined the relationship between 66 type categories measured in first lactation and longevity, determined as number of recorded lactations. The type traits with the strongest correlations with longevity were plumb rear teat position (0.38), sharp dairy character (0.35),

intermediate thurls (0.26), and typical head (0.25). Schaeffer and Burnside (1975) looked at sire proofs for the survival rates of 2-yr-old daughters making a record at 3 and 4 yr of age and resolved that improvement in longevity could best be achieved using type and milk proofs opposed to longevity directly. With the arrival of linear type traits, more research into their correlation with longevity was completed. Several studies highlighted the relevance of many udder characteristics, feet and legs, and dairy character in improving selection for longevity (Rogers et al., 1988; Foster et al., 1989; Short and Lawlor, 1992).

An improvement of the longevity definition was suggested to better direct selection toward increasing the ability of cows to survive irrespective of production (Van Arendonk, 1986). Miller et al. (1967) examined longevity by dividing cows into opportunity groups to enable comparisons before all cows had died and further adjusted longevity for milk production. They found that heritabilities decreased when the effect of first-lactation milk was removed. Later, Ducrocq et al. (1988) suggested 2 measures of longevity: (1) true longevity not adjusted for yield, describing the ability of the cow to remain in the herd, and (2) functional longevity, linearly adjusted for the cow's milk yield relative to the herd, representing the ability to delay involuntary culling. The correction of longevity for milk production should expose differences between animals culled for nonproduction reasons. Given culling levels for production, adjustment of longevity for production was recommended to eliminate bias from culling for production (Dekkers, 1993).

Research over the past decades has shown that longevity is heritable and that selection is possible. Thus, many major countries in dairy breeding have included longevity in routine genetic evaluations (Miglior et al., 2005). Multiple-trait evaluations combining indirect measures of longevity with direct measures are helpful to improve the accuracy of longevity evaluations. There is currently no consensus in the trait definition and methodology for evaluation across countries. The United States considers productive life, which combines direct longevity defined as total months in milk through 84 mo of age, along with SCS, udder, body size, feet and leg composites, and milk, fat, and protein yields (Cruickshank et al., 2002). In Canada, genetic evaluations for direct longevity are from a 5-trait animal model including cow survival from first calving to 120 DIM, from 120 to 240 DIM, from 240 DIM to second calving, survival to third calving, and survival to fourth calving to account for differences in the genetic background of survival at different time points (Sewalem et al., 2007). Complementary indirect longevity evaluations in Canada are based on dairy strength, feet and legs, overall mammary, rump angle, SCS, milking speed, nonreturn rate in cows, and interval from calving to first service (Sewalem et al., 2007). In the future, the incorporation of additional traits relating to longevity, which includes many health traits, may benefit evaluation and selection for longevity.

FERTILITY

Because of the economic importance of reproductive efficiency, much attention has been given to fertility traits and to their relationship with production over the years. Moreover, genetic correlation with productive life indicates that fertility plays a major role in longevity of the cow (VanRaden et al., 2004). The advent of AI activities actualized the problem of fertility, and the possibilities of breeding for reproduction had to be investigated in a completely new light. Principally, the attention and research on fertility has been directed toward female fertility. The consequences and variation in sire fertility are seldom regarded in the genetic improvement of fertility. This is despite the fact that different fertility measures in dairy breeding can be affected by only the cow or bull or a combination of both male and female fertility, such as conception rate. Early measures of female fertility were the number of services required for conception, nonreturns to first service, the interval from calving to first insemination, and calving interval. The disadvantages of interval from calving to first insemination were that it might be influenced by farmer decisions or by seasonal calving. A late insemination could also be the result of estrus detection failure, so that the cow was cycling successfully but did not have the opportunity to conceive. On the other hand, success traits such as nonreturn rate may be affected by culling decisions made after first insemination, lack of recording of natural services, and inseminations made some days after first insemination. One of the earlier studies to address the genetic aspect of the problem of infertility was that of Spielman and Jones (1939), in which a correlation of 0.55 was reported between the reproductive efficiency of the cow and its female descendants. This suggested that the reproductive efficiency of the cow is an important factor in determining the mean reproductive efficiency of its offspring. The possibility of selecting for reproduction efficiency, however, was later questioned by several studies due to the very low heritability estimates reported for most of the considered fertility traits. Trimberger and Davis (1945) stated that even if a certain variation was found between both families and bulls, the number of services required for previous conceptions of the dam could not be used for predicting the breeding efficiency of the daughters. Dunbar and Henderson (1953) reported a

heritability of 0.004 for nonreturn rate at 180 d to first service. In the same study, the heritability estimate for calving interval was zero. Selection for fertility measured by nonreturn to first service, services required per conception, or calving interval was not very effective. Despite a low heritability for these traits, most measures of genetic variation, expressed in this relative way, were substantial and were often almost as large as those for milk yield (Philipsson, 1981). Similarly, the additive genetic variation for reproductive traits is greatly masked by a huge phenotypic variance, and it appeared unwise to ignore reproductive performance in selection programs for dairy cattle (Hermas et al., 1987). Furthermore, Raheja et al. (1989) estimated a heritability of 0.12 for age at first insemination, indicating that selection for this trait would result in a genetic response. Generally, the large residual variation observed for fertility traits is possibly attributable not only to the large effect of the environment and management on these traits but also to the low quality of the data, which represents an issue in the analyses of reproduction data.

The relationship between heifer fertility and cow fertility has been questioned for a long time. Philipsson (1981) reported that the fertility of a virgin heifer, a first-lactation cow, and an older cow might be different traits. Repeatabilities shown by Hansen et al. (1983) for virgin heifers and first-parity fertility suggested that heifer and cow fertility might not be highly related. More recent studies considered the genetic correlations between traits observed in both heifers and older cows and concluded that heifer and cow fertility traits were not genetically the same (Jamrozik et al., 2005).

The correlation between reproduction traits and milk production has been extensively studied. An antagonistic correlation between female fertility and milk production has been reported in several studies over the years (Everett et al., 1966; Miller et al., 1967; Berger et al., 1981; Oltenacu et al., 1991; Dematawewa and Berger, 1998; VanRaden et al., 2004). However, the effect of selection for milk on reproduction was controversial in the beginning because some studies reported little or no relationship between yield and reproduction (Weller, 1989). Even if an antagonistic relationship between female fertility and milk yield existed, the correlated response on fertility due to selection on lactation production would not be significant (Everett et al., 1966; Miller et al., 1967; Shanks et al., 1978). However, because milk yield was the predominant goal in dairy cattle selection, the long-term selection for yield may have caused deterioration of reproductive performance (Nebel and McGilliard, 1993). Later, it became accepted that due to unfavorable genetic correlations, selection for higher yields in dairy cattle has possibly led to a decline in fertility (Pryce et al., 2004) as the reproduction physiology of dairy cattle changed in response to genetic selection for milk production (Lucy, 2001). The inclusion of reproductive measures in the general indices was adopted relatively late, mostly due to data availability. Genetic evaluation for reproduction was first adopted in the Nordic countries, which started to include reproduction traits in national indices in the late 1970s. Female fertility has been included in the total merit index for Norwegian dairy cattle since 1972 by considering the 56-d nonreturn rate in virgin heifers; since 2002, the 56-d nonreturn rates in first-lactation cows have also been considered (Andersen-Ranberg et al., 2005). Nordic countries remained the only ones to consider these traits for many years. According to a survey published in 1994, they were still the only countries to consider fertility, calving performance or stillbirth, and health traits in their total merit index (Philipsson et al., 1994). In a more recent survey referring to data from 2003, Miglior et al. (2005) reported that more countries included fertility in their national selection indices, including several European countries, Australia, New Zealand, and the United States, showing a shift of selection emphasis in the last decade from mainly production to functional traits associated with health and fertility.

Because of the low heritability of fertility traits and the difficulties related to their measurement, indicator traits could be very useful for increasing accuracy of EBV for fertility. Novel phenotypes, such as BCS, have been proposed as indicator traits for fertility. A strong relationship was found between BCS and reproductive measures (Pryce et al., 2001; Veerkamp et al., 2001; Berry et al., 2003; Bastin et al., 2010). Also, some recent studies have explored the possibility of taking advantage of more recently available data, such as the mid-infrared (**MIR**) predicted fatty acid profile in milk. Bastin et al. (2012) observed moderate correlations between C18:1 *cis*-9 fatty acid (an indicator of body fat mobilization) and days open at 5 and 200 DIM (0.39 and −0.38, respectively).

CALVING

Under normal circumstances parturition in cattle should terminate without human interference, leaving a healthy cow and a viable calf. A significant proportion of calvings, however, are assisted to a major degree and could yield a stillborn calf (Meijering, 1984). Stillbirth commonly includes calf mortality shortly before, during, or within 24 to 48 h after parturition (Philipsson, 1976). The selection focus for early-maturing cows over the past years could accentuate calving problems, especially as young animals calve for the first time well

before they reach their mature size. Calving difficulty and calf mortality represent major problems affecting profitability of dairy farming, and the economic loss is associated with various factors. Dystocia was reported to have a negative effect on 305-d milk, fat, and protein yields, days open, number of services, and cow losses (Dematawewa and Berger, 1998). The eventual death of the calf is also costly, especially if the dead calf is female. Several countries reported increasing stillbirth rates for Holsteins between 1985 and the late 1990s (Meyer et al., 2001; Steinbock et al., 2003; Hansen et al., 2004).

Calving ease is a combination of 2 different traits: direct calving ease, which is related to the calf, and maternal calving ease, which expresses how easily the cow gives birth. Pollak and Freeman (1976) reported a heritability of 0.08 for dystocia, defined as prolonged or difficult parturition. A decline in heritability estimates with increasing parity was also observed (0.18, 0.08, and 0.05 for first-calf, second-calf, and older cow records, respectively). Calf size had a heritability of 0.15 and showed a strong genetic correlation with dystocia (0.97). Berger and Freeman (1978) reported that including relationships due to sire and maternal grandsire of bulls with progeny data and accounting for the unequal variance associated with parity of dams increased the precision of individual sire estimates and resulted in a higher heritability for calving difficulty, which was estimated at 0.12. The relationship between dystocia for primiparous and multiparous cows has been controversial. Early studies (Thompson et al., 1981; Boldman and Famula, 1985) reported very strong correlations between dystocia in primiparous and multiparous cows (0.84 and 0.99, respectively). However, Weller et al. (1988) reported that the correlation between first-parity and later-parity sire evaluations was lower than 0.50. Steinbock et al. (2003) estimated heritabilities for stillbirth of 0.04 and 0.03 for the direct and maternal effect, respectively. Heritabilities for calving difficulty were 0.06 for direct effect and 0.05 for maternal effect. At second calving, the corresponding heritabilities for the 2 traits were considerably lower (<0.01). Despite low heritabilities, a substantial genetic variation in both traits, expressed by differences between EBV of bulls, was observed for first calvers. Heringstad et al. (2007) reported strong genetic correlations between direct stillbirth and direct calving difficulty (0.79) and between maternal stillbirth and maternal calving difficulty (0.62). All genetic correlations between direct and maternal effects within or between traits were close to zero, suggesting that bulls should be evaluated both as sire of the calf (direct) and sire of the cow (maternal). Thompson et al. (1981) suggested that selecting for reduced dystocia, considering only the direct effects, would reduce genetic progress for this trait. These conclusions were based on negative correlations found between direct and maternal effects (−0.38 and −0.25 for heifers and cows, respectively). Similar results were also reported by Boldman and Famula (1985), who estimated genetic correlations between direct and maternal effects of −0.40 and 0.07 in heifers and adult cows, respectively. Dekkers (1994) concluded that the optimal breeding strategy for calving ease would be to select sires based on an index that includes EBV for both direct and maternal calving ease along with other traits of economic importance.

Nordic countries were the first to consider calving performance and stillbirth in their national indices (Philipsson et al., 1994). Evaluations for calving ease traits, however, were also available early in other countries. For example, the evaluation of service sire calving ease has been published in the United States since 1978, whereas daughter calving ease was not implemented until 2002 (Shook, 2006). The first sire evaluations for calving ease for bulls used in Ontario, Canada, were published in 1981 (Cady and Burnside, 1982).

HEALTH

Diseases in dairy cattle are a major source of economic loss for dairy producers. Loss is a result of reduced production, death, premature culling of animals, veterinary treatments, lost milk due to antibiotic use, added labor, delayed conception, reduced genetic gains, low milk quality, and increased susceptibility to other diseases. More recently, heightened concerns over antibiotic use and ethical and animal welfare matters have added further demand for health traits to be included in breeding programs. Although there is a large effect of environment and management on disease occurrence, variation between cows can be observed.

Mastitis, the most prominent and economically significant disease affecting the dairy industry, received early attention from animal breeders because reducing its occurrence using all possible approaches was warranted. Unfortunately, mastitis can be caused by different species of bacteria and can occur in one or more quarters of the udder. Management conditions at the herd level can also have a large effect on the presence of mastitis. Lush (1950) first studied the possible inheritance of mastitis resistance by examining cows that developed mastitis at any age and those that had not developed mastitis by the age of 8 yr. Susceptibility to mastitis was found to have a strong genetic background, and Lush concluded that the incidence of mastitis could be reduced by selecting against severely affected cows or those with severely affected sisters or daughters. Legates and Grinnells (1952) also found

mastitis resistance to be heritable based on rating cows as infected or not through the study period.

Casida and Chapman (1951) investigated the incidence of cystic ovaries in a US Holstein herd and estimated a heritability for the occurrence of cystic ovaries some time in a cow's life as 0.43. An inherited component of ketosis, which also caused high economic loss, was also implicated, but Shaw (1956) stated that this component was not of primary importance in most herds and that more reports and evidence were required. These early appraisals of the role of genetics in mastitis and other disease susceptibility were hampered by limited data and highlighted the need for improved phenotypes and methods for earlier evaluation.

Young et al. (1960) studied the genetic relationship between clinical mastitis and the additional phenotypes of bacterial infection, leucocyte count, and udder and hock height alongside clinical diagnoses of mastitis. A significant negative genetic correlation was reported between clinical mastitis and udder height. Most significantly, their results using daughter–dam data indicated that including leucocyte score in an index would increase the efficiency 140% over using clinical mastitis alone. Somatic cell counts were introduced into many milk recording programs in North America and Europe in the late 1970s, raising renewed interest and serious discussion on selection for mastitis resistance. Ali and Shook (1980) demonstrated that a log-transformation of SCC to produce a score (SCS) resulted in a near-normal distribution of SCC and greater heritability. Kennedy et al. (1982) estimated an average heritability of 0.08 for SCS and small, undesirable positive genetic correlations between SCS and yield traits. Heritabilities for SCS reported by Coffey et al. (1985) ranged from 0.09 to 0.29 depending on parity. Additional work by Coffey et al. (1986) approximated genetic correlations between SCS and measures of infection to be between 0.36 and 0.67, providing further evidence that genetic evaluation and selection for decreased SCC may aid in reducing mastitis incidence. Originally purposed as a management tool and milk quality criterion, SCC found its place in genetic selection because of ease of measurement, a moderate heritability, and an association with both clinical and subclinical forms of mastitis. However, Coffey et al. (1986) expressed concern at the time that the consequences on selecting for decreased SCC were not understood and could, in the long term, lead to a reduction in the response to udder infection if SCC were too low. Another apprehension in the implementation of selection for mastitis resistance was the well-known antagonistic genetic relationship between mastitis traits and yield traits (Kennedy et al., 1982; Coffey et al., 1986; Emanuelson et al., 1988). Shook (1989) discussed that the decreasing genetic trend in cow health due to selection for yield was a major concern, especially when considering long-term projections, and this should be a convincing reason to consider mastitis resistance in breeding programs.

In Nordic countries, where only veterinarians are allowed to treat animals, nationwide systems for health data recording were established in the 1970s. These databases contain reliable records for disease treatments on a national scale, and many traits were subsequently added into routine genetic evaluations (Heringstad et al., 2000; Philipsson and Lindhé, 2003). In health data recording programs, a large number of disease traits are recorded, the most prevalent being mastitis and ketosis. Heritabilities of various health traits have been generally low but sufficient to allow for selection for reduced health problems (Emanuelson et al., 1988; Lyons et al., 1991; Simianer et al., 1991; Uribe et al., 1995; Pryce et al., 1997).

In countries with no regulated systems in place for dairy cattle health recording, obtaining sufficient records of health events for genetic evaluation recounts the issues of too few data experienced by early researchers. Zwald et al. (2004) began to look at health data recorded by American dairy producers in on-farm management software programs for mastitis, lameness, cystic ovaries, and metritis. Low heritabilities were found, but these data provide useful information for selection purposes, especially for incidence of any disease in the first 50 d postpartum. In Canada, a national dairy cattle health and disease data management system was started in 2007 for voluntary producer recording of 8 diseases: mastitis, displaced abomasum, ketosis, milk fever, retained placenta, metritis, cystic ovaries, and lameness (Koeck et al., 2012). Producer-recorded health data can be used in genetic evaluations, but sufficient participation and accurate and complete health records are necessary.

Health records could provide important information for genetic selection programs, but low heritabilities, nonnormal distributions, and subjectivity in diagnosis made potential indicator traits appealing. Emanuelson et al. (1988) examined Swedish mastitis treatment records and found enough genetic variation to attain significant genetic improvement if records on enough progeny of a sire were available. They also resolved that SCS should be used in selection. Somatic cell score became the trait contributing to udder health selection indices, with some countries also including evaluations for udder conformation traits, milking speed, clinical mastitis, and dairy form (Miglior et al., 2005). Indicators for selection and the genetic evaluation of metabolic diseases, including ketosis and displaced abomasum, have been discussed by Pryce et al. (2016). For ketosis, acetone was considered to be an indicator for disease,

but low heritabilities estimated for its content in milk suggested little genetic control (Emanuelson and Andersson, 1986; Wood et al., 2004; van der Drift et al., 2012). Another indicator of subclinical ketosis, milk BHB, can be predicted using MIR spectroscopy during milk recording, although with limited accuracy. Heritabilities for milk BHB have ranged from about 0.07 to 0.16 (van der Drift et al., 2012; Koeck et al., 2014; Jamrozik et al., 2016). Milk BHB and other heritable indicators (fat-to-protein ratio and BCS) are genetically correlated with clinical ketosis and displaced abomasum and are used in multitrait genetic evaluations for metabolic diseases (Koeck et al., 2014; Jamrozik et al., 2016). Predictor traits have been an integral component in multiple-trait evaluation of health traits in breeding programs to generate higher EBV reliabilities.

The concern over the genetic decline in dairy cattle health has brought attention to genetic selection for improved health, and research continues on the identification of new traits to aid in this selection goal. Mainly, only individual diseases with high incidence have been included in selection programs thus far, and many health traits would benefit from more records or multitrait evaluation with indicator traits to improve the accuracy of genetic evaluations. Potential future predictor traits for health traits could include on-farm or laboratory measures. Energy balance is associated with many metabolic diseases as well as fat-to-protein ratio (Jamrozik et al., 2016), and it has been examined in regards to change in BCS (Roche et al., 2009). Energy balance can be predicted directly using MIR spectroscopy (McParland et al., 2014) or indirectly from milk fatty acid content (Berry et al., 2013). Other potential areas for exploration could involve information from various on-farm sensor systems, including cow activity and milk traits (Rutten et al., 2013), milk component analysis (Hamann, and Krömker, 1997), and genomic markers, among many others.

WORKABILITY

Traits that facilitate working with cows on the farm are categorized as workability traits; the most important ones are temperament (or, more generally, behavior) and milking speed because they have economic effects on the production system (Schutz and Pajor, 2001). As discussed by Schutz and Pajor (2001), various researchers have contributed to the estimation of genetic parameters for dairy cattle behavior and other workability traits, indicating the potential for genetic selection. Heritability estimates presented for temperament ranged from 0.08 to 0.25. Various decades ago, O'Bleness et al. (1960), Markos and Touchberry (1970), and Touchberry and Markos (1970) indicated a potential to genetically select for the improvement of milking speed scores, milk flow, and time required to milk cows. In addition, differences among breeds were noted. For instance, Burnside et al. (1971) reported genetic variation among breeds for the percentage of cows culled for bad temperament.

Tomaszewski et al. (1975) assessed several measures of milking rate to determine which variable would be most practical as a field measure. Milking rates were estimated by peak flow; average flow; percentage of milk produced in the first 2 min of machine milking; and the amount of milk produced in the first 1 min, first 1.5 min, and first 2 min of milking. They concluded that the percentage of the total milk produced at 2 min of milking was an adequate field measure of milking rate. After that, Miller et al. (1976) reported genetic parameters for various measures of milk flow rate and milking time. They estimated heritabilities for peak rate, average rate, total time, duration of peak rate, and yield during peak rate of 0.47, 0.37, 0.17, 0.10, and 0.07, respectively. Direct selection for peak rate would provide an opportunity to reduce total milking time.

Agyemang et al. (1982) estimated variance components for various workability traits. They also defined some novel workability traits such as overall satisfaction, which was based on the opinion of producers on whether they would like another cow like the one in question. Erf et al. (1992) also defined additional workability traits, including trouble-free workability and overall satisfaction, which had heritabilities of 0.11 and 0.08, respectively. The majority of early studies investigating workability were based on subjective scores. To limit the subjectivity of workability evaluations, Moore et al. (1983) proposed the use of additional objective measures to increase the accuracy of genetic evaluation for milking speed. These were based on the trait "2-min milk" as well as the total milk duration for each animal. Williams et al. (1984) also presented genetic parameters for these traits (e.g., h^2 for 2-min milk = 0.25) in a different population. Meyer and Burnside (1987) suggested the measurement of repeated records for milking speed because genetic and environmental factors that affect milking speed of individual cows may vary during lactations or between subsequent lactations.

Visscher and Goddard (1995) reported heritability estimates for milking speed, temperament, and likeability to be between 0.18 and 0.29. Rupp and Boichard (1999) reported moderate heritabilities for milking ease recorded by producers. Wiggans et al. (2007) and Sewalem et al. (2011) also reported heritabilities for milking temperament and milking speed ranging from 0.13 to 0.22. Analysis of bull proof correlations of temperament and milking speed with other traits gave low correlations with traits such as production,

reproduction, conformation, and auxiliary traits. Various countries have already included milking speed and temperament in their breeding objectives for more than 20 yr (Miglior et al., 2005).

Lin et al. (2013) investigated various indicator traits for feed behavior and its relationship with feed efficiency. The traits studied were number of meals, feeding duration, DMI, eating rate, and average meal size. Kramer et al. (2013) reported updated genetic parameters for general temperament, milking temperament, aggressiveness, rank order in herd, and milking speed, which were low to moderate, indicating the possibility for selection.

Workability traits are becoming more relevant due to robotic milking systems, which are becoming more common in the dairy industry (Chesnais et al., 2016). Traits aimed toward a cow's suitability to automatic milking systems are now under consideration for selection, including milk yield per minute of box time, milking interval (the time between 2 consecutive successful milkings), and habituation of heifers (the time for a primiparous cow to get familiar with automated milking systems; Vosman et al., 2014). Despite the progress made, a better understanding is needed of the genetic mechanisms of various behavior traits that allow dairy cattle to be more adapted to modern production systems.

NOVEL TRAITS

For a long time, selection in dairy cattle focused on the improvement of highly heritable production and conformation traits. As shown in Figure 1, heritabilities for both production and conformation traits are markedly higher than those for traits included later on. Figure 2A presents a schematic representation of how an average selection index has evolved over time in terms of relative emphasis for traits under selection. Selection goals have since broadened to include economically important traits with low heritability, partly due to the realization that such traits can indeed be genetically improved and partly due to the fact that modern and automated technologies provide more data. Selection for production, and partly for conformation, has resulted in indirect negative response in health and fertility, as shown in Figure 2B. To counteract those negative trends, health and fertility were included in selection indices, and their relative emphases have gradually increased at the expense of production and conformation. Since the inclusion of functional traits in selection indices, the detrimental effects of narrow selection goals have been counteracted, and we are currently making genetic progress in all traits of economic interest.

The power of genetic improvement is noticeable, as a desirable genetic progress has been increasingly observed since the inclusion of those traits in selection indices worldwide, especially with the introduction of genomic selection. To ensure continued progress and to develop breeding goals more in line with producer and consumer expectations, the inclusion of novel traits can be considered. Rapid advancements in technology and methodology related to the dairy industry have fueled new opportunities to include new traits in the breeding goal. In particular, the implementation of genomic selection accelerated progress in novel trait selection. In addition, the use of MIR spectroscopy in routine milk testing has expanded in many countries to predict new traits and generate large numbers of phenotypes in an inexpensive manner. The traits considered for selection continue to evolve as the industry changes; many novel traits are currently being considered around the world and are at various stages of development.

Feed Efficiency

Feed represents a large proportion of dairy cattle production expenses. Generally, feed efficiency describes units of product output per unit of feed input, with the units generally being mass, energy, protein, or economic value (VandeHaar et al., 2016). Koch et al. (1963) described a measure of feed efficiency, residual feed intake (**RFI**), that is independent of an animal's body size and production level and is considered to represent the inherent variation in basic metabolic processes that determine efficiency. As reviewed by Connor (2015), heritability estimates for RFI in lactating cows range from 0.01 to 0.40. For some time, feed intake was estimated based on BW and production. However, Gibson (1986) presented a correlation between true feed efficiency and predicted feed efficiency (derived from BW and production) of 0.84, indicating that there could be value to actually measuring feed intake. In the 1990s, there was great interest from the industry in including feed efficiency in dairy breeding objectives, which motivated various organizations to collect individual feed intake records for research and genetic evaluations, as described in various studies (Van Arendonk et al., 1991; Veerkamp, 1998).

Williams et al. (2011) reported that significant variation in RFI exists in growing heifers and that this could be an alternative to indirectly select dairy cows for improved feed efficiency because it is easier to record feed intake in growing heifers. Spurlock et al. (2012) estimated genetic parameters of various traits associated with energy balance and related traits, including DMI, BW, BCS, ECM production, and gross feed efficiency,

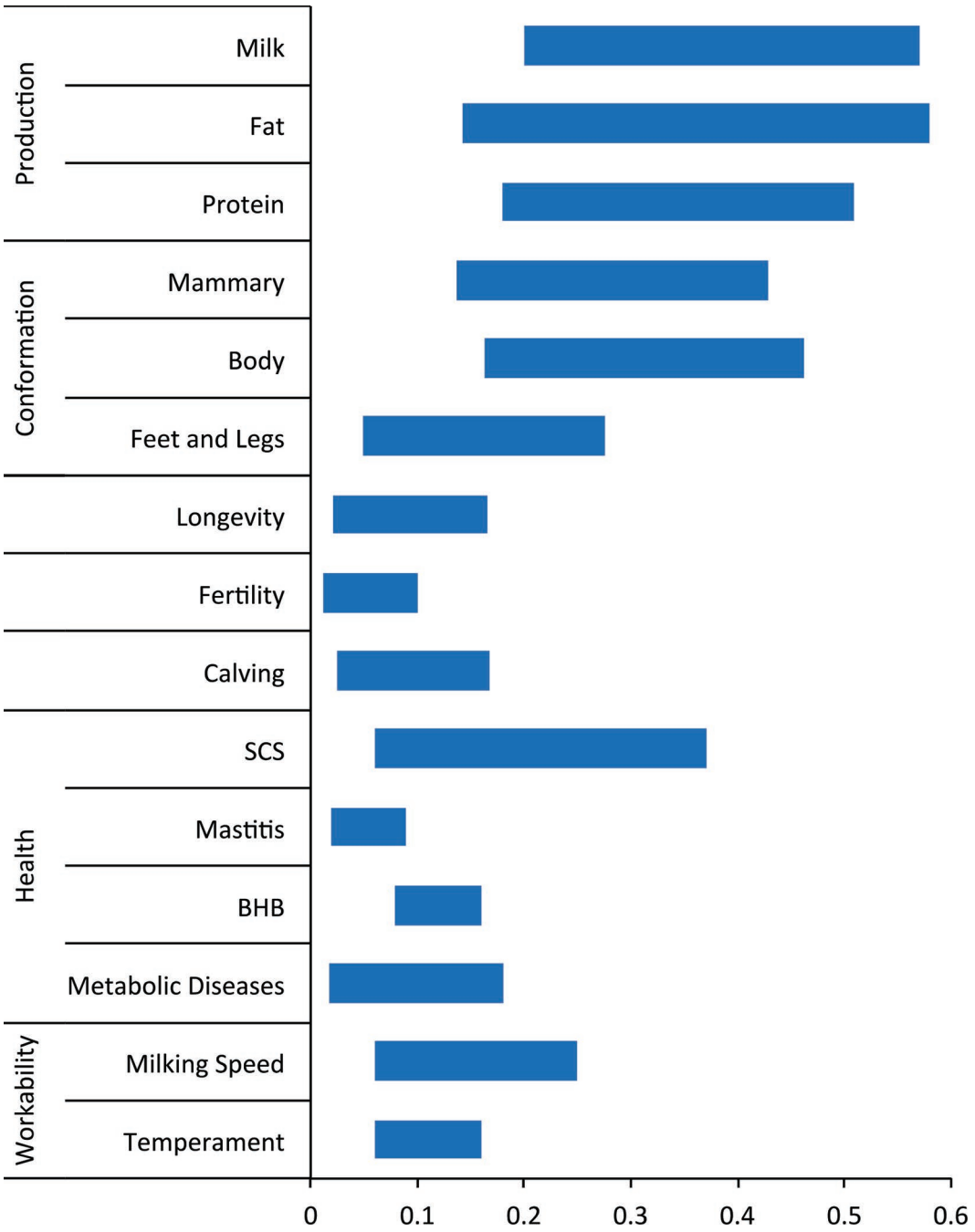

Figure 1. Ranges in heritabilities for various traits used in current Interbull evaluations (April 2017 run, Interbull, Uppsala, Sweden). Heritabilities for metabolic disease are for Nordic countries, the United States, and Canada only; heritabilities for BHB are for Canada and the Netherlands. Color version available online.

and suggested that these traits will likely respond to genetic selection in Holstein cows. A negative genetic correlation was found between gross feed efficiency and energy balance (from −0.73 to −0.99), indicating that selection for more efficient cows would favor a lower energy status.

Genomic selection has been a very important tool in the selection for increased feed efficiency in dairy cows. For instance, Gonzalez-Recio et al. (2014) described the implementation of heifer feed efficiency in the Australian selection index using genomic selection and its effect in the industry. Pryce et al. (2015) described the

implementation of genetic evaluation for Feed Saved, which combines RFI in growing calves and lactating cows with feed required for maintenance predicted from BW as a new indicator of feed efficiency in dairy cows. Since April 2015, Feed Saved has been included as part of the Australian national selection index.

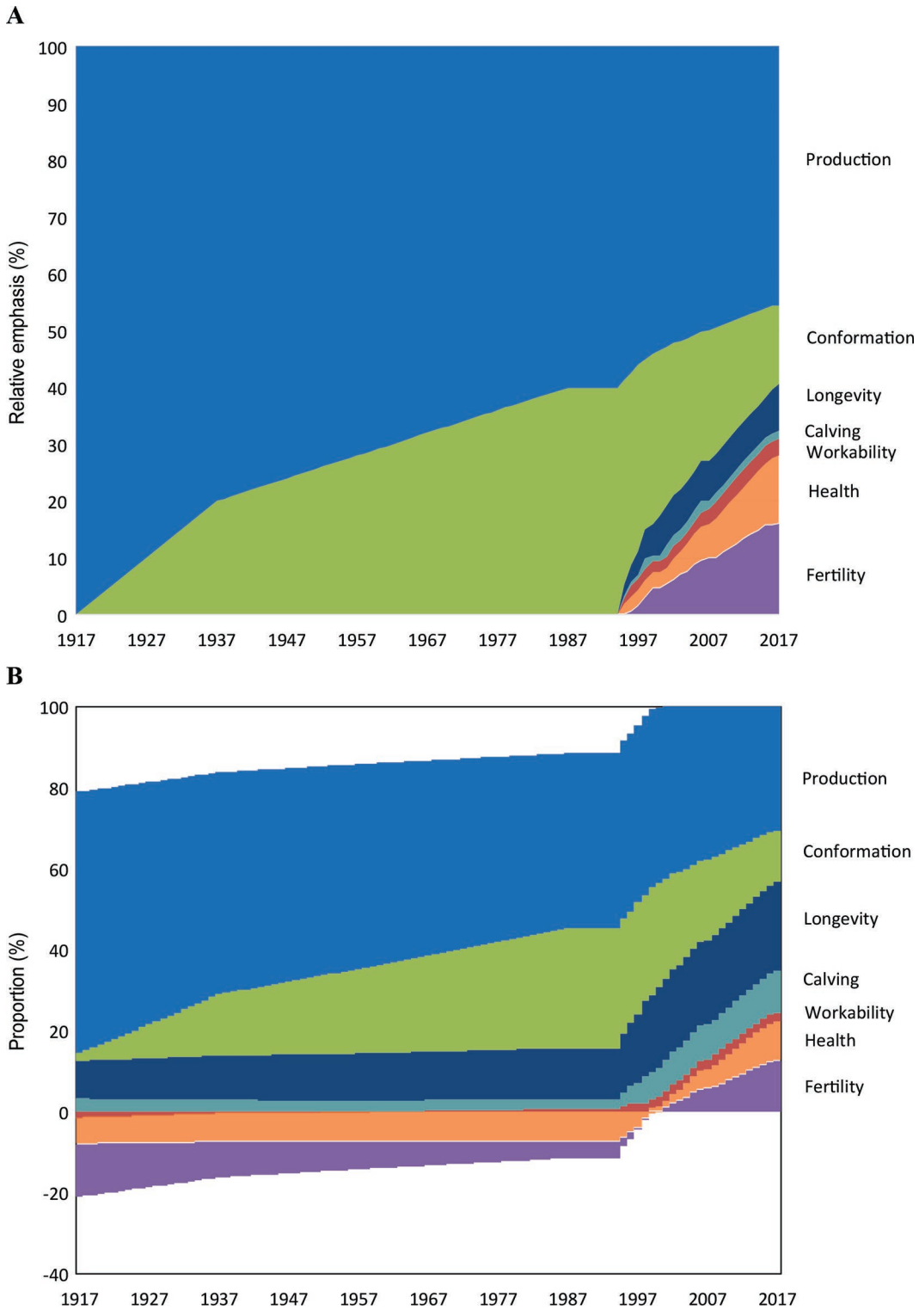

Figure 2. Schematic representation of (A) relative emphasis of traits included in an average selection index over time and (B) proportion of estimated selection response for various trait categories over time (summing to 100%). Color version available online.

Methane Emissions

Mitigation of enteric methane emissions in ruminants has become an important area of research because methane is strongly linked to global warming. Herd et al. (2002) reported variations in enteric methane emissions between animals and breeds and across time, indicating potential for improvement through genetic selection. In a study to predict methane production from dairy and beef cattle, Ellis et al. (2007) presented statistical models of methane production. Over time, various methodologies for measuring and estimating methane production in cattle were developed (Wall et al., 2010; Negussie et al., 2017). Several methane emission proxies and related variables include feed intake and feeding behavior, rumen function, metabolites and microbiome (rumen microbiota and host–microbiome interactions), milk production and composition, hindgut and feces, and measurements at the level of the whole animal, as reviewed by de Haas et al. (2017). de Haas et al. (2011) presented genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. Several studies have confirmed that methane emission is a moderately heritable trait ranging from 0.21 to 0.35 (de Haas et al., 2011; Lassen and Løvendahl, 2016).

Heat Stress

In a world with a changing environment, breeding robust dairy cows will likely become an important activity in the near future. Over time, studies have been performed to better understand dairy cattle adaptation to heat stress. Ravagnolo and Misztal (2000) reported that selection for heat stress is possible and could be particularly effective for environments with a high average temperature–humidity index. Ravagnolo et al. (2000) reported the usefulness of weather data in developing a heat-stress function suitable for studies on genetics of heat stress. More recently, Nguyen et al. (2016) demonstrated that heat tolerance in dairy cattle could be improved using genomic selection, and implementation of genetic selection for heat tolerance in the Australian breeding programs is under discussion. Tolerance to heat stress has been shown to be heritable, ranging from 0.17 to 0.33 (Nguyen et al., 2016).

Hoof Health

In the early 2000s, Warnick et al. (2001) and Booth et al. (2004), respectively, assessed the effect of lameness on milk production and dairy cow survival, indicating the importance of this trait for the dairy industry. Genetic evaluations for feet and leg type traits have been available for several years in many countries. However, van der Waaij et al. (2005) and Chapinal et al. (2013) reported low genetic correlations between infectious claw lesions and feet and leg traits. These results partially explain why selection for improving hoof health by using conformation traits has not been effective so far and indicate the need to include direct indicators of hoof health in selection indices. Koenig et al. (2005) presented genetic parameters for different types of claw and foot disorders and the genetic relationship of disorders with milk yield and selected conformation traits. In the last 2 decades, other major contributions to the understanding of the genetic architecture of various indicators of hoof health and recommendations for genetic selection have been made, such as Boettcher et al. (1998), van der Waaij et al. (2005), Laursen et al. (2009), van der Linde et al. (2010), Oberbauer et al. (2013), and Malchiodi et al. (2017). Heritabilities of individual hoof lesions have ranged from 0.01 to 0.13 (Koenig et al., 2005; van der Spek et al., 2013; Malchiodi et al., 2017). A variety of approaches and phenotypes may be required for the improvement of hoof health. de Mol et al. (2013) investigated the feasibility of implementing a lameness detection model based on daily activity data and concluded that automated lameness detection based on day-to-day variation in behavior is a useful tool for dairy management.

Immune Response

The immune response or ability to resist infections and diseases is a complex trait of great economic importance for the dairy industry. Mallard et al. (1983) studied variation in serum immunoglobulins in Canadian Holsteins and concluded that selection to alter immunoglobulin content and reduce disease may be feasible and should be examined further. Mazengera et al. (1985) reported genetic parameters for bovine serum immunoglobulins, indicating that some variation in the parameters was under genetic control. Thompson-Crispi et al. (2012) estimated genetic parameters for cell-mediated and antibody-mediated immune response traits of Holstein cattle and explored the association of these traits with other economically important traits, including disease resistance. More recently, Denholm et al. (2017) presented genetic and phenotypic parameters for other indicators of immune response. Cellular immune-associated traits are heritable and repeatable. Genetic selection for cellular immune-associated traits could provide a useful tool for improving animal health, fitness, and fertility.

Milk Composition

The genetic variation in the composition of milk is of interest because of its effect on the nutritional value and technological properties of milk. Soyeurt et al. (2011) reported that the prediction of most major fatty acids has become feasible on a large scale via MIR spectroscopy, which can be used in routine genetic evaluation. The fatty acid profile of milk has been shown to be under genetic control. Heritability of milk fatty acid contents tends to decrease with an increase in carbon chain length (Bastin et al., 2011). In addition, SFA are more heritable than UFA (Soyeurt et al., 2008). Therefore, there is potential to alter the fatty acid profile of milk through selection. Some countries, such as Belgium, have started to consider these traits.

Minerals found in milk, such as Se, Ca, K, Zn, Mg, and P, contribute to several vital physiological processes. van Hulzen et al. (2009) observed differences between cows and herds in concentrations of minerals in milk and determined that concentrations of many minerals in milk could be changed by way of nutrition or through genetic selection.

Milk Coagulation Properties

Milk coagulation property traits have become of interest to the dairy industry because they influence the profitability of the cheese sector. Lindström et al. (1984) presented genetic parameters for milk coagulation properties. Subsequently, more than 20 studies have been published on variables influencing milk coagulation and how to genetically select for them, as presented in Bittante et al. (2012). The physical characteristics of milk that are important in cheese manufacturing, such as rennet coagulation time, curd firmness 30 min after rennet addition, and curd firming time, have been studied. A limiting factor in the implementation of routine genetic evaluation for these traits is the time and expense involved in collecting the phenotypes. However, Cecchinato et al. (2009) proposed the use of MIR spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk, which can be more affordably measured.

Reproductive Technology Traits

In the last decades, superovulation and embryo transfer have been widely used around the world to increase the genetic contribution of elite females. König et al. (2007) reported low to moderate maternal heritabilities for number of flushed ova, transferable embryos, degenerated embryos, unfertilized oocytes, and percentage of transferrable embryos. Recently, Jaton et al. (2016) investigated genetic parameters for the number of total and viable embryos produced per flush and concluded that there is genetic variability for these traits and potential for selection. Parker Gaddis et al. (2017) also investigated the genetic components of various traits related to reproductive technologies and found many regions of the genome associated with these traits, some of which correspond with those previously identified for fertility traits already evaluated in dairy cows.

NEXT DECADE

In the 1920s, the 305-d milk yield of an average North American Holstein cow was around 2,000 kg. One century later, the average Holstein cow produces more than 10,000 kg of milk, with fat and protein percentages similar to those of 100 yr ago. This 5-fold increase, attributable to enhanced management, feeding, and genetics, is one of the most successful stories of improvement in livestock production. The dramatic increase, however, has come at a cost in terms of fertility and health. To counteract this decline, selection goals are becoming more comprehensive as new phenotypes become available and cost effective to measure.

A pivotal development in regard to trait selection is the advent of genomics, which has revolutionized the dairy cattle industry and has provided a new opportunity to select for traits that were prohibitively expensive to measure in the past. Selected commercial herds may provide a new source of high-quality phenotypic information, which, in conjunction with genomics, can be used within a breeding program to genetically improve the national herd. The collection of detailed (possibly expensive) phenotypes for a sufficiently large reference population, paired with the corresponding genotypic information of those reference animals, allows accurate estimation of marker effects for a specific trait. Subsequent calculation of genomic breeding values in a group of selection candidates without phenotypes has thus become possible. The advantage is that those detailed phenotypes could have markedly higher heritability than previous related traits. Immune response is an example: estimates of heritability for producer-recorded clinical diseases are very low (mostly <0.05), whereas estimates of heritability for immune response traits are much higher (0.25–0.35). Measuring immune response and genotyping animals in a sufficiently large reference population allow estimation of marker effects, which can then be used to estimate direct genomic breeding values in a group of selection candidates. A further example could be the identification of new fertility phenotypes that are more tightly linked to the female reproduction cycle and embryo survival; the use of current fertility phenotypes (interval traits such as

days open, or conception rate estimates such as 56-d nonreturn rate) has failed to significantly enhance the fertility of our modern lactating cows. If we are able to improve overall cow health, however, fertility may consequently also improve—that is, the healthier the cow, the more fertile it is. Finally, the advent of genomics has also allowed the inclusion of feed efficiency in breeding goals. The ability to decrease feed waste and convert feed into milk more efficiently will have significant advantages for the whole dairy sector while decreasing the environmental impact of dairying.

One century of selection has mostly focused on increasing milk production of the dairy animal. With the exception of fat and protein percentages, milk properties and quality have not been considered in detail thus far. During the last decade, the use of milk MIR spectral data for prediction of fine milk components and milk coagulation properties has added a potential new selection opportunity to enhance the quality of milk for human consumption and cheese-making. This complex objective will have consequences for dairy processors farther down the milk value chain, who will need to communicate with dairy cattle breeders.

CONCLUSIONS

Over the past 100 yr, traits considered for genetic selection in dairy cattle populations have progressed to meet the demands of various stakeholders. At the turn of the 20th century, the focus was fixed on increasing milk production. By the end of the century, the emphasis had shifted toward a more balanced breeding goal and included longevity, fertility, calving, health, and workability traits. This shift represents an increased awareness and recognition of societal influence but also a better understanding of animal physiology. In the near future, fitness, animal health and welfare, milk quality, and environmental sustainability will be included in even more comprehensive breeding goals. In the longer term, on-farm sensors, data loggers, precision measurement techniques, and other technological aids will provide even more data for use in selection, and the difficulty will lie not in measuring phenotypes or gathering data but rather in selecting and weighting traits with producer-minded economic advantages.

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APPENDIX

Table A1. Major milestones in the study of genetic selection of dairy cattle

Date	Milestone	Reference
1920	Breeding decisions are mainly based on milk and fat yields from milk testing programs along with consideration of conformation.	Gowen, 1920; Meade, 1921; Burrington and White, 1925; Graves, 1925; Fohrman, 1926; Copeland, 1927
1930	Classification programs are instituted by breed associations.	Copeland, 1938
1943	Selection index approach is used for simultaneous selection of multiple traits.	Hazel, 1943
1950	Interest increases in the SNF portion of milk due to proposed changes to milk pricing strategies.	Richardson and Folger, 1950; Johnson, 1957
1970	Fertility and health traits are evaluated in Nordic countries.	Heringstad et al., 2000; Philipsson and Lindhé, 2003
1970	Calving traits are introduced.	Philipsson, 1976; Pollak and Freeman, 1976
1970	Milk protein contents measured in milk recording programs and genetic evaluations are available.	Biggs, 1967; Shook, 2006
1980	Somatic cell counts are used in selection for udder health indices.	Ali and Shook, 1980; Kennedy et al., 1982; Coffey et al., 1985, 1986
1980	Linear type appraisals for cattle are used.	Thompson et al., 1983
1990	Workability and longevity traits are added to national genetic evaluations.	Burnside et al., 1971; Tomaszewski et al., 1975; Moore et al., 1983; Meyer and Burnside, 1987
2000	Fertility traits are considered by many non-Nordic countries.	Miglior et al., 2005
2010s	Health trait evaluations using producer-recorded health data and their indicators are initiated in several non-Nordic countries.	Zwald et al., 2004; Koeck et al., 2012; van der Drift et al., 2012; Koeck et al., 2014; Jamrozik et al., 2016; Pryce et al., 2016
2010s	Novel traits (including feed efficiency, milk coagulation properties, milk fatty acid contents, hoof health, and automatic milking systems) are implemented by different countries.	Ravagnolo and Misztal, 2000; Thompson-Crispi et al., 2012; Pryce et al., 2015; Chesnais et al., 2016; Lassen and Løvendahl, 2016; de Haas et al., 2017; Malchiodi et al., 2017

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A 100-Year Review: Methods and impact of genetic selection in dairy cattle—From daughter–dam comparisons to deep learning algorithms[1]

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ABSTRACT

In the early 1900s, breed society herdbooks had been established and milk-recording programs were in their infancy. Farmers wanted to improve the productivity of their cattle, but the foundations of population genetics, quantitative genetics, and animal breeding had not been laid. Early animal breeders struggled to identify genetically superior families using performance records that were influenced by local environmental conditions and herd-specific management practices. Daughter–dam comparisons were used for more than 30 yr and, although genetic progress was minimal, the attention given to performance recording, genetic theory, and statistical methods paid off in future years. Contemporary (herdmate) comparison methods allowed more accurate accounting for environmental factors and genetic progress began to accelerate when these methods were coupled with artificial insemination and progeny testing. Advances in computing facilitated the implementation of mixed linear models that used pedigree and performance data optimally and enabled accurate selection decisions. Sequencing of the bovine genome led to a revolution in dairy cattle breeding, and the pace of scientific discovery and genetic progress accelerated rapidly. Pedigree-based models have given way to whole-genome prediction, and Bayesian regression models and machine learning algorithms have joined mixed linear models in the toolbox of modern animal breeders. Future developments will likely include elucidation of the mechanisms of genetic inheritance and epigenetic modification in key biological pathways, and genomic data will be used with data from on-farm sensors to facilitate precision management on modern dairy farms.

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Key words: genetic selection, dairy cattle, genomic selection, statistical models

THE BUILDING BLOCKS

Performance Recording

Pedigree records and performance data were the key building blocks in developing effective genetic selection programs in the pre-genomic era, as noted in Appendix Table A1. Pedigree records traced back to the origin of breed societies in the late 1800s, and widespread collection of performance data began shortly thereafter, with the encouragement of early dairy industry pioneers such as W. D. Hoard. The first statewide association for recording milk weights and analyzing butterfat samples was formed in Michigan in 1905, and by 1908, the United States Department of Agriculture (USDA) Bureau of Animal Industry began organizing local and state cow testing associations into the national Dairy Herd Improvement Association (DHIA). Responsibility for this effort was transferred to federal extension workers in 1914, and participation in milk testing grew rapidly (VanRaden and Miller, 2008), as shown in Figure 1.

Monthly DHIA testing was the norm for many decades, but now about two-thirds of dairy farms use labor-efficient a.m./p.m. testing plans, in which milk samples are taken at alternate times each month. Future strategies that focus on more frequent DHIA sampling of recently fresh cows or cows in the highest-producing pens may provide more useful data for cows that are at peak efficiency and at the greatest risk for common health disorders. Electronic measurement of data, via radiofrequency identification (RFID) sensors and inline sampling systems, has replaced manual entry of pedigree and performance data, as shown in Figure 2.

Local bull associations were common during the 1920s and 1930s, until the widespread adoption of AI in the 1940s, when dozens of regional AI cooperatives were formed. Because virtually all traits of interest in dairy cattle are sex-limited, genetic evaluation of a bull's own

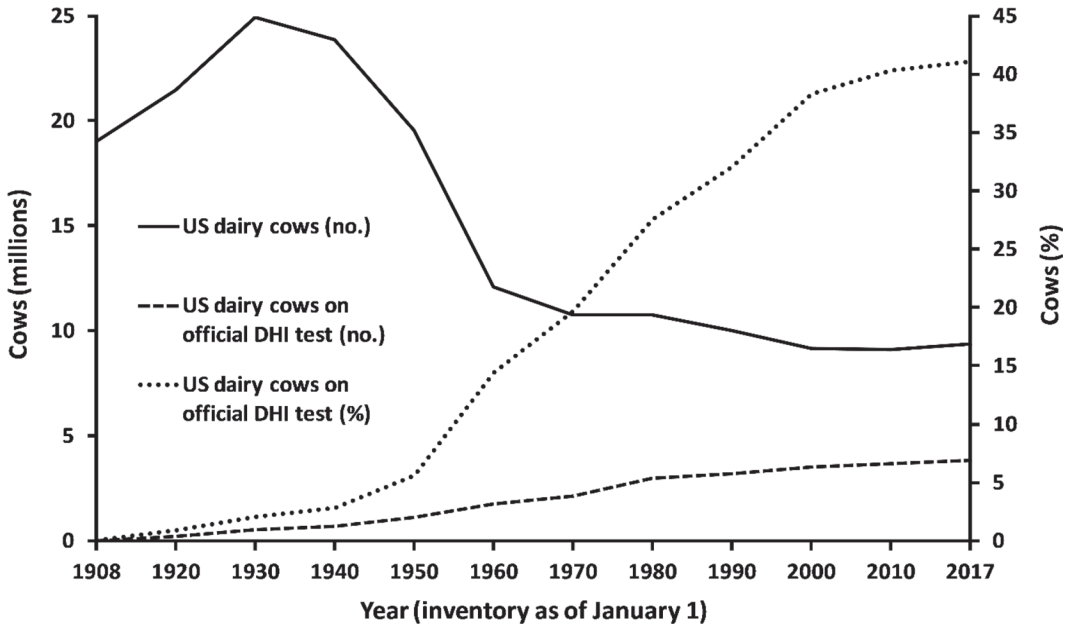
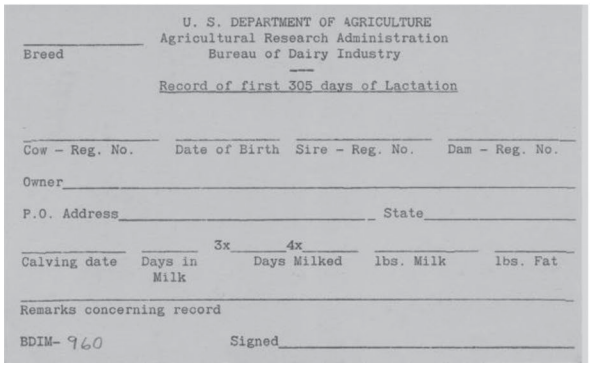


Figure 1. Participation in milk recording programs in the United States, from 1908 to 2017.



U. S. DEPARTMENT OF AGRICULTURE
Agricultural Research Administration
Breed Bureau of Dairy Industry

Record of first 305 days of Lactation

Cow – Reg. No. | Date of Birth | Sire – Reg. No. | Dam – Reg. No.

Owner

P.O. Address State

Calving date | Days in Milk | 3x Days Milked 4x | lbs. Milk | lbs. Fat

Remarks concerning record

BDIM- 960 Signed

Figure 2. Recording of performance data for dairy cows then (1936, left panels) and now (2017, right panel).

phenotypes is not useful, and strategies for estimating a bull's genetic superiority or inferiority based on the performance of his offspring were needed.

Pedigree Data

Despite the fact that dairy cattle breed societies assigned unique identification numbers to individual cows and bulls as early as the late 1800s, a large proportion of nonregistered animals ("grades") were not included in breed society herdbooks. An alternative identification method was needed, and USDA introduced metal ear tags with unique numbers in 1936. These evolved into the 9-digit tag series (e.g., 35ABC1234) introduced by the Animal and Plant Health Inspection Service (APHIS) and National Association of Animal Breeders (NAAB) in 1955, which are still used for many cows today. The American ID series, introduced in 1998, features a 2-character breed code, 3-character country code, and 12-digit identification number (e.g., HOUSA00035ABC1234 or HO840012345678910). This system was designed to be unique worldwide and to include both registered and grade animals, and it allows multiple identification codes for individual animals to be cross-referenced to a single unique number.

EARLY METHODS TO PREDICT BREEDING VALUES

Daughter–Dam Comparison

The lactation performance of a cow was long thought to be influenced by heredity, and early selection decisions were based simply on an individual cow's phenotype for milk or butter yield. The idea of comparing a cow's milk production with that of her dam emerged near the turn of the 20th century. Several indices were proposed for this purpose (Davidson, 1925; Graves, 1925; Yapp, 1925; Goodale, 1927; Gowen, 1930; Bonnier, 1936; Allen, 1944) and their relative accuracy was compared by Edwards (1932). In practice, the earliest known daughter–dam differences in the United States were computed by individual bull associations around 1915, based on a handful of sires with a few offspring apiece—this was the first serious attempt to improve dairy cattle by selection. By 1927, approximately 250 cooperative dairy bull associations, representing more than 6,000 farmers, provided data to the USDA and, for the next 4 decades, the USDA computed daughter–dam comparisons for dairy bulls and mailed the results to their owners. Artificial insemination became available in the late 1930s, and with it, the opportunity for superior bulls to produce hundreds or thousands of offspring in many herds. Large groups of daughters performing under a variety of management and environmental conditions greatly enhanced the accuracy of genetic predictions. During this period, the work of giants such as R. A. Fisher (1918, 1930) and J. B. S. Haldane (1932) laid the foundations of population and quantitative genetics, which allowed pioneers such as Sewall Wright (1932) and Jay Lush (1931, 1933) to develop the science of animal breeding and the statistical methodologies needed for accurate evaluation of dairy sires. Various indices based on daughter–dam comparisons were developed, including those of Wright (1932) and Lush et al. (1941).

Daughter–dam comparisons facilitated genetic evaluation of bulls that were used in multiple herds, as long as performance data were available for the dam and her daughters. This method accounted for herd-specific management practices and local environmental conditions if the dam and daughter were housed in the same herd. Changes in management or environmental conditions that occurred in the time between dam's and daughter's performance were ignored. Relationships between sires and their mates were not considered, and this assumption was sometimes violated if the bull was used in his herd of origin. Variation in the phenotypic performance of the dam, relative to her actual genetic merit, was a huge source of error in the resulting predictions. Genetic trends over time were ignored, but genetic progress was negligible in most herds at the time. An important limitation was that sire evaluations were not regressed to the mean, so bulls evaluated based on only a few daughter–dam pairs were more likely to have extremely high or low genetic predictions. During this period, methods were developed to standardize records for lactation length (305 d), milking frequency (2×), and age at calving (mature equivalent). Adjustments for season of calving were also developed, but differences in environmental conditions between years were generally ignored.

Selection Index

Hazel and Lush (1942) introduced the selection index for EBV for individual traits, and this method was used by Lush (1944) to derive weights for various sources of information in daughter–dam comparisons. The EBV of a selection candidate was predicted using multiple linear regression, where each independent variable represented individual or mean performance for a specific type of relative, such as dam, sire, maternal half-siblings, paternal half-siblings, or progeny. The regression coefficients represented index weights, which were a function of genetic relationships and the amount of information contributed by the phenotypic record or average (e.g., number of lactations or number of offspring). The amount of information from various types

of relatives often differed between selection candidates, so index weights were adjusted for the number of relatives or lactations contributing to mean performance, based on heritability and repeatability parameters.

Contemporary (Herdmate) Comparisons

Contemporary comparisons represented a huge leap in the accuracy of genetic evaluations because of their ability to account for the specific management and environmental conditions under which phenotypes were expressed (Robertson et al., 1956). Robertson and Rendel (1954) are credited with introducing contemporary comparisons, and Henderson et al. (1954) formally published the herdmate comparison model in the same year. However, Searle (1964) noted that this method had been used in New Zealand before either publication. The concept of contemporaries or herdmates exposed to similar management and environmental conditions is much like that of an epidemiological "cohort," in which patients are grouped based on commonalities in demographic features (e.g., age, sex, or geographical region) and lifestyle characteristics (e.g., exercise regimen or tobacco usage). A critical consideration in designing contemporary groups is the balance between a precise definition of the cow's environmental conditions and the need for enough herdmates to provide an accurate estimate of the contemporary group effect.

Progeny testing became widespread during the era of daughter–dam comparisons. However, the introduction of contemporary comparisons allowed AI centers to fully capture the benefits of distributing the semen of young bulls to dozens or hundreds of herds with different geographical locations, environmental conditions, and management practices. Contemporary comparisons were enhanced by regressing average daughter contemporary deviations (now known as daughter yield deviations) toward zero, based on heritability and number of progeny, because mean deviations for bulls with few offspring have larger variance than mean deviations for bulls with many offspring. Some contemporary comparison models also included a herd by sire interaction adjustment to limit the effect of a single herd on a sire's EBV.

Cornell University implemented a regional sire evaluation system based on contemporary comparisons in the mid-1950s (Henderson, 1956), in which records were weighted based on the number of lactations per cow and a repeatability parameter. However, information about the number of daughters or contemporaries was not used when combining daughter contemporary deviations to compute the sire's EBV. The contemporary comparison method was applied by the USDA in 1961, replacing the daughter–dam comparison system. This model allowed the inclusion of cows for which performance records of the dam were unknown. Herd-year-season contemporary groups were based on a 5-mo moving average, and herdmate averages were adjusted for seasonal effects. As in the Cornell model, sire effects were regressed to the mean, so a bull could not rank highly unless he had a significant number of daughters. Records of cows that were culled or sold for dairy purposes were extended to 305 d, whereas longer records were truncated at 305 d.

Other adjustments were implemented at this time, including factors for extending short lactations to 305 d that were specific to breed, region, season, and parity, and records were weighted by length of lactation. A time lag between the cow's calving date and initiation of the sire summary ensured that records from culled cows with short lactations did not bias the genetic evaluations of their sires. This was an obvious limitation as regards timeliness of data entering the genetic evaluation system, at least until 1975, when records in progress became available for all cows in the herd. Estimates of sires' genetic merit were published as the predicted difference (**PD**) in performance of their daughters relative to contemporaries in a typical herd. The term "repeatability" (later "reliability") was used to denote the accuracy of a bull's PD, and it indicated the level of confidence a farmer should have when purchasing the bull's semen. This method, which was used until 1973, allowed the inclusion of more data, tended to be less biased, and provided a cow index for ranking elite females.

Several competing methods for sire evaluation were introduced during this period. Most were closely related to each other and to the weighted least-squares approaches of C. R. Henderson (1952, 1963) and Cunningham (1965), as well as simplified versions of the best linear unbiased prediction (BLUP) models described in subsequent sections (Thompson, 1976). The cumulative difference method of Bar-Anan and Sacks (1974) is essentially equivalent to the contemporary comparison method but with an adjustment for the genetic level of sires of the cow's contemporaries. The term "cumulative" recognized that performance data of a bull's daughters accumulate over time, resulting in increased accuracy of predictions, and this method was the basis of the modified cumulative difference method proposed by Dempfle (1976).

Genetic evaluations of dairy sires were unified at USDA in 1968 (Plowman and McDaniel, 1968), when dairy cattle breed associations discontinued their own sire rankings for production traits. In 1972, the USDA Division of Dairy Herd Improvement Investigations was renamed as the USDA-ARS Animal Improvement Programs Laboratory (**AIPL**)—this laboratory set the

global standard for translational research on genetic evaluation of dairy cattle for the next 45 years.

Modified Contemporary Comparison

In 1974, the modified contemporary comparison (**MCC**) method was introduced (Dickinson et al., 1976; Norman et al., 1976). In this model, a bull's PD represented a weighted average of his pedigree value and the deviation in performance of his daughters from their contemporaries. In previous methods, a bull's pedigree information was generally discarded when data from milking daughters became available. The MCC method also allowed the inclusion of sire and maternal grandsire pedigrees. The genetic merit of competing sires within a given herd (i.e., sires of contemporaries) was taken into account, and this approach could better accommodate genetic trends over time (Norman et al., 1972). These features of the MCC method were increasingly important, because modern selection tools and advanced reproductive technologies now allowed some farmers to make more rapid genetic progress than their peers (McDaniel et al., 1974). In addition, positive assortative mating had become popular, as farmers "mated the best to the best" to improve their herds (Norman et al., 1987). The first 5 lactation records from a given cow were included in the MCC model, which provided a more accurate picture of an animal's genetic superiority or inferiority in lifetime productivity. Contemporary groups differed for primiparous and multiparous cows within a herd. As previously, a bull's evaluation was regressed based on heritability, number of daughters, and lactations per daughter, but regression was toward his pedigree value, rather than the population mean.

The MCC method produced results that were nearly identical to those of BLUP in a sire model, but with substantially lower computing requirements. The practice of resetting the genetic base was initiated during this time, so farmers would be reminded to raise their sire selection standards as the breed made genetic progress. However, periodic resetting of the genetic base "forgives" undesirable genetic trends that may occur as correlated responses to selection (e.g., female fertility) or biases in the perceived value of certain traits (e.g., stature). The MCC method was widely accepted by pedigree breeders and AI studs, and it led to impressive annual genetic gains of about 45 kg of milk per cow per lactation. Another innovation during this period was the incorporation of pricing data for milk, fat, and protein, so that estimates of genetic merit could be expressed as the financial gain or loss relative to an average sire of the same breed (**PD$**). Cow indices became widely used during the MCC era; these represented a weighted average of the cow's modified contemporary deviation and her sire's PD (and later her dam's cow index), with weights depending on the amount of information contributing to each component.

LINEAR MODELS

Mixed Linear Models

Henderson (1953) advocated the use of statistical models to partition genetic and environmental variance components and predict the genetic merit of dairy sires, and this led to the development of BLUP methodology. Despite its theoretical appeal, computing limitations prevented implementation of BLUP until 1972, when Cornell University implemented BLUP in a sire model; this model was later modified to include genetic relationships among sires.

A mixed linear model is expressed most succinctly in matrix notation as

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e},$$

where $\mathbf{y}$ is a vector of phenotypic measurements on a group of animals; $\mathbf{b}$ is a vector of continuous or categorical fixed effects that are known to influence the phenotype, such as age at calving or herd-year-season contemporary group, as one would encounter in a traditional least-squares analysis; $\mathbf{u}$ is a vector of random effects, such as sire breeding values; $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices that map the phenotypic observations in $\mathbf{y}$ to the fixed and random effects in $\mathbf{b}$ and $\mathbf{u}$, respectively, and $\mathbf{e}$ is a vector of random residual effects, such as temporary environmental conditions or measurement error. The variance components σ_u^2 and σ_e^2, corresponding to the random effects $\mathbf{u}$ and $\mathbf{e}$, can be estimated using a variety of methods, such as maximum likelihood (Harville, 1977).

Sire and Maternal Grandsire Models

If the vector $\mathbf{u}$ in the mixed model equations comprises the breeding values of dairy sires and $\mathbf{y}$ contains the lactation records of their daughters, the aforementioned mixed linear model would be considered as a "sire model." If we specify that $\mathbf{G} = N\left(0, \mathbf{I}\sigma_u^2\right)$, this model assumes that sires are unrelated to each other, and the resulting sire EBV are regressed toward the population mean in proportion to the magnitude of σ_u^2 relative to σ_e^2. The assumption that sires are unrelated to each other is highly unrealistic, given the widespread use of AI and embryo transfer, which lead to large families of paternal half-siblings and small families of

full-siblings, respectively. The concept of modeling correlations between the elements of **u** when specifying **G** is straightforward, and in this application pedigree information was used to derive a matrix of expected additive genetic relationships, where $\mathbf{G} = N\left(0, \mathbf{A}\sigma_u^2\right)$. The resulting **A** matrix is very large, of the order of the number of elements of **u**, and it could not be inverted with computational resources available at the time. Henderson (1976) developed a set of rules for constructing $\mathbf{A}^{-1}$ directly, without building **A**. This allowed more precise modeling of relationships among sires than the MCC model, as well as relationships between sires and cows or relationships between sires and maternal grandsires (Henderson, 1975). Later, this method was extended to allow efficient construction of $\mathbf{A}^{-1}$ in the presence of inbreeding (Tier, 1990).

In the sire model implemented for the Northeast AI Sire Comparison at Cornell University in 1972, the vector **b** included the fixed effects of herd-year-season of calving and genetic group of the sire, where the latter was based on birth year of the bull and the AI organization from which he came. The idea was that all young bulls purchased by a given AI center in a given year were of similar genetic merit, which facilitated the assumption that the sires in **u** represented independent (unrelated) samples from the same distribution. Only first-lactation records of AI daughters were used, although this restriction was later relaxed if the additional records were from the same herd (Ufford et al., 1979). Random mating between sires and dams was assumed, and maternal relationships between cows were ignored.

To address the naïve assumption that sires were randomly mated to dams, Quaas et al. (1979) proposed a maternal grandsire model. This model included an additional random effect, which represented the additive genetic merit of the maternal grandsire, as well as an additional fixed effect, which represented the genetic group of the maternal grandsire. Although this was a positive step in addressing assortative mating, it still assumed that each mate of a given bull represented a random sample of all daughters of that maternal grandsire. Maternal relationships between dams were ignored and the model did not add value in cases where the maternal grandsire was unknown. A comprehensive examination of assortative mating for milk yield by Norman et al. (1987) showed that herds with higher average genetic level consistently used genetically superior bulls. However, the primary concern was bias due to within-herd assortative mating, which was not common at the time (Norman et al., 1987), and few AI bulls were affected negatively in the national sire evaluation system.

Animal Model

The inability of sire or maternal grandsire models to fully account for nonrandom mating of sires with expensive semen to cows and heifers with highest perceived value within a given herd was well known. In addition, farmers who wished to market superior breeding stock were no longer content with a genetic evaluation system that focused on sires and treated cows as a by-product. In 1989, AIPL scientists introduced the "animal model" (Wiggans and VanRaden, 1989), which used all known relationships between cows and their maternal and paternal ancestors. In this model, the additive genetic effects of animals represent an infinite number of alleles with very small effects—the so-called infinitesimal model of inheritance.

Once implemented, by using an iteration on data algorithm and the supercomputer at Cornell University, the animal model became the global standard for genetic evaluation of dairy cattle. The statistical methodology, which had been derived almost 3 decades earlier, allowed precise accounting of the genetic merit of mates and provided a consistent framework for simultaneous evaluation of male and females. The breeding value of an individual animal is represented as the sum of one-half of the additive genetic merit of its sire, one-half of the additive genetic merit of its dam, and a Mendelian sampling term that represents its deviation from the average additive genetic merit of its full-siblings due to random sampling of alleles represented in the gametes. All known relationships are considered in the **A** matrix, so the performance of one animal contributes to the EBV of all known paternal and maternal relatives, with degree of the contribution depending on proximity of the relationship. Users typically provide at least 4 or 5 generations of pedigree data, and pedigrees are rarely traced beyond the 1970s, when herdbook records were computerized. When pedigree data are missing, unknown (phantom) parent groups (Westell et al., 1988) can be used to account for differences in the genetic merit of missing ancestors.

In the USDA animal model, management groups were defined according to parity (first vs. later), registry status (registered vs. grade), and 2-mo time blocks within herd-year. As in previous systems, adjustments were used to account for age, milking frequency, and length of lactation, and these factors were specific to breed and geographical region. Records in progress have been used in the United States since 1975; this increased genetic progress by up to 10% by reducing the time lag between data collection and breeding value prediction (Powell et al., 1975). Incomplete lactation records were projected to a 305-d basis once the cow had completed 2 or 3 monthly DHI tests, to produce

timely genetic predictions and enable rapid selection decisions for cows and their sires. Data collection ratings (DCR) were introduced by USDA in 1998; these are based on the number and spacing of test-day milk records relative to standard monthly supervised recording of all milkings per day, which receives a score of 100. The DCR system allows weighting of records according to their expected value in genetic evaluations, and they can be used as a guide to reimburse farmers who provide high quality data.

The accuracy of EBV produced by an animal model can be calculated from the elements of the inverse of the mixed model coefficient matrix, but this is computationally infeasible, so approximations are used (Harris and Johnson, 1998). A practical approach is to sum the number of daughter equivalents that contribute to the genetic prediction of a given animal (VanRaden and Wiggans, 1991), where the quantities of information from the animal's descendants, own phenotypic records, and ancestors (noting that siblings and cousins contribute through the animal's parents) are counted when computing reliability values.

Test-Day Model

In 1993, Cornell University was granted a US patent for the "test-day model," in which the performance of an animal relative to its herdmates was evaluated using daily milk weights from the herd's monthly test, rather than standardized 305-d lactation yields. This model was introduced for routine genetic evaluations in several countries (e.g., Canada, Germany) in which the genetic evaluation center obtained a license or successfully challenged the patent. However, because of this patent, a test-day model was not implemented for routine genetic evaluations in the United States. The Cornell patent was controversial because many organizations (including USDA) had been providing information for decades about the performance of an individual cow relative to her herdmates on a given test date, and Australia had formally implemented a test-day genetic evaluation model in 1984. However, no one had previously considered patenting this relatively well known statistical process (Rothschild and Newman, 2002). An interesting feature of test-day models is their ability to produce genetic evaluations for lactation persistency; for example, the ratio of expected milk yield at 280 d versus 60 d postpartum. Animals with greater lactation persistency may be more likely to remain healthy throughout the lactation and might be able to meet their nutritional needs with a less expensive ration because they do not experience the extremes of DMI or negative energy balance of their less-persistent contemporaries.

Random Regression Models and Covariance Functions

Data that are collected over time, such as test-day milk weights of lactating cows or periodic body weights of growing heifers, are often analyzed using a random regression model (Henderson, 1982; Ali and Schaeffer, 1987; Jamrozik et al., 1997). Functions such as Legendre polynomials or splines can be used to describe the trajectory of genetic, permanent environment, and temporary environment effects during the lactation. Numerous linear and nonlinear functions have been proposed for modeling these effects. For example, the Ali and Schaeffer (1987) model included a random herd-test date contemporary group effect, as well as fixed (overall mean) and random (additive and permanent environmental) regression coefficients corresponding to 4 functions of the time during lactation when the cow's milk weight was recorded. In that study, the residual variance was assumed fixed throughout the lactation but, in general, random regression models can provide estimates of the genetic, permanent environmental, and residual variances (as well as heritability and repeatability) at any time point during the lactation. The EBV of selection candidates can be computed at various time points during the lactation, and random regression models offer greater flexibility in accommodating variation in the frequency of milk recording between farms.

A similar approach, known as covariance functions (Kirkpatrick et al., 1990), can be used to analyze longitudinal data and explain the interrelationships between genetic and environmental factors over time. These models can be computationally demanding, and one must ensure that trajectories of additive genetic, permanent environment, and temporary environment effects are modeled appropriately. The goal of modeling the trajectory of genetic, permanent environment, and temporary environment effects precisely using a complicated function with 4 or 5 parameters must be balanced with the reality that parameter estimates will have large standard errors when applied to monthly DHIA records with only 8 to 10 data points per cow per lactation.

Random regression models and covariance functions can provide insight about the trajectory of biological processes during the lactation (e.g., milk fat synthesis, body tissue deposition). In addition, these models can provide information about correlated responses to selection for traits expressed over time, such as the effect of selection for peak milk yield in early lactation on milk composition in late lactation. The results of random regression models or covariance functions can also be used to facilitate the development of efficient data

collection protocols that maximize genetic progress per dollar invested in measuring phenotypes.

Multiple-Trait Models

Harvey and Lush (1952) introduced the first selection indices to combine production and conformation traits in cattle, following the work of Hazel and Lush (1942) and Hazel (1943), who defined an animal's aggregate genotype as a linear combination of the additive genetic values and economic values of traits that comprise the overall breeding goal. The number and definition of traits in the aggregate genotype or breeding goal may differ from the number and definition of traits in the selection index, particularly if some traits are difficult or expensive to measure (e.g., feed efficiency), or if selection relies on correlated traits for which phenotypes are more readily available.

Most models for estimation of breeding values can be extended to incorporate multiple traits (Henderson, 1976). Genetic correlations between traits represent the extent to which genetic superiority for one trait tends to be inherited with genetic superiority or inferiority for another trait in the breeding goal. Such correlations can be due to pleiotropy (one gene affecting several traits) or they can be induced by selection. Permanent environmental correlations measure the extent to which nongenetic factors occurring at some point during an animal's life may affect multiple phenotypes measured in subsequent months or years, whereas temporary environmental (residual) correlations acknowledge the extent to which current management practices, environmental conditions, or recording errors affect more than one trait.

Examples of favorable genetic correlations in dairy cattle include milk yield with longevity or body condition score with female fertility, whereas examples of unfavorable genetic correlations include milk yield with female fertility or milk yield with mastitis. Enough genetic variation exists within the population to find specific individuals or families that excel for traits that are negatively correlated, such as high milk yield with good female fertility. Multiple-trait models enhance the accuracy of genetic predictions by bringing additional phenotypes from positively or negatively correlated traits into the analysis. In addition, multiple-trait models help alleviate selection bias if phenotypic data for the trait upon which historical selection decisions were made are available (Pollak et al., 1984). However, the primary advantage of a multiple-trait model is its ability to assess interrelationships between traits in the breeding goal; this information is critical for projecting the desirable and undesirable correlated responses that will occur due to selection on EBV for various traits.

Genotype by Environment Interactions

Generally speaking, genotype by environment ($\mathbf{G}\times\mathbf{E}$) interactions for economically important traits in dairy production systems in temperate environments are small, at least compared with the interactions that plant breeders consider when matching lines or varieties with photoperiod, temperature, moisture, and soil conditions. Important G×E interactions exist between temperate and tropical environments, so farmers in countries such as Brazil or Thailand tend to avoid purebred cattle from the common European breeds and prefer cows with 12.5 to 37.5% inheritance of breeds that are adapted to local temperature-humidity conditions, tick-borne illnesses, and infectious diseases.

Multiple-trait models are often used to assess G×E interactions. For example, one can consider milk production in a confined herd with a TMR and milk production in a pasture-based herd with rotational grazing as separate but correlated traits (Weigel et al., 1999). Phenotypes of the same animals in both production systems (as a plant breeder would do by planting the same variety in different fields) are not necessary, because genetic relationships between cows in different systems allow partitioning of phenotypic covariances between environments into their genetic and environmental components.

Reaction norm models, which are conceptually similar to covariance functions, can describe the trajectory of genetic or environmental effects across some gradient, usually a gradient that spans overall management level or specific environmental conditions (Strandberg et al., 2009). A conceptually similar approach was used by Ravagnolo et al. (2000) when modeling the effect of heat stress on milk yield and fertility using temperature-humidity index (**THI**) data from local weather stations. Each animal is hypothesized to have a specific intercept for the onset of heat stress—the THI at which a decline in milk yield or fertility is observed for a particular cow. In addition, each animal is assumed to have a specific slope, which represents the rate of decline in milk yield or fertility per additional increment of THI beyond that animal's point of onset. Similar analyses have been carried out in Australia to quantify the ability of individual animals or sire families to cope with the effects of climate change (Garner et al., 2016). A challenge with the implementation of heat stress, climate adaptation, or other reaction norm models is the presentation of results. Sire EBV for every trait in each of low, medium, or high THI environments would not be sensible due to information overload, but electronic distribution of results could be simple if EBV were customized to end users' local environmental and production conditions. Customizing EBV or selection index weights to local

environmental and herd management conditions could provide additional benefits beyond precise modeling of G×E. For example, it would reduce the tendency for all farmers in a given region or country to select the same sires, thereby addressing the challenge of controlling inbreeding and maintaining genetic diversity.

INTERNATIONAL COMPARISONS

Holstein-Friesian Strain Comparison

A massive cattle breeding trial by the Food and Agriculture Organization (FAO) of the United Nations in the 1970s involved mating 30,000 Polish Black and White cows on 70 state-owned farms to international sires. Approximately 80,000 doses of semen were sourced from young (unproven) Holstein AI bulls in 10 countries, although it was difficult to ensure that these bulls represented a random sample of the country's Holstein population. This study led to great interest in the international trade of dairy sire semen, particularly the export of semen of North American Holstein bulls to Europe and other continents.

Conversion Equations

Early genetic comparisons of dairy sires from different countries used regression-based "conversion equations." The EBV of bulls with milking daughters in multiple countries, usually the country of origin and one or more importing countries, were used to develop conversion equations. The regression model included an intercept (difference in mean) and a slope coefficient (difference in scale), but the accuracy of converted EBV were generally poor, due to large standard errors of the intercept and slope coefficients, unless a large number of bulls had milking daughters in both countries.

Multiple-Trait Across-Country Evaluation

In 1995, the International Bull Evaluation Service (Interbull; Uppsala, Sweden) introduced the multiple-trait across-country evaluation (**MACE**) method as a replacement for conversion equations (Schaeffer, 1994). This linear model approach allowed the Interbull Centre to generate EBV for every bull in every participating country simultaneously. The input data were daughter yield deviations or deregressed EBV (with ancestral influence removed) from every country in which the bull had milk-recorded daughters, and these were weighted by the number of progeny per country. More than two dozen countries currently participate in Interbull sire evaluations, and the service includes production, type, fertility, calving, longevity, health, and workability traits for every major dairy cattle breed. Estimated genetic correlations for milk yield between North American and European countries tend to be high, in the range of 0.85 to 0.95, whereas those with Australia, New Zealand, and other countries with grazing-based production systems can be 0.75 or lower. Genetic correlations for conformation and fitness traits vary widely, due to differences in trait definitions. The influence of factors such as heat stress or parasite resistance is largely unknown, due to the absence of tropical or subtropical countries in the Interbull analyses.

Member countries have provided their national bull EBV and pedigree files to Interbull free of charge for more than 2 decades, and Interbull staff have carried out pedigree-based meta-analyses using the MACE methodology. Predictions for young genome-tested bulls can be computed with genomic MACE (Sullivan and VanRaden, 2009), but most countries publish predictions derived from genotype exchanges; for example, the North American Consortium (which includes Great Britain, Italy, Switzerland, Germany, and Japan), Eurogenomics (for Holsteins), or Intergenomics (for Brown Swiss). Exchanging genotypes and pedigrees is simpler than sharing and standardizing phenotypes measured in various ways under different conditions, and breeders from more than 50 countries have obtained genomic predictions derived from the North American reference population.

NONLINEAR MODELS

Threshold Models

Threshold models, introduced to the field of animal breeding by Gianola and Foulley (1983), allow proper modeling of binary or categorical traits, such as stillbirth or dystocia. Normality assumptions are violated, but a link function (e.g., probit, logit) matches observed binary or categorical phenotypes with sire EBV on an underlying "liability" scale. The area under the curve of a normal distribution is modeled such that if a sire's EBV (λ) is less than the first threshold, it is assigned to category 1, whereas if λ falls between the first and second thresholds, it is assigned to category 2, and so on. Threshold models are commonly applied to calving traits, often in conjunction with maternal effects models, and their usage is generally limited to sire models (rather than animal models). In general, threshold models lead to slightly more precise EBV than can be obtained by fitting binary or categorical phenotypes with a conventional linear model.

Survival Analysis

Failure-time (survival analysis) methods, such as Cox or Weibull proportional hazards models, are used widely in epidemiology to account for the presence of "censored" observations; that is, measurements of time to an event for which the starting or ending point (or both) is unknown. An example is longevity or length of productive life (PL), which is measured as time from first calving until death or culling due to illness, injury, or infertility. Cows that are still alive have observations that are right-censored, because their date of death or culling is unknown, as do cows that are sold to another herd for dairy purposes. Likewise, phenotypes for days open, a common measure of female fertility that is computed as time from calving until pregnancy, are right-censored for cows that have not yet become pregnant and for nonpregnant cows that left the herd for reasons other than infertility. Simplistic approaches, such as assuming a large and arbitrary value for days open of nonpregnant cows or longevity of living cows, have been implemented in many genetic evaluation systems (VanRaden and Klaaskate, 1993). Ducrocq et al. (1988) extended the Weibull proportional hazards model to include random additive genetic effects and relationships, which allowed computation of sire EBV for survival. Proper modeling of right-censored records allowed the inclusion of massive numbers of animals that were still alive, leading to more timely and accurate results. Previous studies allowed an opportunity period (e.g., 84 mo) for cows to fully express phenotypes for productive life or lifetime net profit (e.g., Cassell et al., 1993), but by the time studies were completed and manuscripts were published, the youngest cows were born more than a decade earlier. Another advantage of this method is the ability to use time-sensitive covariates, which allows more precise modeling of management and environmental factors that can change over time.

GENOMIC SELECTION

Marker-Assisted Selection

As shown in Figure 3, tremendous genetic progress was achieved by selecting for EBV computed under the assumption of polygenic inheritance and the infinitesimal model (i.e., the concept that most traits are affected by dozens or hundreds of genes, each having a very small effect). Nonetheless, technologies for assessing variation at the genome level, such as RFLP or microsatellite markers, allowed geneticists to pursue underlying functional mutations or QTL with large effects. Initial expectations were highly unrealistic, with many researchers and funding agencies believing one could find "the gene" that causes high milk production, exceptional female fertility, or appealing physical conformation. The number of functional mutations affecting quantitative traits that have been mapped precisely and for which the mode of inheritance has been fully characterized is negligible, and the effect of single-gene selection has been limited to genetic defects that are inherited in a simple Mendelian manner.

From the late 1980s to early 2000s, various methodologies were developed for marker-assisted selection. Information about QTL that were identified by various methods were incorporated into linear models for

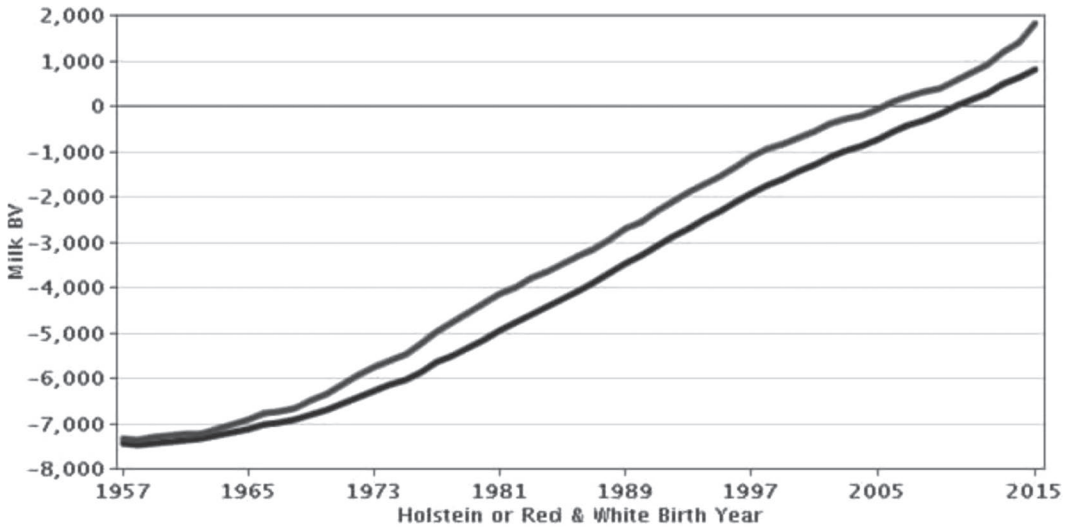


Figure 3. Genetic trend for milk yield [milk breeding value (BV)] in United States Holstein sires (upper line) and cows (lower line), for birth years from 1957 to 2015.

genetic evaluation, typically as fixed effects. One represented the EBV of a selection candidate as the sum of estimated effects for QTL1, QTL2, QTL3, . . ., and a polygenic EBV that represented unknown loci dispersed throughout the genome that were accommodated by the relationship matrix, **A**. Gains in genetic progress due to marker-assisted selection failed to meet expectations, as reviewed by Dekkers (2004), particularly when the causative mutation was unknown and selection relied on markers in population-wide linkage disequilibrium, or when selection was carried out within families using markers in population-wide linkage equilibrium. The effects of significant markers were often overestimated (Beavis, 1998), and many QTL with small effects were missed due to stringent significance thresholds (Lande and Thompson, 1990).

Whole-Genome Selection

The seminal genomic selection papers of Nejati-Javaremi et al. (1997) and Meuwissen et al. (2001), coupled with the development of inexpensive high-throughput genotyping platforms for SNP markers (Matukumalli et al., 2009), revolutionized dairy cattle breeding. Dozens of methods and algorithms were developed for whole-genome selection in plants and animals (de Los Campos et al., 2013), and dairy cattle breeders were at the forefront of this movement (VanRaden, 2008; VanRaden et al., 2009; Wiggans et al., 2017). Additional benefits, such as genome-based discovery of missing ancestors, can further enhance genetic progress. Early computational and statistical hurdles associated with whole-genome selection were formidable, given the problem of estimating a large number (p) of SNP effects from the phenotypic data of a smaller number (n) of genotyped individuals.

BLUP Models

Mixed linear models have been used to estimate SNP effects, where the vector **u** contains SNP markers that are assumed to represent a sample from a normal distribution; this provides BLUP estimates of SNP effects that can be summed over the genome to obtain genomic EBV of new selection candidates (SNP-BLUP; Meuwissen et al., 2001). Equivalently, a genomic relationship matrix (**G**) can be constructed from SNP genotypes, and this replaces the pedigree-based relationship matrix (**A**) in BLUP when computing genomic EBV (**GBLUP**). Initially, SNP-BLUP was more computationally demanding than GBLUP, because the number of SNP exceeded the number of genotyped animals with phenotypic records. However, the training populations in major dairy breeds now consist of tens of thousands of genotyped bulls with progeny data or hundreds of thousands of genotyped cows with performance records. The dimension of the mixed model coefficient matrix in GBLUP is of the order of the number of genotyped animals, which is growing very rapidly and often exceeds the number of SNP. Nonetheless, GBLUP is appealing due to its familiarity and ease of implementation among animal breeders who have been using BLUP for decades. The rapid growth in genotyped animals is due to the availability of inexpensive, low-density SNP panels, which typically feature 5,000 to 25,000 SNP dispersed evenly across the genome. These low-density genotypes can be matched with medium-density (50,000 to 100,000 SNP) or high-density (500,000 to 800,000 SNP) genotypes of ancestors, and missing SNP on the low-density panel are filled in with 95 to 99% accuracy using genotype imputation algorithms (Habier et al., 2009; Weigel et al., 2010).

Single-Step GBLUP

Legarra et al. (2009) and Misztal et al. (2009) solved the perplexing challenge of analyzing phenotypes from genotyped and nongenotyped animals simultaneously when computing genomic predictions. Before this development, direct genomic predictions (direct genomic values, DGV) were derived from associations between SNP genotypes and corresponding phenotypes in the subset of genotyped animals. In a subsequent step, the DGV were combined with pedigree-based EBV of the same selection candidates, using a selection index or weighted average. An initial challenge with single-step GBLUP (**ssGBLUP**), as proposed by Legarra et al. (2009), was that tricks to enhance its computational efficiency, such as Henderson's (1976) rapid method to create $\mathbf{A}^{-1}$ from pedigrees, were lacking. In ssGBLUP, one must create the inverse of a matrix (**H**) that includes blocks for genome-based relationships among genotyped animals, pedigree-based relationships among nongenotyped animals, and pedigree-based relationships between genotyped and nongenotyped animals. Legarra et al. (2014) developed an efficient method for building $\mathbf{H}^{-1}$, and ssGBLUP can now be applied to relatively large data sets comprising genotyped and nongenotyped animals.

Bayesian Regression Models

Another set of models for genomic prediction was developed using Bayesian regression. Ordinary least-squares regression cannot accommodate a situation in which the number of explanatory variables (SNP) exceeds the number of data points (animals with phe-

notypes), but in Bayesian regression models, the SNP effects are treated as random samples from an underlying distribution. Bayes A (Meuwissen et al., 2001) assumes SNP effects are sampled from a t-distribution with thick tails, such that most SNP have very small effects but a few SNP (presumably those in linkage disequilibrium with nearby QTL) can have large effects. A similar method, known as Bayes B (Meuwissen et al., 2001), assumes that SNP effects represent a mixture of 2 distributions, where a fraction (π) of the markers have zero effect on the phenotype and the remaining fraction ($1 - \pi$) have effects that follow a t-distribution. The fractional parameter, π, can be fixed in advance arbitrarily or estimated from the data using a method such as Bayes Cπ (Habier et al., 2011). Erbe et al. (2012) subsequently developed Bayes R, which features a mixture of normal distributions and accommodates SNP with zero, small, medium, and large effects. Bayesian regression methods tend to outperform GBLUP if QTL with moderate or large effects exist, whereas GBLUP performs very well in situations where inheritance approaches the infinitesimal model. A concern for the future is whether these models can provide robust estimates of breeding values for selection candidates when only a handful of animals are chosen for propagation with advanced reproductive technologies. Can our statistical models function properly with this extreme level of selection intensity?

Machine Learning Methods

Machine learning is a branch of artificial intelligence that focuses on prediction of outcomes for unobserved individuals (unlabeled data) by applying highly flexible algorithms to the known attributes (features) and outcomes of observed individuals (labeled data). Outcomes can be continuous, categorical, or binary. In animal breeding, labeled data correspond to the reference population or training set of older animals with genotypes and phenotypes, whereas unlabeled data correspond to the validation population or testing set of selection candidates with genotypes only. The features used for prediction are SNP genotypes. Countless machine learning algorithms exist, and no single method provides universally superior predictions—the optimal method and its parameters vary from one application to the next.

As the popularity of machine learning has exploded in other fields, it has gained footing in genomic prediction of livestock as well. Machine learning algorithms are widely known for their ability to discover patterns in large, messy data sets, even when data regarding some potential explanatory variables are missing. Long et al. (2007) was among the first animal breeders to apply machine learning to genomic prediction, using a filter-wrapper method for SNP-based classification of health traits in broiler chickens. A subsequent study by González-Recio et al. (2010) focused on a boosting algorithm for genomic prediction of the lifetime net merit of Holstein sires, whereas Okut et al. (2011) used an artificial neural network to predict the body mass index of mice using dense molecular markers. Yao et al. (2013) showed the tremendous flexibility of machine learning methods by using a random forest algorithm to identify potentially additive and epistatic QTL affecting residual feed intake in dairy cattle. More recently, Ehret et al. (2015) used an artificial neural network to predict milk production breeding values of Holstein-Friesian and Fleckvieh sires in Germany.

Machine learning, particularly deep learning algorithms for implementation of multi-layer artificial neural networks, has great potential for enhancing genomic selection and dairy herd management. The ability of these algorithms to discover intricate patterns in messy data and predict outcomes more effectively than conventional statistical methods has been demonstrated in a variety of fields. Powerful algorithms are readily available in commercial and public domain software, but they are "black box" in nature. The end user must understand basic concepts, such as how to construct training and testing sets that are independent and appropriate for the intended use, how to tune the parameters of a given model or algorithm, and how to avoid over-fitting the training data and making unrealistic conclusions about the model's predictive ability in future applications. The flexibility of machine learning algorithms may be valuable when incorporating biological knowledge gleaned from designed experiments, along with massive quantities of genomic and phenotypic data, for predicting the breeding values of selection candidates.

Inbreeding

Inbreeding coefficients are used to monitor the loss of genetic diversity within a breed over time and to account for the effects of inbreeding depression when computing genetic evaluations. Expected future inbreeding has been calculated by the USDA since 1998, by measuring the relationship between each bull and a sample of females from the same breed, and this statistic can be used to identify "outcross" bulls that are lowly related to the breed. Since 2005, USDA genetic evaluations have been adjusted for differences between the inbreeding of actual milk-recorded daughters and that of expected future mates, which can occur if a bull's original mates are not a random sample of the breed. Genomic measures of inbreeding, such as percent heterozygosity

or runs of homozygosity, can provide more precise measures of similarity at the genome level. Genome-based predictions of the inbreeding of hypothetical calves from a given cow and her prospective mates can facilitate mate allocation decisions, and genomic data can provide new insights into the genetic basis of inherited defects and inbreeding depression (VanRaden et al., 2011). Assessment of breed composition using genomic data is now routine, but effective methods for utilizing genomic data in crossbreeding systems are lacking. Loss of within-breed genetic diversity remains a concern, and stewards of the breeds should monitor the balance between rapid genetic progress and maintenance of diversity. There is no reason for a single Holstein bull to sire >3,000 progeny-tested sons that dominate the bull barns of AI studs on every continent, yet this has happened in practice. And although methods for constraining the rate of change in additive genetic relationships over time are available, based on optimal contribution theory (Meuwissen, 1997), these methods are not used widely in practice. Implementation of farm, region, or production system-specific EBV and selection indices would effectively address the issues of inbreeding and genetic diversity, while also capturing the benefits of G×E associated with local adaptation.

Phenotype Prediction and Management Diagnostics

Animal breeders have focused, almost obsessively, on expected performance of the offspring of selection candidates in the next generation. Performance of animals in the current generation is often an afterthought. They also have a tendency to eliminate, via data editing, exceptions that seem to arise from nongenetic causes. For example, cows that calve with twins are usually removed from dystocia and stillbirth analyses, cows that die in early lactation (before the first DHIA test) are removed from milk yield evaluations, and cows that are culled before the end of the opportunity period for a given disease may be discarded from health trait analyses. However, farmers must manage their businesses based on the income and expenses of all animals in the current generation, including those that animal breeders would consider as exceptions. Equations for predicting future phenotypes, such as estimated relative producing ability (ERPA) or most probable producing ability (MPPA), can be computed easily from a cow's EBV and corresponding estimates of permanent environmental effects and other relevant explanatory variables. Predicted future phenotypes can incorporate nonadditive genetic effects, which are ignored in pedigree-based or genomic applications of BLUP, and this could become particularly informative regarding specific mutations and their mode of action. Values of MPPA, ERPA, and similar metrics have been provided to farmers for decades in reports from dairy records processing centers, but this information is rarely used when making culling and management decisions.

Now that genomic testing is widespread, with tens of thousands of calves tested each month, the utility of predicted future phenotypes has increased dramatically. Well-managed herds with modern facilities have excess heifer calves relative to the number of replacements needed to maintain herd size, and feed, labor, and housing costs associated with rearing a heifer until first calving often exceed the animal's market value. Culling inferior heifer calves based on predicted future phenotypes, perhaps by repurposing them for beef production, is a common and economically sensible practice (Weigel et al., 2012). Culling decisions can be carried out using EBV, but genetic predisposition is an incomplete predictor of the future phenotype of a calf with significant lung damage due to respiratory disease, for example. Predicted phenotypes form the basis of genome-guided dairy herd management—the bovine equivalent of personalized medicine—as described by Weigel et al. (2017) when predicting hyperketonemia phenotypes of early postpartum Holstein cows.

An overlooked application of predicted phenotypes is the opportunity to use genomic data for evaluation or benchmarking of herd management practices. Genomic testing can describe the genetic predisposition of the calves, heifers, or cows on a given farm, and this information can be used to quantify the extent to which the farm's housing, heat abatement, forage quality, ration formulation, breeding program, health protocols, and other management practices allow these animals to fully express their genetic superiority. For example, one might use genomic predictions for early postpartum health disorders (Vukasinovic et al., 2017) to assess a herd's transition cow management, or one might regress daily milk weights of mid-lactation cows on genomic predictions for milk yield to assess a herd's nutrition program.

SUMMARY

Over the past 100 years, genetic selection programs evolved from an infancy of pedigree recording, performance recording, and daughter–dam comparisons, to an adulthood of animal model BLUP, whole-genome prediction, nonlinear models, and machine learning algorithms. Grosu et al. (2014) provided a comprehensive review of these developments and their effect on dairy cattle improvement programs worldwide, whereas this review focuses primarily on the United States. Every scientific advancement by a dairy cattle breeder in the past century was built upon the shoulders of his or

her predecessors, and collaborations with colleagues in genetics, statistics, and computer science have yielded remarkable returns. Furthermore, virtually every scientific advancement by a dairy cattle breeder in the past century was developed to solve a practical problem that affected dairy farmers, address a potential threat that could harm dairy farmers, or capitalize on an opportunity that might benefit dairy farmers. This was precisely the goal of legislators who conceived the idea of the land-grant university system and a network of federal agricultural research institutes, as well as the expectation of taxpayers who were asked to fund these endeavors. The discoveries of the next 100 years cannot be imagined at present, but we can hope that similar successes will be achieved in producing research results that will lead to healthy animals, vibrant farms, satisfied consumers, and a sustainable food production system.

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APPENDIX

Table A1. Timeline of important discoveries and developments in genetic selection

Date	Milestone	Reference
1868–1917	Growth of pedigree data in herdbooks established by breed societies.	VanRaden and Miller, 2008
1905–1917	Expansion of milk recording programs that originated in Michigan.	VanRaden and Miller, 2008
1915–1917	USDA begins evaluating dairy bulls by daughter–dam comparison method first proposed in Denmark.	VanRaden and Miller, 2008
1918–1931	R. A. Fisher and J. B. S. Haldane lay the foundations of population and quantitative genetics.	Fisher, 1918; Haldane, 1932
1920s	Local bull associations are formed with assistance of USDA Division of Dairy Herd Improvement investigations.	VanRaden and Miller, 2008
1931–1941	S. Wright and J. L. Lush develop the science of animal breeding and statistical methods for sire evaluation.	Lush, 1931; Wright, 1932
1934–1942	L. N. Hazel and J. L. Lush introduce the selection index method for estimating breeding values of dairy sires.	Hazel and Lush, 1942
1940s	Artificial insemination (AI) is adopted and regional AI cooperatives are formed.	VanRaden and Miller, 2008
1954–1956	A. Robertson, J. M. Rendel, and C. R. Henderson propose a herdmate comparison, which is soon implemented by Cornell University for sire evaluations.	Henderson et al., 1954; Robertson and Rendel, 1954; Searle, 1964
1961	USDA adopts herdmate comparison for routine genetic evaluation of dairy sires.	VanRaden and Miller, 2008
1964	Publication of high-index (elite) cow lists to facilitate selection of bull dams.	Powell and Norman, 2006
1970s	Polish breeding trial by Food and Agriculture Organization of the United Nations stimulates international semen trade.	Grosu et al., 2014

Continued

Table A1 (Continued). Timeline of important discoveries and developments in genetic selection

Date	Milestone	Reference
1972	USDA Animal Improvement Programs laboratory formed. Cornell implements BLUP sire model for prediction.	Grosu et al., 2014
1974	USDA introduces the modified contemporary comparison model to compute predicted differences for males and cow indexes for females.	Norman et al., 1976
1976	C. R. Henderson develops a rapid method to construct the inverse of the additive genetic relationship matrix.	Henderson, 1976
1977	D. A. Harville proposes maximum likelihood estimation of variance components.	Harville, 1977
1987–1997	L. R. Schaeffer and colleagues propose random regression for implementation of test-day models.	Ali and Schaeffer, 1987
1989	USDA implements an animal model for computing predicted transmitting abilities of dairy cattle.	Wiggans and VanRaden, 1989
1995	International Bull Evaluation Service provides multiple-trait across-country evaluation of dairy sires.	Schaeffer, 1994
2001	T. H. E. Meuwissen, B. Hayes, and M. E. Goddard propose whole-genome selection with dense molecular markers.	Nejati-Javaremi et al., 1997; Meuwissen et al., 2001
2009	Sequencing of the bovine genome is completed and routine whole-genomic selection is implemented by USDA.	VanRaden, 2008; Matukumalli et al., 2009
2008–2017	D. Gianola and colleagues apply machine learning algorithms to genomic prediction of livestock.	Long et al., 2007; González-Recio et al., 2010
2009–2014	A. Legarra, I. Misztal, and I. Aguilar develop single-step genomic BLUP.	Legarra et al., 2009, 2014; Misztal et al., 2009

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A 100-Year Review: A century of dairy heifer research[1,2]

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ABSTRACT

The years 1917 to 2017 saw many advances in research related to the dairy heifer, and the *Journal of Dairy Science* currently publishes more than 20 articles per year focused on heifers. In general, nutrition and management changes made in rearing the dairy heifer have been tremendous in the past century. The earliest literature on the growing heifer identified costs of feeding and implications of growth on future productivity as major concepts requiring further study to improve the overall sustainability of the dairy herd. Research into growth rates and standards for body size and stature have been instrumental in developing rearing programs that provide heifers with adequate nutrients to support growth and improve milk production in first lactation. Nutrient requirements, most notably for protein but also for energy, minerals, and vitamins, have been researched extensively. Scientific evaluation of heifer programs also encouraged a dramatic shift toward a lower average age at first calving over the past 30 yr. Calving at 22 to 24 mo best balances the cost of growing heifers with their production and lifetime income potential. Increasingly, farms have become more progressive in adopting management practices based on the physiology and nutrient needs of the heifer while refining key economic strategies to be successful. Research published in the *Journal of Dairy Science* has an integral role in the progress of dairy heifer programs around the world.
Key words: dairy heifer, growth, age at first calving, heifer nutrition

INTRODUCTION

The years 1917 to 2017 saw many great advances in research related to the dairy heifer, and in this time the industry has made many improvements to the way we grow and manage heifers (Appendix Table A1). In the earliest years of the century, the *Journal of Dairy Science* (**JDS**) had very few heifer publications—sometimes <1 or 2 per year related to the growing heifer. However, the later years, notably the past 20 yr, have seen a dramatic increase in heifer research publications, with 20 or more per year. This review includes publications appearing in JDS since its beginning that had the term *dairy heifer* in the title or key words. Papers focusing on preweaned calves and treatments applied to heifers after their first calving were excluded. Topics related to breeding and reproduction, welfare, disease, and housing and facilities are not covered to limit duplication with other articles in this issue of JDS.

In the earliest literature on the growing heifer, researchers identified costs of feeding and implications of growth on future productivity as major concepts requiring further study to improve the overall sustainability of the dairy herd. The status of knowledge of the practical feeding of dairy heifers at the time JDS was established was well summarized by Henry and Morrison (1915; page 426): "The rearing of the heifer after 6 to 8 months of age is an easy task, and perhaps because of this many are stunted for lack of suitable feed." The authors subsequently described the feeding of heifers in approximately half a page, clearly indicating opportunity for conducting and reporting additional research on growth, nutrition, and management.

GROWTH

Growth has been a fundamental outcome of interest in heifer nutrition and management research over the years. Eckles (1920) and Ragsdale (1934) published the first growth standards for dairy heifers at the University of Missouri; standards from USDA Beltsville (Matthews and Fohrman, 1954) and the University of Nebraska (Davis and Hathaway, 1956) followed. Most were derived from a single experiment station herd over

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[2]Mention of any trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.
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a period of years, yet all were quite similar in their outcome. In many early heifer studies, researchers characterized growth relative to a "standard" or "normal" heifer using 1 of these reports. There are even instances in which a control group was not included in an experiment because comparisons could be made with such a standard (Maynard and Norris, 1923). Also of interest, in this early period of research many articles contained pictures of the animals on study to supplement growth data with a visual representation of the form of growth. These practices emphasize 2 important concepts of this early work: experiments consistently contained a very small number of animals, and appropriate controls rarely were included.

In one of the earliest reports of growth, McCandlish published measurements from birth to production age and showed that BW consistently increased proportionally to the product of height, depth, and width (McCandlish, 1922). Likewise, height:weight ratio was suggested to maintain a constant proportionality independent of age (Brody and Ragsdale, 1922). Much later, Swanson et al. (1967) published an estimate of optimal growth patterns for dairy heifers. Twenty years after this, Heinrichs and Hargrove (1987) published Holstein heifer weight and height growth standards derived from population studies that showed that heifers were larger than previously published standards, which likely represented changes in breeding programs over time. In addition, they published population growth studies of other dairy breeds from 2 to 24 mo of age (Heinrichs and Hargrove, 1991, 1994). These were (and still are) the most comprehensive publications of growth standards for these breeds.

Summaries of data by Kertz et al. (1998) provided descriptions of Holstein growth through the heifer development phase. Growth rate was fastest in the first 6 mo of life, and feed cost per unit of BW or withers height was lowest during this time. Heinrichs et al. (1992) showed equations predicting BW from other body measurements and published a modified heart girth to BW equation, likely reflecting changes in conformation from early standards (Ragsdale, 1934; USDA/Matthews and Fohrman, 1954). Taking this concept further, Oliveira et al. (2013) developed an equation using heart girth to estimate BW for crossbred Holstein-zebu heifers in Brazil.

Early studies began to relate growth to production and later to economics. Growth from birth to 2 yr was uncorrelated with first-lactation or lifetime-lactation performance (Davis and Willett, 1938). In contrast, Plum et al. (1952) documented a positive relationship between growth of chest girth and butterfat production; however, this relationship was suggested to have been attributable to environmental factors instead of genetics. This same study reported a positive genetic correlation between growth in withers height and butterfat production. Touchberry (1951) and Blackmore et al. (1958) reported a negative genetic association between body size measurements and milk production, with the exception of a positive correlation between withers height and production in the latter study. With the limited data available, it was noted that there was a correlation (44 comparisons, $r = +0.40$) between BW gain during the last 2 mo of pregnancy and subsequent lactation performance regardless of season of calving (Blackmore et al., 1958).

Further studies on growth looked at the genetic relationships of growth and production. Koenen and Groen (1996) estimated the genetic relationship between BW at first calving and other growth patterns and found that estimated mature BW was negatively correlated with rate of maturation, whereas BW at first calving had a strong correlation with mature BW and a negative correlation with maturation rate. Coffey et al. (2006) studied growth of dairy heifers from 2 genetic lines in the United Kingdom: selection for maximum production of fat and protein (select) or average production (control). Under the same management, heifers from the select line grew faster and were heavier at first calving. However, Van de Stroet et al. (2016) showed that larger heifers were not superior in production compared with average-sized animals.

Swanson published a series of papers in the 1960s reporting heifer growth and its relationship to future production (Swanson and Hinton, 1964; Swanson et al., 1967; Swanson, 1971). This helped support his definition that an optimal growth pattern for dairy heifers will develop their full lactation potential at the desired age with minimum expense. Many of these early growth studies used twins to minimize genetic differences, and many of these studies were done with a limited number of animals, often with a variety of breeds. Swanson and Hinton (1964) restricted growth of twin heifers by 25%, and although first-lactation production was 78 to 95% that of the normal-growth heifers, in second and third lactations the pairmates produced almost alike, showing no harmful effect of subnormal growth after the first lactation. However, size of the restricted-growth animals was smaller through maturity. Swanson et al. (1967) studied slowly grown heifers versus those grown at normal rates of gain. Their results showed that prepartum supplementation of the slowly grown heifers allowed them to attain, if not exceed, normal lactation performance. On this basis, liberal prepartum feeding for 9 to 12 wk before expected calving was recommended for heifers if they were substandard in size.

Gardner et al. (1977) published the first work on accelerated growth and early breeding of heifers. They in-

creased ADG from weaning to breeding and accelerated heifers calved at 19.7 mo, 7.2 mo earlier than control heifers. Accelerated heifers produced significantly less milk in first lactation and numerically less in following lactations. Interestingly, the authors provided little to no discussion of the results. Kertz et al. (1987) showed that growth of Holstein heifers can be accelerated up to 1 kg of ADG from 3 to 12 mo of age without excessive fattening. Several studies evaluated prepubertal ADG and first-lactation milk production, often with differing conclusions. Zanton and Heinrichs (2005) conducted a meta-analysis of heifer growth and production studies and found a quadratic relationship, which demonstrated that the conclusions of each study were tied to the ADG chosen. The reported optimal ADG was 799 g/d before puberty.

AGE AT CALVING

In reference to growth and management effects on age at first calving (**AFC**), a progression of research has demonstrated the economic importance of this aspect of heifer raising. In the first year of JDS, cows calving at later than 30 mo of age were shown to have produced more milk in 2 lactations than cows calving earlier than 30 mo did in 3 lactations (White, 1917). This was shown by following 10 cows that calved for the first time over an 8.3-yr period and were not randomly assigned to early or late treatments. In an observational study, Plum and Lush (1934) reported that average AFC in the Iowa Cow Testing Association registry was 27.1 mo, with a median age of 25.33 mo. These data also showed that half of the purebred calves in this analysis were born to dams that were <52 mo of age. Similarly, the average age of a cow was reported to be 56.4 mo in the Iowa Cow Testing Association using broader inclusion criteria than were used previously (Cannon and Hansen, 1939). These data indicated that an average cow spends a substantial proportion of her life in the unproductive state. Thus, management factors that reduced the time or increased the efficiency of raising heifers from weaning to first calving without negatively affecting future productivity could positively influence profitability of dairy farms. It should be noted that much of the research in the area of age at calving does not take into account the length or number of lactations or age of animal life.

In a review of heifer growth and production research, Schultz (1969) stated that there appeared to be a trend in more recent publications to revise the breeding age downward. Schultz discussed research on the effects of alterations in the rearing system from the "standard" or "normal" procedures to make recommendations for lowering AFC. Powell (1985) published a review of AFC trends covering 1960 to 1982. Means and distributions were similar for all breeds, and the distribution of AFC was skewed toward higher AFC for all breeds. Ayrshires had the highest mean AFC, and Jerseys had the lowest. Mean AFC changed little from the early 1960s to the early 1980s, although it peaked slightly around 1976. Powell's (1985) data showed that AFC decreased about 3 d/yr from the mid 1970s through 1982. Later work showed that trend (AFC decreasing 3 d/yr) continuing in the Holstein breed from 1985 to 1990 (Heinrichs and Vazquez-Anon, 1993).

Breeding heifers at a younger age coupled with a proper rearing program offers a promising approach to increase profit for the dairy industry. Lin et al. (1986) showed that breeding heifers as early as 350 d of age had no adverse effects on calving ease or retained placenta but did result in calves that were 1.2 kg lighter at birth. Heifers of the 350-d breeding group had lower milk production but were the same size at all points as heifers bred at 462 d. When lactation performance was analyzed, a reduction of 1 mo in AFC increased 3-parity and 61-mo total milk by 427 and 554 kg, respectively (Lin et al., 1988). Gardner et al. (1988) observed no differences in reproduction or production in heifers managed to achieve early or late AFC. Results from these studies suggested that early breeding was a viable and practical approach to improving profitability. The industry responded with a strong trend in reducing AFC from this point onward. However, Mohd Nor et al. (2013) showed that calving earlier on Dutch farms resulted in less milk in first lactation, indicating that management changes must accompany breeding at earlier ages.

Heinrichs et al. (1994) reported results of the National Dairy Heifer Evaluation Project, the first epidemiological study of the national dairy industry conducted by USDA. In this survey, operations with mean BW >545 kg at first calving and AFC ≤27 mo had higher milk production. Hare et al. (2006) examined AFC from 1980 through 2004 and found that it declined progressively with time for all 5 breeds studied. Jerseys had the lowest AFC, and it declined from 26.5 mo in 1980 to 24 mo in 2004. Ayrshires had the highest AFC at 29 mo in 1980 and 28.5 mo in 2004. Holstein AFC was 28 mo in 1980 and decreased to 25.5 mo by 2004. The distribution also shifted to the left during this period, with almost 20% of heifers calving at 24 mo in 2004 compared with approximately 8% in 1980.

Results of studies conducted in multiple states and countries investigating optimum AFC are remarkably consistent. Gill and Allaire (1976) using Ohio data showed that optimal AFC for total lifetime production was 22.5 to 23.5 mo. Hoffman and Funk (1992) proposed that 22 to 24 mo was the best AFC; calving ear-

lier resulted in reduced milk yield. In Italian Holstein-Friesians, an AFC between 23 and 24 mo was found to best balance rearing costs and milk yield (Pirlo et al., 2000). In California Holsteins, the highest economic return was generated for AFC from 23 to 24.5 mo when considering milk production, stillbirths, reproduction, and health (Ettema and Santos, 2004). Nilforooshan and Edriss (2004) observed that milk yield in Iranian Holsteins increased as AFC increased from 21 to 24 mo but decreased as AFC extended past 24 mo; they determined that the optimum AFC was 24 mo. In Pennsylvania the most efficient heifer-raising programs had AFC of 23.7 mo and first-lactation animals producing 88.42% of the milk produced by multiparous cows (Heinrichs et al., 2013).

Simerl et al. (1991) showed that Florida herds were calving at 22.2 mo of age without issues at parturition. Bach et al. (2008) compared 47 dairy herds in Spain that fed the same lactation ration but were under individual management and had a range of 13 kg/d in average milk production. Four factors, including AFC of heifers, accounted for more than 50% of the variation in milk production observed between these herds. Mohd Nor et al. (2013) introduced relative AFC (the difference between AFC of an individual and median AFC of the herd) as a new way of evaluating the effect of AFC. Dutch heifers calving 1 mo earlier than the median AFC produced 90 kg less milk per 305 d; those calving 1 mo later produced 86 kg more. This measurement assumed that heifers had the same management within a herd, and a heifer's deviation from the median reflected her development. Thus, earlier breeding without adjusting management to ensure adequate development was shown to reduce first-lactation production. Krpálková et al. (2014) reported that AFC <23 mo did not negatively affect milk yield or reproduction parameters during the first 3 lactations. Other than the normal known factors, AFC in heifers and level of milk production during gestation in cows were indicated as decisive determinants of calf birth size (Kamal et al., 2014). Thus, we can see the progression of a downward trend in AFC worldwide. Current genetics of the Holstein breed and management of commercial farms will allow the breed to reach at least 22 mo AFC on average.

NUTRIENT REQUIREMENTS

Determining nutrient requirements of heifers was the subject of much research throughout the first 100 yr of JDS. Early on, the maintenance energy requirement of young growing Holsteins was determined to be approximately 90% of that previously reported by Armsby (Eckles et al., 1927). Eckles suggested that this discrepancy was attributable to Armsby's (1917) use of beef cattle in deriving maintenance values. Mixed diets marginally improved heifer growth and improved first- and second-lactation production compared with alfalfa-only diets when heifers were fed the test diets starting at about 315 kg of BW (Woll, 1918). Supplementing a winter ration of alfalfa hay with barley grain increased winter growth compared with heifers fed alfalfa hay only (Willard, 1932). In this study, growth was compared with Eckles' "normal." Further study at the Wyoming station demonstrated that increased consumption of alfalfa hay was obtainable by increasing the hay allowance and decreasing the grain allowance (Willard, 1938).

Feedstuff digestibility for Holstein heifers fed diets that contained no roughage (Mead and Goss, 1935) was shown to be consistent with what would be calculated from ingredient digestibility coefficients determined from the book values of Henry and Morrison (1923), which had been determined when forage was fed. Differing hay quality grades affected gains and feed efficiency (Kelkar and Gullickson, 1950; Gordon et al., 1954, 1956); however, it was noted that the current chemical and physical analyses were insufficient to completely characterize the value of hays for heifer growth (Gordon et al., 1952). Donker et al. (1968) published a method for evaluating forage energy production formulas by comparing various published energy intake estimates with heifer requirements. They reported that of the 9 equations used to estimate forage energy, all were inadequate for differentiating the ability of hay qualities to stimulate heifer growth. Corn silage was found to be inferior to grass silage as the sole source of forage, although heifer growth was improved if a small amount of grass hay was added to either ration (Keener et al., 1958).

Reid and Robb (1971) evaluated the relationship of body composition to energy intake and energetic efficiency. Their work showed that body protein and fat were linearly related to empty BW; furthermore, body protein and fat explained 99.7 and 96.1% of the variation in empty BW, respectively. They used 61 heifers from 1 d to 14 mo of age and provided a wide range of body composition data covering 4 different breeds and crosses fed a variety of nutritional regimens. In the same year, Swanson (1971) investigated feed energy requirements of heifers under different rates of growth. Data for this meta-analysis came from 16 experiments involving more than 900 heifers in 267 experimental groups, within which heifers had been fed to produce different growth rates. He published a prediction equation based on diet TDN and BW that was reported to apply to all dairy breeds from 4 to 22 mo of age. Fox et al. (1999) evaluated the applicability of the beef NRC (1996) equations for predicting growth requirements,

target weights based on mature size, and energy reserves in dairy cattle. Coefficients of the equations were modified, but the methods were accurate when predictions were compared with actual observations for heifers >100 kg. With slight modifications, this system was adopted by the next edition of the dairy NRC (2001).

Protein

Early research evaluated using homegrown protein supplements to reduce costs of providing protein to growing heifers. Hilton et al. (1932) demonstrated that raw soybeans could effectively replace linseed oil meal when fed for approximately 1 yr in a diet containing alfalfa hay as the source of forage. Subsequently, heifers were found to grow equally well on diets containing either steam-dried or flame-dried fish meal (Berry and Manning, 1936). Results based on weight gains alone provide little insight into the nutritional mechanisms affecting an increased ADG or the effect of protein quality on ADG. Hart et al. (1939) initiated studies that evaluated the ability of the growing ruminant animal to use NPN supplements to increase the CP content of a CP-deficient diet. In one of the first studies of its kind, a rumen-fistulated Holstein heifer was used to demonstrate the conversion of dietary urea to protein in the rumen (Wegner et al., 1941a). Further work with the fistulated Holstein heifer demonstrated that conversion of urea to protein was affected by the protein (Wegner et al., 1941b), starch (Mills et al., 1942), and soluble sugar (molasses; Mills et al., 1944) contents of the basal diet. Growth studies showed that although urea could be used as a source of CP, the N from urea was not used as effectively as the N from soybean meal (Bohman et al., 1954; Lassiter et al., 1958) or when urea was included in a molasses-containing diet compared with a corn-containing diet (Bohman et al., 1954; King et al., 1957). In heifers fed low-protein diets, feeding high energy markedly increased the retention of N that was available for growth (Lofgreen et al., 1951). Later researchers from New Hampshire also studied urea utilization. Using 3 sets of twins in a respiration chamber, Colovos et al. (1963) showed that including urea in limited amounts was beneficial in higher forage rations.

Some interesting work from the late 1970s and early 1980s evaluated unusual alternative protein and energy sources. Johnson and McCormick (1975) fed municipal waste with sorghum silage, and Cross and Jenny (1976) fed turkey litter silage. Block and Shellenberger (1980) fed wood fines, and Keys and Smith (1981) fed poultry litter. All found adequate growth when diets were balanced; however, this line of research did not seem to go further, likely because of the questionable welfare of heifers fed these types of alternative feedstuffs.

Since the 1990s a great deal of dairy heifer nutrition research has been conducted, much of it related to updating protein requirements with little emphasis on energy. In general, these studies had reasonably large numbers of animals and adequate controls, unlike some of the earlier work. A variety of research groups studied total protein intake as it relates to diet energy. Lammers and Heinrichs (2000) used a CP:ME ratio of 46:1, 54:1, or 61:1 g/Mcal and found a linear increase in ADG, feed efficiency, and structural growth with increasing CP:ME ratio. Hoffman et al. (2001) investigated 8, 11, 13, or 15% CP in similar energy diets; structural growth, N retention, and diet digestibility were maximized at 13% CP. Gabler and Heinrichs (2003) fed a CP:ME ratio of 48.3, 59.1, 67.5, or 76.5 g/Mcal at DMI allowing an ADG of 800 g/d; feed efficiency and height improved linearly with increasing CP:ME ratio. Marini and Van Amburgh (2005) observed that low-N diets reduced urine excretion of N, whereas growth rates were maintained. These studies showed that altering the CP:energy ratio of heifer diets improved growth and feed efficiency.

Research on the effects of protein degradability on growth has begun relatively recently. Petit and Yu (1993) fed heifers fresh grass (timothy-clover mix) ad libitum with no supplemental N, a high-NPN molasses block, or high-RUP block containing animal proteins. Protein supplementation increased ADG, but no difference was observed between blocks, so the authors concluded that RUP was not the limiting factor. Casper et al. (1994), using 3-mo-old Holstein heifers, found that ADG improved in diets with extruded soybean meal, with greater improvement in diets containing barley versus corn. They recommended 35 to 40% RUP for heifers 3 to 6 mo of age. Bethard et al. (1997) used 32 heifers fed rations formulated for an ADG of 0.6 or 0.9 kg/d with 30 or 50% of CP as RUP from age 6 to 13 mo and found improved feed efficiency for high-energy and high-RUP diets. However, Steen et al. (1992) and Swartz et al. (1991) found no growth advantage to increasing the undegradable protein intake of heifers, as was noted in the 1989 NRC (NRC, 1989). Daccarett et al. (1993) fed Holstein heifers 107 or 124% of NRC requirements for CP and ME from 3 to 6 mo of age and 100 or 115% from 6 to 24 mo and demonstrated that feeding in excess of NRC (1989) recommendations increased BW and height without fattening. Van Amburgh et al. (1998) evaluated NRC (1989) and Cornell Net Carbohydrate and Protein System recommendations using 273 Holsteins with ADG treatments of 0.6, 0.8, or 1.0 kg/d from 90 to 320 kg of BW. Actual ADG

were 0.68, 0.83, and 0.94 kg/d and were not affected by feeding plant and animal or plant and urea protein sources. They also showed, consistent with several previous studies, that NRC underestimated RDP and overestimated RUP requirements. Zanton and Heinrichs (2008a) published a meta-analysis of N utilization and excretion in weaned dairy calves that summarized 10 experiments with 57 different treatments. True N digestibility was estimated at 96.4%, and endogenous, nondietary fecal N excretion was 6.51 g of N/kg of DMI. Diet CP of 14.2% and N intake of 1.84 g/kg of metabolic BW maximized gross N efficiency. They concluded that reductions in fecal N excretion would be most achievable through reduced DMI while feeding a high enough concentration of energy to meet the needs of the animal. Reducing urinary N excretion is best accomplished by decreasing N intake. In a follow-up study (Zanton and Heinrichs, 2009), Holsteins of 362 kg of BW and 12.3 mo of age were fed 25 or 75% forage diets with 0.94, 1.62, 2.30, or 2.96 g of N/kg of metabolic BW and equal energy intakes. Their results suggested that feeding 1.67 g of N/kg of metabolic BW optimized N efficiency regardless of the forage level in the ration. Lascano et al. (2012) used those levels of N to evaluate starch digestibility and found that high starch decreased ADF and increased hemicellulose digestibility. Lascano et al. (2016) concluded that low fiber levels in low- and high-forage diets increased utilization of nutrients under limit-feeding conditions when provided with high RDP. Furthermore, Zanton and Heinrichs (2016) reported that heifers limit-fed high-energy diets had increased digestibility and N retention and that younger animals were more responsive to this feeding strategy.

Vitamins and Minerals

The early research on vitamins for dairy heifers occurred closely in parallel with the development of the understanding of the importance of vitamins in overall nutrition. The first evidence that the knowledge of the chemical composition of feeds was incomplete was determined when researchers fed rations comprising a single plant source (corn, wheat, or oats) to dairy heifers, resulting in differences in offspring vigor and milk production capacity even though the chemical compositions of the rations were, in all known respects, identical (Hart et al., 1911). Several studies were subsequently reported detailing vitamin requirements of heifers. Vitamin A was shown to be indispensable for the normal growth and development of calves (Jones et al., 1926). However, calves could grow and reproduce normally on a ration that contained vitamin B insufficient to support the growth and well-being of rats; the deficit in dietary vitamin B was proposed to be synthesized by the rumen bacteria (Bechdel et al., 1926). Likewise, heifers were shown to grow normally for 1 yr on a ration that resulted in death from scurvy of guinea pigs in less than 30 d (Thurston et al., 1926), indicating that heifers can synthesize sufficient vitamin C. Calves grown from birth to first calving in the dark grew normally (Gullickson and Eckles, 1927). However, these calves were provided with ad libitum access to timothy hay, which was subsequently shown to prevent rickets when sun cured but not when cured in the dark (Huffman et al., 1935). The forage component of the heifer diet was suggested to be a significant contributor to the vitamin and mineral requirements of heifers when a diet devoid of roughage was able to support normal growth when supplemented with cod liver oil and alfalfa ash (Mead and Regan, 1931; Wise et al., 1939). Previously, it was unclear whether the deficiency in a roughage-free diet was attributable to a lack of bulk in the diet or a vitamin or mineral deficiency (McCandlish, 1923). More recently, biotin supplementation of heifers precalving and during first lactation did not affect incidence of lameness (though it did for cows of greater parity; Hedges et al., 2001), and no improvement in immune function was detected in heifers fed a source of vitamin C after being transported for 2.3 h (Tyler and Cummins, 2003). In addition, studies showed that vitamin D could be administered effectively using mucosal absorption (Okura et al., 2004; Rivera et al., 2005).

As bone diseases were not uncommon in developing dairy heifers, a description was made of normal bone from 60 to 180 d (Kruger and Bechdel, 1928). Bone meal supplemented to heifers for approximately 18 mo was said to be without effect compared with when heifers were supplemented with common salt (Salmon and Eaton, 1925). Diets low in P (0.2% DM) resulted in reduced ADG; however, reproduction was not compromised compared with a group fed a diet with adequate P (0.4% DM; Huffman et al., 1933). Noller et al. (1977) evaluated the ability to meet P requirements from natural feedstuffs and the adequacy of minimums for P recommended by NRC (1971) and determined that those requirements (0.22%) were adequate based on mineral status of the heifers and conception rates. Subsequently, Esser et al. (2009) fed diets with (0.39% P) or without (0.29% P) supplemental P from 4 to 21 mo of age and found no effect of supplementation on frame measurements, bone density, or markers of bone metabolism. Similarly, Bjelland et al. (2011) found similar ADG and body measurements at 22 mo with or without supplemental P (0.4 or 0.3% DM) from 4 to 22 mo of age. No difference was observed in heifer reproductive performance (services per conception, age

at conception, or dystocia) or in first-lactation milk production and reproduction; however, heifers fed supplemental P excreted more P (29.9 vs. 24.2 g/d). Ray et al. (2012) evaluated disappearance of phytate from the large intestine using heifers with ruminal and ileal cannulas using ileal infusions of 0, 5, 15, or 25 g of phytate/d. Ileally infused phytate was degraded in the large intestine, but degradability was not dependent on the amount of phytate.

Studies showed little difference in the absorption of Cu from sulfate, proteinate, carbonate, or Cu-AA complex (Du et al., 1996; Ward et al., 1996; Yost et al., 2002); however, when S and Mo were included in the diet, the proteinate form had better absorption than sulfate and was stored more efficiently than carbonate (Ward et al., 1996). Supplementing Cu in the form of sulfate or Lys also made little difference in growth, though Cu-Lys enhanced liver and plasma Cu concentrations in heifers with low Cu status (Rabiansky et al., 1999). Holstein heifers supplemented with Cu sulfate or proteinate prepartum and in early lactation had improved plasma and liver Cu status and a less severe response to an *Escherichia coli* mastitis challenge in one study (Scaletti et al., 2003) and lower bacteria count in their milk in another study (Scaletti and Harmon, 2012). Sumner et al. (2007) fed 0, 5, 10, or 15 mg of Cr/d from 11 to 14 mo of age; increasing Cr increased glucose concentration and utilization and decreased insulin and nonesterified fatty acid (**NEFA**) concentration. Spears et al. (2012) reported that feeding Cr propionate increased tissue sensitivity to insulin and recommended providing 3 mg/d or 0.47 mg/kg of DM.

In a survey of 22 herds in Finland, Jukola et al. (1996) found that serum Se and vitamin E were adequate in heifers and that supplementation was not needed. However, supplementing vitamin E at 3,000 IU/d beginning 2 mo before calving increased blood, liver, milk, and follicular fluid levels of vitamin E in other reports (Bouwstra et al., 2008; Dobbelaar et al., 2010). In addition, Ceballos-Marquez et al. (2010) found that supplementing Se to Chilean Holstein-Friesian heifers precalving via a single barium selenite injection or Se yeast at 3 mg/head daily for 45 d increased blood glutathione peroxidase activity and tended to reduce IMI and quarters with high SCC at calving.

Kincaid et al. (1997) fed weaned calves (6–12 wk of age) starter containing Zn at various concentrations from Zn-Met, Zn-Lys, or ZnO. No effects were observed on BW, DMI, or immune functions, but Met or Lys forms were absorbed and retained better than oxide. Feeding vitamin E, Zn, and Fe for 42 d before calving had no effect on the incidence of retained fetal membranes, but supplementing Zn or vitamin E before calving reduced days to first estrus (Campbell and Miller, 1998). Beef heifers fed a basal diet containing 15.8 mg of Mn/kg of DM or supplemented with 50 mg of Mn/kg of DM had no difference in blood concentration of Mn and grew similarly; calves born to unsupplemented heifers had lower blood Mn and presented signs of Mn deficiency (Hansen et al., 2006). Weiss et al. (2010) reported that feeding supplemental Fe for 60 d before calving resulted in no differences in measures of Fe status or in milk production, but SCC was reduced with supplemental Fe.

Nennich et al. (2005) compiled data from 4 universities to update prediction equations for nutrient excretion by dairy heifers, including total manure, total DM, N, P, and K. Current accurate predictions are important because farms use these equations to comply with environmental regulations. Manure excretion for heifers was predicted based on BW and DMI using previously published data sets. Lascano et al. (2015) reported that dietary fiber modulated ammonia emissions differently on diets extremely high (~80%) and low (~20%) in forage and that methane concentration was correlated with odor intensity. Methane emissions were not reduced when corn silage with differing NDF and starch concentrations was used or when oil from extruded linseed meal was incorporated into heifer diets (Hammond et al., 2015).

MANAGEMENT

Management systems and alterations in management have long been a topic of heifer research, as reviewed by Clark et al. (1984), who summarized much of the early feeding and management research in a review of the NC-119 cooperative research group that studies dairy heifers.

Feeding

Stair-step compensatory growth strategies have fed above or below recommendations for 2 to 3 mo and then reversed, supporting lower growth and then allowing feed-efficient, compensatory growth when animals were on a higher plane of nutrition (Choi et al., 1997; Park et al., 1998). Feed efficiency with the stair-step program was improved compared with controls (Park et al., 1998) and enhanced by including lasalocid in the diet (Ford and Park, 2001). Heifers on a compensatory program also had greater milk yield in first and second lactation (Ford and Park, 2001).

Some interest was focused on the proposal that more frequent feeding of the growing ruminant would produce faster weight gains. Clark and Keener (1962) suggested that as a result of frequent feeding, specific enzyme pathways for the metabolism of end products

of rumination may be altered because of a change in the concentration ratios of these metabolites. In Missouri, Campbell et al. (1963) used 40 Guernsey heifers to study urea feeding and indicated that rumen adaptation to urea may be related to increased frequency of feeding. Later work (Swanson and Hinton, 1964) showed no advantage of feeding more than once a day.

Montgomery and Baumgardt (1965a, b) used heifers as a model for feed intake studies. Quigley et al. (1986a,b) evaluated factors affecting intake of heifers in intensive management and developed an equation to predict daily DMI of heifers weighing 100 to 400 kg. Intake of DM and digestible energy was affected by BW, BW gain, ration ADF, NDF, bulk density, and ambient temperature. Hoffman et al. (2008) evaluated previously published DMI prediction equations (Quigley et al., 1986b; NRC, 2001) using observed daily pen DMI over 28 mo (n = 9,275) and found that both equations predicted DMI reasonably well in the middle of the growth period, but both were less precise for lighter and heavier heifers. Alternative prediction equations were derived, and greatest precision was obtained with an exponential equation that included adjustment for diet NDF.

Lammers et al. (1999) adopted a concept from the beef industry and fed heifers a high-energy diet but limited DMI to meet their requirements for growth. This began a long series of studies evaluating limit feeding of heifers in Pennsylvania and Wisconsin, with additional work done in Ontario and South Dakota. Limit feeding offers a way to improve feed efficiency and reduce nutrient losses while meeting the nutrient needs of the growing heifer. Hoffman et al. (2007) fed an ad libitum (control) diet or more nutrient-dense diets at 80 or 90% of ad libitum DMI and found no difference in weight gain, structural growth, or 150-d milk production. Limit-fed heifers had higher feed efficiency and lower manure DM excretion. Zanton and Heinrichs (2008b) fed 14.5-mo-old heifers a high-forage diet at 1.25, 1.50, 1.75, or 2.00% BW and concluded that limit feeding increased nutrient utilization efficiency but that DMI below 1.5% of BW reduced efficiency. Green et al. (2013) measured feeding behavior in 1,049 heifers from 5 to 9 mo of age and reported that the more efficient heifers (lower residual feed intake) consumed less feed, ate more slowly, spent less time eating, had longer meals, and consumed more feed during the night and less during the afternoon. Greter et al. (2013) reported that straw with either long or short particle length increased intake when heifers were limit fed an energy-dense diet and that they preferred long straw when given the opportunity. Suarez-Mena et al. (2013) observed that chewing activities were affected by forage concentration and by the addition of distillers grains. Higher forage inclusion increased eating time and rumen digesta weight and volume, whereas feeding distillers grains increased rumination time in limit-fed heifers. Greter et al. (2015) reported that limit-fed heifers were more motivated to access a low-quality feedstuff such as straw, possibly in response to a lack of satiety or need for forage. Miller-Cushon et al. (2015) observed that the method of transitioning heifers to a novel TMR influenced sorting behavior. In their study, heifers fed a novel ration sorted against long particles and thus received a lower NDF diet.

As an alternative to limit feeding an energy-dense diet, Coblentz et al. (2015) demonstrated that low-energy forages (eastern gamagrass haylage, chopped wheat straw, or chopped corn fodder) offered to Holstein heifers for ad libitum intake as diluting agents reduced caloric density and DMI, with heifers sorting the straw diet and, more severely, the chopped corn fodder diet.

Transition to Lactation

When conducting research into the transition period, many studies included both primiparous and multiparous cows. This has increased our understanding of the similarities and differences between heifers entering their first lactation and more mature animals. Drenching with propylene glycol (Grummer et al., 1994) or feeding diets with greater nutrient density (Minor et al., 1998; Vandehaar et al., 1999) can reduce plasma NEFA concentrations and improve energy balance (Rabelo et al., 2003) when DMI declines before calving. Although first-lactation heifers have a lower risk of ketosis and other illnesses associated with the transition period than older cows (Duffield et al., 1998b, 1999), prepartum monensin supplementation decreased BHB in early lactation regardless of parity (Duffield et al., 1998a; Petersson-Wolfe et al., 2007). In a New York study, increases in NEFA and BHB pre- and postpartum had negative effects on milk production and reproductive performance except in postpartum heifers. Heifers with NEFA $\geq$0.57 mEq/L had 488 kg more 305-d mature-equivalent milk and 403 kg more milk when BHB was $\geq$9 mg/dL (Ospina et al., 2010). Heifers also seem to respond differently than cows to anionic salts. Moore et al. (2000) found that compared with +15 or 0, diets with a DCAD balance of −15 mEq/100 g of DM reduced DMI, energy balance, and BW gain and elevated liver triglyceride in heifers when fed for 24 d before calving. Hayirli et al. (2002) evaluated 16 studies from 8 universities and found that heifer DMI before calving was fairly constant from 3 to 1 wk before calving, at around 1.7 to 1.8% of BW, but decreased to 1.23% of BW in the final week. Less overall depression

in DMI may be one factor contributing to a lower risk of health problems in the periparturient period for heifers compared with cows. Heifer DMI was also found to be more sensitive to increased ether extract in the diet compared with cows.

Daniels et al. (2007) demonstrated that milking heifers for 3 wk before their expected due date resulted in less edema, improved milk production and DMI, and lowered SCC after calving. In addition, training heifers to the parlor 2 wk before calving improved milk let down and reduced distress, but heifer temperament influenced the response to milking (Sutherland and Huddart, 2012). Kutzer et al. (2015) showed that training heifers habituated them to the milking routine but did not influence milk production.

Hoof Health

A study of lameness in Dutch herds (Amory et al., 2006) found that farms that fed corn silage to heifers had increased lameness in the milking herd compared with those that did not feed corn silage. In addition, heifers diagnosed with repeated cases of digital dermatitis during the rearing period showed decreased conception rates and less milk in first lactation (Gomez et al., 2015).

Danish researchers developed an oligofructose overload model for studying laminitis (Thoefner et al., 2004). This model successfully induced acute acidosis and enabled investigation of the clinical progression of symptoms and behavior and histological examination of lesions in hoof tissue (Thoefner et al., 2005; Danscher et al., 2009, 2010; Niss et al., 2009). This group also found that lameness, lesions, and abnormal conformation identified 41 d before calving persisted throughout first lactation (Capion et al., 2008). In addition, herd of origin and sample location affected strength of the suspensory tissue supporting the pedal bone within the claw more than induced laminitis (Danscher et al., 2010).

MODELING TO IMPROVE HEIFER MANAGEMENT DECISIONS

Modeling heifer programs has been a more recent endeavor that thus far has considered age at calving, breeding, and growth in the context of making effective management decisions. Mourits and colleagues described (Mourits et al., 1997) and then developed (Mourits et al., 2000) a dynamic programming model that would help evaluate heifer management decisions. With prepubertal ADG set at 900 g/d and maximum postpubertal ADG at 1,100 g, the model was optimized at 20.5 mo AFC and BW at calving of 563 kg. Key factors in sensitivity analysis were growth rate and reproductive performance. Penn State researchers developed a tool to collect and calculate the costs of raising a dairy heifer (Gabler et al., 2000), which averaged approximately $1,100 ($1.50/d) at that time; feed costs were 62% of the total cost. This was followed by a multiple-component analysis of factors affecting the cost of raising heifers, which determined that herd cull rate and AFC had the greatest effect on the total cost (Tozer and Heinrichs, 2001). Another study incorporated nutrient variation in ration formulation for heifers (Tozer, 2000), which reduced diet cost and overfeeding of CP compared with more conventional methods of linear programming or adjusting requirements or rations to include a safety margin. Another published model found economic advantages to delaying the replacement of a culled cow with a heifer when fixed costs and net returns per space in the herd were low and seasonality of cow performance was high (de Vries, 2004).

SUMMARY AND FUTURE DIRECTIONS

The objective of rearing dairy replacement heifers has been described as minimizing required inputs while returning the most profitable outputs (Hoffman and Funk, 1992). To this, modern objectives must add limiting environmental impact and protecting animal welfare. To reduce inputs, a fundamental method is reducing rearing time. Early in the century and up to the 1980s, only modest progress was made in understanding dairy replacement heifer nutrient requirements and management in relation to improving growth rates and reducing AFC. However, in the past 30 yr, there has been consistent progress toward optimizing heifer growth rates and reducing AFC. It will be interesting to see how far and how long this trend continues until it plateaus. Continued research in this area should include information related to length and number of lactations as well as heifer age in the analysis.

In general, nutrition and management changes made in rearing the dairy heifer have been tremendous in the past 100 yr. The minimal amount of published heifer research likely slowed the rate of progress in the first half of the century. However, the second half has enjoyed significant basic nutrition research and applied management studies that have propelled our knowledge of the dairy heifer forward. Increasingly, farms have become more progressive in adopting management practices based on the physiology and nutrient needs of the heifer while refining key economic strategies to be successful. There remains opportunity for research to better understand the energy requirements and feed efficiency of heifers as they continue to mature at

younger ages of life. Along with the continuing growth in farm size and custom heifer raising, the heifer operation will increasingly be managed as its own enterprise on individual farms. By integrating reproductive (sexed semen), genetic (genomic testing and strategic culling), nutrition (limit feeding or other systems) with relation to environmental impacts, and management technologies (animal handling and behavior with an emphasis on animal well-being), the heifer operation will be a critical control point for accelerating whole-farm efficiency and sustainability. Research published in JDS has clearly had and will continue to have a tremendous influence on the progress of dairy heifer programs around the world.

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APPENDIX

Table A1. Major milestones in the study of dairy heifer nutrition and management

Date	Milestone	Reference
1920	First growth standards for dairy heifers are published.	Eckles, 1920
1926	Research shows that vitamin A is essential, B is produced by rumen bacteria, and heifers can synthesize vitamin C.	Bechdel et al., 1926; Jones et al., 1926; Thurston et al., 1926
1939	The first studies using NPN to supplement protein in growing heifer diets are conducted.	Hart et al., 1939
1951	Feeding high energy could improve nitrogen retention of low-protein diets.	Lofgreen et al., 1951
1954	Beltsville heifer growth standards are published.	Matthews and Fohrman, 1954
1967	A series of papers is published describing optimal heifer growth to develop full lactation potential with minimum expense.	Swanson et al., 1967
1969	Schultz identifies a trend for earlier age at breeding and describes changes in the rearing system that would enable producers to reduce age at first calving.	Schultz, 1969
1971	A growth prediction equation is published based on diet energy and animal BW for heifers of all breeds from 4 to 22 mo of age.	Swanson, 1971
1976	Optimal age at first calving for total lifetime production is reported to be 22.5 to 23.5 mo.	Gill and Allaire, 1976
1977	The first study of accelerated growth and early breeding is published by Gardner et al. Heifers on the early breeding treatment calve at 19.7 mo of age.	Gardner et al., 1977
1986	Quigley and colleagues develop the first predictions of DMI for heifers.	Quigley et al., 1986a,b
1987	Growth standards based on population surveys are published for all dairy breeds.	Heinrichs and Hargrove, 1987
1988	Research shows that earlier age at first calving could be accomplished without hurting production and reproduction. From this point forward, average age at first calving declines in the industry.	Gardner et al., 1988; Lin et al., 1988
1992	The equation relating BW to heart girth is updated.	Heinrichs et al., 1992
1999	Limit feeding is introduced as a strategy for improving feed efficiency of dairy heifers.	Lammers et al., 1999
1999	Equations for beef cattle are adapted to develop targeted growth equations for dairy heifers based on mature size.	Fox et al., 1999
2004	The average age at first calving is 25.5 mo for Holsteins in the United States.	Hare et al., 2006
2005	A meta-analysis shows that the optimal ADG before puberty is 799 g/d.	Zanton and Heinrichs, 2005
2009	The optimal nitrogen intake for heifers is 1.67 g/kg of metabolic BW.	Zanton and Heinrichs, 2009

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A 100-Year Review: Calf nutrition and management[1]

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ABSTRACT

The first calf paper, published in the May 1919 issue of the *Journal of Dairy Science* (JDS), described factors affecting birth body weight of different breeds of calves. Other studies were done on nonmilk ingredients, growth charts were developed, and early weaning was followed to conserve milk fed to calves. Calf papers did not report use of statistics to control or record variation or to determine whether treatment means were different. Many experiments were more observational than comparative. Typically fewer than 5 calves, and sometimes 1 or 2 calves, were used per treatment. During the next 20 yr, calf studies increased and included colostrum feeding, milk and milk replacer feeding, minerals and vitamins, and fats and oils. Many concepts fundamental to current knowledge and understanding of digestion, rumen development, and milk replacer formulation were developed during this period. In addition, the concept of using antibiotic growth promoters in dairy calf diets was first evaluated and developed during the 1950s. During the 20-yr period of January 1957 through December 1976, a large number of universities in the United States and 1 in Canada contributed almost 150 papers on a variety of calf-related topics. These topics included genetics, physiology of the calf, review of calf immunity, antibiotic feeding, and milk replacer ingredients. This became the golden era of calf rumen development studies, which also engendered studies of calf starter rations and ingredients. A classic review of management, feeding, and housing studies summarized research related to calf feeding and management systems up to that point with an emphasis on maintaining calf growth and health while reducing labor and feed costs. It was also during this period that metric measurements replaced English units. In the 20-yr period from 1977 to 1996, more than 400 articles on calf nutrition and management were published in JDS. With the growing research interest in calves, a paper outlining standardized procedures for conducting and reporting data from calf experiments was first published. A very active area of calf nutrition research from the late 1970s to the mid 1980s was colostrum quality, feeding, and preservation; more than 60 such research articles were published in the journal during this time. Various nonmilk protein sources were evaluated. Extensive studies were done evaluating trace and major mineral requirements in calves along with some vitamin studies. Throughout the 1970s, 1980s, and 1990s, the primary objective of most calf research was how to wean healthy, adequately grown calves at an early age—generally less than 30 d of age. This program was reviewed in a 1979 publication. Research on calf starter ingredients, nutrient composition, and additives was minimal in the 1980s and 1990s given the importance of starter intake to the success of early weaning, but the role of water intake in starter intake and growth was established. Research on issues with calves continued to increase during the last 20-yr period as evidenced by publication of more than 580 articles in JDS as well as many more in other refereed journals. In addition to papers contributed by several universities in the United States and Canada, the number of papers authored by scientists at universities and institutes in other countries increased dramatically during this period. Factors influencing colostral antibody absorption, heat treatment of colostrum, and efficacy of colostrum supplements and replacers were reported. Most studies in this period related to nutrition. Studies were published supporting greater neonatal growth rates from feeding more milk replacer but with a higher crude protein content than traditional. Protein energy effects on growth and body composition were evaluated in concert with greater growth rates. Milk and nonmilk protein sources in milk replacers along with AA supplementation were evalu-

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ated. Limited studies were done with fat sources and fatty acid supplementation along with trace minerals and fat-soluble vitamins. Waste milk feeding and heat treatment became more prevalent. Studies established starter ingredient palatability and use of forage when fed with pelleted starters. With the advent of automatic milk and milk replacer feeders, factors influencing how and when to wean were established. Research programs established factors affecting calf behavior and welfare. Several databases were evaluated along with various published studies, and established calf growth during the first 2 mo was subsequently reflected in first- and later-lactation milk production of those calves. A new area of calf research that emerged from 1997 on was the effects of maternal environment and nutrition on calf health, growth, and future productivity. From a mechanistic standpoint, the field of epigenetics seems likely to explain many of these phenomena. Some possibilities for future calf nutrition and management were elaborated.

Key words: calf, nutrition, management, growth

INTRODUCTION

When dairy calves are born they have no innate immunity, nor are they functioning ruminants. They face the 2 major challenges of acquiring immunity and initially feeding as a nonruminant while rumen development and function are established. A calf depends on colostrum from its dam to initially acquire immunity through absorption of antibodies before it later begins to synthesize its own antibodies. A calf's liquid diet provides the majority of nutrition until the calf begins to consume enough dry diet, which will contribute to development of its rumen and allow it to be weaned from the liquid diet. Meeting these challenges involves myriad nutrition and management practices that have been researched over the past 100 yr and that have largely been published in the *Journal of Dairy Science* (**JDS**) by the American Dairy Science Association. In 1906, the USDA estimated that there were about 18 million dairy cows in the United States, with an average of about 4 cows on 15 million dairy farms (Jones, 2006). In 2015, there were 9.317 million dairy cows in the United States (USDA, 2016), with an average of about 214 cows on 43,584 commercial dairy herds (Olson, 2016). As the number of cows per herd increased over the past 100+ yr, feeding and management practices have changed (see Appendix Table A1). Newer nutrition and management information generated by scientific studies with calves has increasingly been adopted. This review succinctly addresses key studies in 20-yr segments.

1917 TO 1936

During the first 20 yr of the journal, 16 different research units contributed papers on various topics in calf nutrition, management, and growth. C. H. Eckles of the University of Missouri published the first paper on calves in the May 1919 issue using records of 654 animals describing birth BW of calves of different breeds (Eckles, 1919). Birth BW of first-, second-, ninth-, and tenth-parity dams was less than birth BW of third- through eighth-parity dams. Males were 5 to 8% heavier than females. Length of gestation was not related to birth BW. Using a subset of 29 cows fed low or high levels of nutrients, Eckles reported that nutrition of the dam did not affect calf birth BW. Eckles moved to the University of Minnesota and in the first 20 yr of the journal published 7 other papers on topics of milk substitutes, feeding yeast, calves not requiring sunlight (vitamins were supplemented), vitamin A being required, and vitamin C and B vitamins not being required.

In the July 1919 issue, R. E. Caldwell from Purdue University stated in his introduction that 19% of nutrients consumed by Americans came from milk and milk products (Caldwell, 1919). Additionally, he wrote of the need for food conservation in the years after World War I and thus the need to reduce milk fed to calves. He evaluated liquid and dried blood and boiled clover juice as milk substitutes using both N balance studies and measurements of BW gain in 4 calves. They were not adequate replacements for milk.

In the November 1919 issue, W. B. Nevens from the University of Nebraska evaluated the use of self-feeders for grain mixes fed to 5 calves (Nevens, 1919). Self-feeders were used for other farm animals, and ways to conserve labor were needed because dairy farm laborers were scarce. Self-feeders were satisfactory.

In the first 20 yr of the journal, A. C. McCandlish from Iowa State College was the most prolific author of calf papers, with 9 articles on calves plus many more on other topics. His first article, in May 1922, was based on 369 gestations over 14 yr (McCandlish, 1922a). He reported that calves born in winter were heavier than calves born in summer. Also, hip width growth was greater than height growth when expressed as percentage change. Growth charts to calving were first reported in the journal in May 1922 (McCandlish, 1922b). He described construction of a metabolism crate for calves (McCandlish and Ely, 1922). He reported in the September and November 1923 issues that calves prefer whole corn and whole oats to ground grains but that older, weaned calves preferred ground grains (McCandlish, 1923a,b). He also evaluated self-feeders for dry feeds and several other topics. In the introduction to

his paper in the July 1922 issue, McCandlish (1922b) wrote, "Many calves are improperly fed from birth, while in an even larger number of cases the calves are reared well until weaning then when they are turned loose to rustle for themselves." This situation is still prevalent in the industry today, and published research on the postweaned period until 4 or 5 mo of age is lacking.

In September 1923, L. A. Maynard and L. C. Norris from Cornell University described feeding whole milk for 1 mo followed by a 24% CP grain gruel for 1 mo as a way to conserve milk and wean calves early (Maynard and Norris, 1923). In many of the early publications, authors wrote of feeding calves milk for the first month or so of life followed by feeding liquid skim milk until weaning as a way to conserve milk. Liquid skim milk was referenced as fairly abundant and free or low cost and was suggested as a good feed when combined with grains, if available. However, M. H. Berry at the University of Maryland reported that skim milk added to grains did not enhance growth in 2- to 5-mo-old calves (Berry, 1932). Those calves were fed 0.91 to 1.82 kg of dry feed with hay.

F. W. Atkeson, T. R. Warren, and G. C. Anderson from the Idaho Experiment Station in Moscow reported water intake in 30 heifers from birth to 24 mo of age (Atkeson et al., 1934). They reported the water requirements by age and in relation to DM intake. Water consumption was approximately 7 times DM (kg of water/kg of DM) consumed preweaning (before 6 mo of age) and approximately 4 times DM consumed until 26 mo of age.

In the first 20 yr of the journal, calf papers did not report use of statistics to control or record variation and separate treatment means. Many experiments did not have 2 treatments or controls and were more observational than comparative. Typically fewer than 5, and sometimes 1 or 2, calves were used per treatment. Despite low numbers of calves in experiments, many researchers followed performance of calves beyond 6 mo of age. Experimental methods were often poorly described. Two experimental methods that are odd for us today but that were used in the early years of the journal were photographing calves to describe their look or condition and palpating the abomasum to check for curd formation and disappearance.

Other than those mentioned above, institutions with calf papers in the first 20 yr of the journal included the USDA, Beltsville, Maryland; Pennsylvania State College; University of California, Davis; Michigan Agricultural Experiment Station, Lansing; Massachusetts Agricultural Experiment Station, Amherst; Alaska Agricultural Experiment Station, Kodiak; Panhandle Agricultural Experiment Station, Goodwell, Oklahoma; and Ohio Agricultural Experiment Station, Wooster.

1937 to 1956

The pace of scientific inquiry during the second 20 yr of JDS (1937–1956) was affected by world events and redirection of public funding during the period of World War II. Nonetheless, numerous critical discoveries were made and published in the journal during this period. Many concepts fundamental to current knowledge and understanding of digestion, rumen development, and milk replacer formulation were developed during this period. In addition, the concept of using antibiotic growth promoters in dairy calf diets was first evaluated and developed during the 1950s.

Antibiotic Feeding

An area of tremendous research during these 20 yr was use of antibiotics in liquid diets and solid feeds for calves. At least 24 papers studied effects of various antibiotics, particularly Aureomycin, on growth, health, feed efficiency, and rumen development. In some cases, improvements in growth rates approaching 30% were reported (Loosli and Wallace, 1950; Rusoff et al., 1951). Research in feeding antibiotics to calves as growth promoters followed similar work in monogastrics in the 1950s. Much of the work was conducted by E. E. Bartley and colleagues at Kansas State University. These studies consistently showed improved growth and feed efficiency, reduced incidence of scours, and improved health when Aureomycin was included in calf diets. However, as pointed out by Murley et al. (1952), "Although the incidence of diarrhea was slightly lower among Aureomycin-fed calves, the comparatively infrequent occurrence of this disorder in all groups indicates that diminished scouring is not the sole cause of the improved growth resulting from feeding of the antibiotic. Moreover, whether the improved general appearance and condition of hair coat readily apparent among Aureomycin-fed calves was due to increased feed intake and reduced scouring or to other factors is not clear."

Colostrum Feeding and Milk Digestibilities

The importance of colostrum feeding to the health and growth of the calf was well established by 1937. A series of important papers published in JDS by Parrish and colleagues at Kansas State between 1947 and 1953 reported the composition of colostrum. Some of these results are consistent with contemporary data—for example, first-milking colostrum nutrient content

reported by Parrish et al. (1950). Another important finding from the group was that digestibility of fat and lactose by calves was consistently >95% from 1 to 2 d of age through 14 to 17 d of age. On the other hand, protein digestibility averaged 92% during 1 to 2 d of age and then declined to 83 and 86% during 3 to 4 and 5 to 8 d of age, respectively, and then increased to 93% during 14 to 17 d of age.

Milk and Milk Replacer

Replacing whole milk in the diets of young dairy calves has long been an interest of calf researchers and a consistent theme in JDS. Very early work in the 1920s described replacement of milk at 4 wk of age with a gruel consisting of vegetable ingredients and blood meal as a high-quality protein source. Actual replacement of liquid milk with a liquid was not part of these early research trials. Development of modern products to effectively replace whole milk in the diets of young calves began with work conducted by Wiese et al. (1947) and Williams and Knodt (1949, 1950). Critical discoveries were made in areas including fat homogenization and sources, vitamin and mineral supplementation in replacement diets, and use of high-quality ingredients with high digestibility. Early diets incorporated CN, skim milk, and whey as primary protein sources. Inclusion of vegetable ingredients, including corn distillers solubles, beet pulp, oat flour, wheat red dog, banana meal, raw soybeans, and wood flock, likely impaired growth to some degree in early formulations. Nonmilk proteins included blood meal, blood flour, meat scraps, fish meal, and soy flour. These ingredients undoubtedly also affected digestion, particularly in very young calves. Initial attempts to replace whole milk were generally unsuccessful; however, continued development of technology improved nutrition so that calves fed these formulas performed acceptably. An important goal was to replace dried skim milk, which was the primary base for early successful replacement formulas. Many current papers published in JDS continue to attempt to replace milk proteins in milk replacer formulations.

Fats and Oils

Filled milks are skim milk compounded with any fat or oil other than milk fat to resemble milk or cream. Use of filled milks including different fats and oils to replace whole milk in the diets of young calves was a topic of some interest as attempts were made to replace whole milk. The dramatic effects of choice of fat source in milk replacers were perhaps best described by Gullickson et al. (1942). These researchers added fat sources (3.5%) to skim milk (96.5%) and fed calves for a few days to 6 mo of age. All fats were homogenized into skim milk before feeding. The researchers reported that calves fed corn, cottonseed, or soybean oils grew poorly and were unthrifty, listless, and emaciated. Many of these calves died or were taken off treatment due to poor health. Calves fed butterfat, lard, or tallow performed well, although the amount of carcass fat in calves fed lard or tallow was less than that in calves fed milk fat. Bate et al. (1946) and Barker et al. (1952) commented that homogenization of soybean oil improved growth, health, and hair coats of calves and ameliorated hair loss caused when unhomogenized fat and skim milk were fed. The inclusion of emulsifying agents with vegetable oils and skim milk was reported by Huff et al. (1951) and Kastelic et al. (1950), who also reported that fat globule size played an important role in fat digestion by calves.

Rumen Development

Perhaps the most significant contribution by researchers in the area of calf and heifer research during the period of 1937 to 1956 was the dramatic expansion in our understanding of ruminal development. Many of the fundamental principles regarding maturation of the ruminant gastrointestinal tract were reported during this time frame. Indeed, many of these papers are still referenced in papers published in the journal today.

Much of the work published during this period originated from The Ohio State University by workers including H. R. Conrad, J. W. Hibbs, W. D. Pounden, and T. S. Sutton. These researchers documented changes in populations of ruminal bacteria affected by age of calf and intake of dry feed (Pounden and Hibbs, 1948a,b). The concept of rumen inoculation with cud from mature animals was also tested extensively during the period (Pounden and Hibbs, 1948a; Conrad et al., 1950). Hibbs and colleagues also developed a high roughage system that relied on limited milk feeding and large amounts of high-quality forage and limited concentrates in the diet of young calves.

The critical role of VFA in physical and metabolic development of the rumen was reported by Warner et al. (1956) at Cornell University. This study clearly showed that extended milk intake slowed the rate of rumen development and that feeding forage did not stimulate maturation of the rumen wall to the same extent as calf starter. The authors hypothesized, “Because grain has been as effective as hay in stimulating the early growth of forestomach papillae and tissue, while milk was ineffective, it appears that dry feed per se is essential for the development of the fore-stomachs. Hay, because of its greater bulk, increased the capacity of the fore-stomachs without affecting the amount of tis-

sue growth." They went on to suggest, "It seems logical that chemical products resulting from microbiological breakdown of dry feed might stimulate the development of tissue." Other work during this period documented temporal changes in ruminal concentrations of VFA and in vitro cellulose digestibility (Lengemann and Allen, 1955; McCarthy and Kesler, 1956) as well as changes in circulating concentrations of metabolic end products (McCarthy and Kesler, 1956).

Vitamins and Minerals

No area of calf and heifer research garnered more interest in JDS from 1937 to 1956 than vitamin and mineral nutrition in young calf diets. We counted 77 papers in the journal that referenced some aspect of vitamin or mineral nutrition in calf diets. Of particular interest was the role of vitamin A and carotene in calf health and growth.

Typically, diets almost devoid of vitamin A were fed to calves until signs of deficiency (loss of appetite, scouring with eventual loss of BW, and blindness) were observed. Then, various repletion diets were offered. Clinical outcomes included ophthalmoscopic evaluations and the animals' ability to avoid obstacles in dim light. Sources of carotene and vitamin A in several studies included carrots, alfalfa, and fish oils.

Colostrum was increasingly recognized as a source of important nutrients for the newborn calf in addition to providing passive immunity by absorption of Ig. Limited placental transmission of fat-soluble vitamins was also recognized, and dietary inclusion or injections were used to treat neonatal vitamin deficiencies. Several researchers reported that the vitamin A concentration in colostrum was 10 to 100 times greater than that in milk produced later in lactation (e.g., Stewart and McCallum, 1938) and that diet influenced the concentration of colostral vitamin A (Stewart and McCallum, 1942). Transmission of vitamin A to the calf via colostrum was also reported.

The role of adequate vitamin and mineral nutrition in the health of very young calves was increasingly clear in research conducted from 1937 to 1956. As Lundquist and Phillips (1943) stated, "It has been demonstrated that adequate vitamin A and nicotinic acid are the essential factors in the control of calf scours; the administration of these substances has controlled all types of scours thus far encountered, except that which accompanied septicemia in the newborn."

Mineral nutrition was also an important topic during this period of scientific inquiry. Papers evaluated the nutrition of magnesium, fluoride, manganese, molybdenum, and iron. The recognition of widespread cobalt deficiency in soils in certain regions of the United States and dietary amelioration of Co deficiency during the 1940s prompted research into the tolerance of calves to supplemental dietary Co (Keener et al., 1949).

As mentioned previously, proper statistical methods were not universally applied, though more papers included statistical methods later in the period. Typically, results from individual animals were reported, and studies often included fewer than 12 animals.

1957 to 1976

During the 20-yr period of January 1957 through December 1976, a large number of universities in the United States and 1 in Canada contributed almost 150 papers on a variety of calf-related topics. Published studies investigated factors affecting calf birth weight, including genetics, cross-breeding programs, and breed. Foote et al. (1959) evaluated effects of genetics and maternal environment on gestation length and birth weight. Other studies from Illinois showed that birth weight and production were genetically independent (Legault and Touchberry, 1962). This work was a continuation of earlier breeding and genetics studies focused on calf birth weight and subsequent growth rates. Boyd and Hafs (1965) noted the increased use of beef bulls (Angus) in the dairy industry; 13% of AI-bred dairy cattle were bred to beef bulls to reduce calf size. In addition, they showed that calves sired by Holstein bulls from Holstein cows were larger than those sired by Angus bulls. They also showed that certain Holstein bulls sired smaller calves than others and suggested that AI organizations evaluate and rank bulls based on birth size of their offspring. The effect of animal growth on experimental design was noted in several publications as it was related to the presentation of data in future research publications (Martin et al., 1962). Iowa State researchers concluded that calf nutrition experiments should be designed to prevent confounding breed, sex, and inbreeding effects with ration effects; a portion of these effects can be removed by using birth weight as the independent variable in an analysis of covariance (Martin et al., 1962).

During this 20-yr period, a variety of published papers looked at the physiology of the calf, both using the calf as an example animal species and seeking to understand the calf's uniqueness. Some of these studies included blood and blood constituents, pancreatic physiology, thyroid uptake of iodine, adrenalin metabolism, glucose metabolism, and nonesterified fatty acids. Several physiology papers used radioisotope methods and relatively low numbers of calves. Their findings were important and well done, utilizing techniques that today are not available or often possible. In many cases the low numbers of animals used was well justified due

to the laborious and complex methods used. Their results were valid and remain valid today.

In 1969, Butler published a review of calf immunity that gave an up-to-date and complete summary of early-life immunity, IgG absorption, and related health research. This was a landmark paper covering the state of knowledge to that point and is still a very useful reference (Butler, 1969).

Research studying feeding antibiotics (primarily in milk replacers) to calves continued during this period. Researchers from Iowa State and Michigan State observed increased intake and growth in calves fed antibiotics (Bush et al., 1959). Results indicated that antibiotics had little effect on overall digestion and protein metabolism (Lassiter et al., 1958). The establishment of adult-type organisms and cellulose digestion were shown to be slow to develop and were hindered by Aureomycin and liberal milk feeding (Lengemann and Allen, 1959). Other studies showed that antibiotic feeding promoted animal growth and reduced diarrhea. Tennessee researchers published that the most effective method of feeding calves antibiotics was via milk and that after weaning the effects of antibiotic feeding were minimized (Swanson and Harris, 1958).

A large number of studies focused on rumen development during this time. Work from Warner's laboratory at Cornell hypothesized that rumen papillae growth resulted from metabolism of specific compounds by the rumen wall or their effect on blood flow in the rumen (Sander et al., 1959). Others studied rumen development using various VFA salts and manipulating rumen pH. Tennessee workers studying hay feeding and diet type indicated that more than half of the calves in a particular study were ruminating effectively at 2 wk of age (Swanson and Harris, 1958). The introduction of plastic sponges as a nonnutritive bulk source into the reticulorumen promoted increased rumen capacity and muscular development (Tamate et al., 1962). Feeding hay and starter in addition to milk resulted in forestomach development, which included papillary and muscular growth of the reticulorumen and pigment deposition in the rumen mucosa (Tamate et al., 1962). Conrad et al. (1958b) studied rumen development with the use of isotope marker techniques. Purdue researchers studied rumen development and noted that some feeding systems resulted in hyperkeratosis of the rumen, erosion of the rumen wall, and rumen plaques (Noller et al., 1962).

Oklahoma researchers (Gilliland et al., 1962) fed VFA salts to promote rumen development. Hodson et al. (1965) published classical work looking at butyrate absorption by the rumen wall mucosa. They used very complex physiological procedures such as rumen-cannulated calves with portal vein catheters and exteriorized carotid or femoral arterial catheters to study ketone formation during passage of butyrate through the rumen wall using C^{14} butyrate. These Iowa State researchers noted the conversion of butyrate to BHB in the rumen mucosa, which resulted in little butyrate in the blood after butyrate infusion in the rumen. Kansas researchers conducted corroborating work related to rumen VFA absorption studies using C^{14}. These data indicated that the very young calf can absorb and metabolize VFA whether the acids are produced in the forestomach or large intestine (Liang et al., 1967). Some researchers noted parallelisms between changes in ammonia-N in the rumen and blood urea and ammonia-N. Others looked at inoculation of a calf rumen with rumen contents of older animals, which resulted in establishing active populations of ciliate protozoa in early-weaned calves.

Milk replacer ingredients also were studied during this period to improve the quality and diversity of ingredients in replacers. Addition of lecithin improved digestibility of fat sources in milk replacers (Hopkins et al., 1959). Alternative protein sources, including soy and fish (Bouchard et al., 1973), as well as various fat sources and the level of fat feeding (Huber et al., 1968) were investigated. Other work looked at adding dried skim milk to calf starter. Swanson et al. (1969) concluded that the more efficient use of diets containing liquid milk was explained by the fact that liquid milk bypassing the rumen results in more readily metabolizable energy than does dry milk in the concentrate, which must go through the rumen. Various milk feeding systems were researched during this period. Ad libitum versus limited milk replacer feeding was studied in beef and dairy steers for veal production. Once-daily milk feeding compared with twice-daily feeding showed no increase in digestive disorders and did not reduce the growth and vigor of calves (Willett et al., 1969). Other milk feeding studies looked at the level of solids in milk replacer. We still see papers today that corroborate many of these findings.

Workers at several universities, including Kansas, Pennsylvania, and Minnesota, studied calf starter. Protein requirements in starters were discussed in light of the then-current NRC recommendations for protein and energy allowances for young calves (Morrill and Melton, 1973). They reported no differences between once- and twice-daily feeding of milk and 13 or 16.2% CP in starter with calves growing at 750 to 850 g/d after 6 wk of age. They studied AA level in the blood as an outcome variable. One later study found that total free AA in the blood were affected by protein-to-energy ratios in the diet and that these also affected rumen development (Traub and Kesler, 1972). Results of many studies showed that the optimum protein-to-energy

ratio in the young calf would be 46:1 or slightly less and helped establish protein requirements of the young calf (Brown and Lassiter, 1962; Bryant et al., 1967). Protein levels and hay versus grain feeding were studied by researchers in Kansas (Bartley, 1973). Protein levels of 12 and 16% starter were shown to be adequate with whole-milk feeding by researchers from Iowa State. In addition, several studies concluded that 16% CP in starters under various feeding systems resulted in adequate growth over other levels studied.

Various feed ingredients were studied for calf starters during these years, including dry skim milk, rapeseed, cottonseed meal, sunflower, meat solubles, and soy products. Grain processing effects and fiber levels also were investigated. In addition to growth measurements, feed preference studies were often part of these starter comparisons. Also, some vitamin and mineral studies were done during this period to help refine requirements of the young calf.

During this 20-yr period a continuation of papers by J. W. Hibbs and H. R. Conrad related to the Ohio high-roughage system for calf feeding (Conrad et al., 1958a; Hibbs et al., 1961). These studies looked at a variety of forage and fiber feeding systems for calves. They focused on feeding silages, pasture, hay, alfalfa pellets, and high-fiber by-products for young calves through 3 to 4 mo of age. Their studies were done on both Holstein and Jersey calves and often included digestibility of fiber (cellulose) and N, with implications to rumen physiology and development and efficiency of feed utilization. Other researchers studied calves on pasture from 6 to 20 wk of age (Kesler, 1962). Many of these studies found that feed intake, rates of gain, and feed efficiency were superior for calves fed higher fiber feeds and high-quality forages as long as rumen development was adequately addressed. Feed efficiency as TDN per kilogram of BW gain was the endpoint being studied in several studies comparing milk feeding levels or calf starters (Macleod et al., 1970).

Finally, a classic review of management, feeding, and housing studies was published (Appleman and Owen, 1975). This review summarized research related to calf feeding and management systems up to that point with an emphasis on maintaining calf growth and health while reducing labor and feed costs. Annual calf death losses were in the range of 6 to 15% during that period, as taken from various studies. Appleman and Owen (1975) discussed new work on nutrient levels in the diet and feeding systems, including feeding acidified milk with weaning at 12 to 28 d. Another management paper that was referenced for many years was a survey of calf mortality and factors affecting it on a large number of farms in New York (Hartman et al., 1974). An interesting side note is that papers published during this period reported results in English units rather than metric units.

1977 to 1996

In the 20-yr period from 1977 to 1996, more than 400 articles on calf nutrition and management were published in JDS. With the growing research interest in calves, the North Central Regional Research Committee on Improving Large Dairy Herd Management published a paper outlining the standardized procedures for conducting and reporting data from calf experiments (Larson et al., 1977). Many of the guidelines are still in use today, aiding the interpretation of nutrition and management data across research studies.

Colostrum

An active area of calf nutrition research from the late 1970s to mid 1980s was colostrum quality, feeding, and preservation; more than 60 such research articles were published in the journal during this time. A series of articles in 1979 by Stott and colleagues at the University of Arizona on timing of first colostrum feeding, rate and amount of Ig absorption, and method of feeding colostrum described the basic principles and practices essential for successful passive immunity in calves (Stott et al., 1979a,b,c,d). Bush and Staley (1980) presented a detailed description of Ig absorption from the intestinal tract, cessation of absorption, and factors affecting absorption in a symposium paper. The amount of Ig in the serum of the calf at 24 h of age is in positive linear relationship with the amount and concentration of colostral Ig fed. Immunoglobulin concentration in the first feeding of colostrum is more important than the volume of colostrum fed. Fleenor and Stott (1980) developed a simple and rapid method based on specific gravity for determining Ig concentration in colostrum.

From late 1970 to early 1980, more than 20 research articles on preservation and feeding of excess colostrum or unsalable milk appeared in the journal. The review on preservation and feeding of surplus colostrum by Foley and Otterby (1978) and the 25-yr summary of calf nutrition and management (Otterby and Linn, 1981) summarized fresh and preserved colostrum feeding. Fermented colostrum, acid-preserved fermented colostrum, and unsaleable milk all supported good growth and health in calves when fed during the preweaning period. The review on feeding fresh and fermented mastitic milk to calves by Kesler (1981) found no differences in calf growth, performance, or health disorders with feeding mastitic liquid feeds compared with nonmastitic liquid feeds.

A National Dairy Heifer Evaluation Project surveyed 1,811 farms in 1991 and 1992 (Heinrichs et al., 1995). The percentage of farms feeding various liquid feeds to calves after the first 24 h of age was 80% for fresh or soured colostrum, 70% for whole milk, 65% for milk replacer, and about 55% each for mastitic and antibiotic milk. Larger farms fed more mastitic and antibiotic milk than smaller farms that fed more milk replacer.

Milk Replacer Protein

Replacing a portion of milk protein in milk replacers with vegetable and fish protein was shown in several studies during the 1980s to result in poor calf performance and increase the incidence of diarrhea in calves, particularly in calves less than 3 wk of age (Campos et al., 1982). The lack of clot formation in the abomasum—and thus a fast passage rate of proteins and other nutrients from the abomasum into the intestine when soy, fish, and other alternative proteins were fed—was thought to be the cause of diarrhea and reduced performance. The prevailing premise at this time was that the lack of clot formation indicated a low-quality milk replacer (Gaudreau and Brisson, 1980). Although the nonclotting premise may have been true for some protein sources, it was not for whey proteins, which were becoming the major protein source in milk replacers in the 1980s (Petit et al., 1989).

Seegraber and Morrill (1982) found that antigenicity and other factors in soy flour and soy concentrate caused morphological changes to the intestinal villi, impairing absorption of protein and other nutrients and leading to increased diarrhea. The changes in intestinal villi of calves fed soy protein were similar to those associated with viral infections in calves, celiac disease in man, and transmissible gastroenteritis in pigs. Silva et al. (1986) also found low weight gain, diarrhea, and villus atrophy in calves when soy protein concentrate accounted for 66% of the total protein in milk replacer. Despite considerable research in the 1980s, no alternative protein source emerged as an equivalent to milk protein in supporting good growth and health in calves, especially calves less than 3 wk of age.

In the 1990s, plasma protein started to be evaluated as a partial substitute for milk protein in milk replacer. Advantages of plasma protein over other protein sources were that it provided (1) highly digestible protein, (2) a desirable balance of AA, and (3) Ig localized in the intestine to protect against certain bacteria and viruses. Morrill et al. (1995) reported that either bovine or porcine plasma was an acceptable replacement for 25% of the milk protein in milk replacer for calves weaned at 42 d of age. Calves fed plasma containing milk replacer gained equal to all calves fed milk protein replacer the first 3 wk of feeding but significantly better than all calves fed milk protein wk 4 through 6. Quigley and Bernard (1996) also replaced 25% of the protein in milk replacer with bovine plasma and achieved growth and performance equal to that of calves fed an all milk protein milk replacer through 56 d of age.

AA

Supplementation of milk replacer or milk diets with AA received very little attention during the period 1977 to 1996. Foldager et al. (1977) reported that Met intakes of calves fed milk replacer should be 2.75 to 2.95 g/100 g of CP ingested. Tzeng and Davis (1980) reported that requirements for DL-Met ranged from 0.17 to 0.23 g/d per kilogram of BW and requirements for Lys ranged from 0.27 to 0.31 g/d per kilogram of BW in neonatal calves.

Fat

Very little research on fat sources in milk replacers was conducted during the 1980s and early 1990s. Jenkins et al. (1986) evaluated several lipid sources and forms of fat for addition to milk replacer and concluded that tallow and canola oil resulted in the best DMI and performance of calves. Oils high in linoleic acid (e.g., corn) caused diarrhea, whereas oils high in oleic acid (e.g., canola) did not. Fats and oils with high amounts of free fatty acids reduced milk replacer intake, fatty acid digestion in the calf, and overall calf performance.

Calves housed in outdoor hutches supplemented with extra fat in cold weather increased BW gains during the first month of life (Jaster et al., 1992). Scibilia et al. (1987) estimated that the maintenance energy requirement increased 32% as the environmental temperature decreased from 10°C to −4°C.

Minerals

Two locations conducted most of the calf mineral research in the 1980s and early 1990s. Research at the Animal Research Center, Agriculture Canada in Ottawa led by Jenkins, Hidiroglou, and Kramer focused on trace minerals in the preweaned calf, whereas research at the University of Georgia led by Miller and Neathery examined both macro- and trace-mineral toxicity in postweaned calves. A synopsis of the research follows.

Trace Minerals in Milk Replacer

Copper toxicity in calves occurred at 1,000 mg/kg in milk replacer (Jenkins and Hidiroglou, 1989). The safe

upper level of Cu in milk replacer for feeding to calves was 50 mg/kg (Jenkins and Hidiroglou, 1989). Jenkins and Hidiroglou (1990) suggested a 10-mg/kg I level in milk replacer because feeding 50 mg/kg of I or more significantly increased I content of nonthyroid tissues. Iron concentrations of 5,000 mg/kg in milk replacer reduced feed intake and weight gain of preruminant calves (Jenkins and Hidiroglou, 1987). Consumption of milk replacer containing 1,000 mg/kg of Mn reduced feed intake and weight gain of calves, whereas 5,000 mg/kg of Mn in milk replacer was toxic and resulted in calf deaths (Jenkins and Hidiroglou, 1991). Calves readily absorbed and metabolized Se from sodium selenate or selenite and tolerated up to 5 mg/kg of Se in milk replacer (Jenkins and Hidiroglou, 1986). Above 500 mg/kg of Zn in milk replacer, calves reduced feed intake and weight gain (Jenkins and Hidiroglou, 1991). Forty milligrams per kilogram of Zn in milk replacer was adequate; increased concentrations provided no additional benefit in calf performance.

Macrominerals in Diets of Weaned Calves

Adding 5% fat to a calf starter did not affect Ca absorption (Fielding et al., 1985). The primary factor affecting Ca absorption is Ca content of the feed, with increasing amounts decreasing apparent absorption. Up to 1.31% Ca can be included in starter with no reduction in feed intake or performance (Alfaro et al., 1988). Calves adapted quickly to a diet deficient in Cl (0.038%) by reducing Cl in plasma, saliva, and urine (Burkhalter et al., 1980). Magnesium toxicity was exhibited (reduced feed intake, diarrhea) in calves fed diets containing more than 1% Mg, but no effects on performance were found with 0.25 or 0.7% Mg diets (Quillian et al., 1980). Miller et al. (1987a) suggested that P content of diets fed to 2- to 4-mo-old calves not be less than 0.32% of the diet DM. In palatability studies on K supplements, 90-d-old calves found $KHCO_3$ and KCH_3CO_2 more palatable than KCl and found K_2CO_3 the least palatable (Neathery et al., 1980).

Trace Minerals in Diets of Weaned Calves

Aluminum at 0.2% in calf starter significantly reduced feed intake (17%) and weight gain (47%) of weaned calves (Crowe et al., 1990). Miller et al. (1991) reported that calves fed starters containing more than 500 mg/kg of supplemental Fe from ferrous carbonate tended to have lower intakes than calves fed starter with no supplemental Fe, but no adverse health effects were observed in calves fed the higher concentrations of Fe. Manganese absorption from a purified diet or corn–skim milk diet was the same in calves, but absorption of Mn from the same diets fed to rats was much greater from the corn–skim milk diet than from the purified diet (King et al., 1980). Fermentation of feeds in the rumen was thought to have produced products that aided Mn absorption postruminally. Intestinal absorption of Mn from diets is very low, and Miller et al. (1987b) showed that absorption is what limits Mn in body tissues and not metabolism. Lead toxicity occurred in 74-d-old calves fed diets containing 500, 1,500, or 4,500 mg/kg of Pb (Logner et al., 1984). Death loss was 25 and 100% within 10 d after feeding diets containing 1,500 and 4,500 mg/kg of Pb, respectively.

Vitamins

The importance of vitamin E in calf health and performance received the most attention from 1980 to 1996. Kansas State researchers provided recommendations of 125 to 250 IU of vitamin E/calf per day for good health and performance from birth to 6 mo of age (Reddy et al., 1987). Vitamins A, C, D, and K also were evaluated during this period, but to a lesser extent than vitamin E.

Calf Starter

Throughout the 1970s, 1980s, and 1990s, the primary objective of most calf research was to examine how to wean healthy, adequately grown calves at an early age (generally <30 d of age). Kertz et al. (1979) summarized 30 experiments published in JDS during the 1970s and 3 yr of calf research data from a commercial facility to show that calves could achieve adequate growth and development for weaning at 28 d of age. In studies where calf growth was considered adequate or better (>0.32 kg/d) for early weaning, the quality and amount of calf starter consumed was the major factor contributing to the success of early weaning. Jenny and O'Dell (1981) fed calves milk replacer at 8 or 10% of BW with DM concentrations of 10, 15, and 20% from birth to 6 wk of age to show the effect of the liquid feeding program on starter consumption. Starter was withheld from calves the first 3 wk but offered ad libitum to all calves from 4 to 12 wk. Average daily gain of calves fed milk replacer at 8 or 10% of BW was the same (0.67 and 0.68 kg/d) from 4 to 12 wk of age. The DM concentration of milk replacer had no effect on gain during wk 4 to 6; however, during wk 7 to 8, immediately postweaning, ADG were 0.56, 0.60, and 0.38 kg for calves fed milk replacer at 10, 15, or 20% DM, respectively. Intake of starter during wk 4 to 6 decreased as milk replacer DM increased. By 12 wk of age with unrestricted access to starter, ADG of calves fed 10, 15, or 20% of DM milk replacer were similar.

Research on calf starter ingredients, nutrient composition, and additives was minimal in the 1980s and 1990s given the importance of starter intake to the success of early weaning. Klein et al. (1987) showed that calves could be weaned at 17 d of age when fed a milk ingredient pelleted prestarter (22% CP, 12% fat, and 0% fiber) until 4 wk of age. A starter containing 10 or 20% alfalfa also was fed the first 4 wk and then as the only starter from wk 5 to 10. Calf weight gain at 10 wk of age was slightly less for calves weaned at 17 d compared with 28 d, but at 6 mo of age BW gain of calves was not different. Feeding a highly digestible prestarter supported early rumen development and demonstrated that VFA content in the rumen is more likely responsible for rumen development than physical fiber.

Calf Starter Protein and Energy

Luchini et al. (1991) reported no advantage in calf performance to feeding a 25% versus 20% CP starter to calves weaned at 26 or 42 d of age. Akayezu et al. (1994) fed calf starters with 15.0, 16.8, 19.6, or 22.4% CP to calves from 4 to 56 d of age. Daily gain of calves increased linearly with increasing CP in the starter; however, after weaning, calves fed the 19.6% CP starter had the best growth performance.

Soybeans add both protein and energy to a calf starter; however, processing is required to remove some of the antinutritional factors. Raw soybeans and soybeans roasted at 138, 171, or 191°C were included in calf starters for feeding from birth through 8 wk of age (Abdelgadir et al., 1984). Calves fed soybeans roasted at 191°C consumed the most starter and gained the most during the 8 wk, but only slightly better than calves fed starter with soybeans roasted at 171°C. Calves fed starter containing raw soybeans had the lowest feed intake, lowest weight gain, and highest fecal score. Reddy et al. (1993) found that roasting soybeans at 146°C followed by 30 min of steeping resulted in the best calf performance. Luchini et al. (1993) reported that adding 5% fat to a starter had no effect on intake or calf performance through 42 d of age. Although fat in milk replacer and starter can be a good source of energy for calves, feeding a high-fat milk replacer with a high-fat calf starter depressed feed intake and reduce calf performance (Kuehn et al., 1994). High-fat milk replacer (21.6% DM) depressed DMI before and after weaning, and high-fat calf starter (7.3% DM) depressed DMI after weaning. Before weaning, calves gained the most BW when fed the low-fat (15.6% DM) milk replacer; after weaning, calves fed the low-fat (3.7% DM) calf starter gained the most BW.

Water

Providing free access to water early in life encourages calf dry feed intake. In a study where free-choice water was withheld the first 4 wk of life, calves fed milk replacer had a 38% lower BW gain and 31% reduction in calf starter intake compared with calves fed milk replacer with ad libitum water (Kertz et al., 1984). Very few studies consider and report free water intake as a factor in response to dietary treatments.

1997 to 2016

Research on issues with calves continued to increase during this 20-yr period, as evidenced by publication of more than 580 articles in JDS as well as many more in other refereed journals. In addition to papers contributed by several universities in the United States and Canada, the number of papers authored by scientists at universities and institutes in other countries increased dramatically during the period January 1997 through December 2016.

Increased interest in calves likely resulted in part from changes in the industry, with larger farms (USDA, 2002) and more custom calf-raising enterprises (Walker et al., 2012). Publication of the seventh revised version of *Nutrient Requirements of Dairy Cattle* (NRC, 2001), which contained an entire chapter on calf nutrition along with a mathematical model for calculating calf requirements, helped to emphasize variable nutrient needs for growth and cold stress. Kertz and Chester-Jones (2004) published an updated review on guidelines for measuring and reporting necessary background information by researchers, which improved the quality of many research studies; however, this remains an area for improvement.

Colostrum

During the period 1997 to 2016, research continued to define factors that affect attainment of adequate passive immunity, emphasizing the mass of colostral IgG consumed early after birth as affected by timing of feeding, amount fed, and colostrum quality (Morin et al., 1997). A major research theme involved transmission of disease organisms from dam to calf via colostrum or waste milk, related to biosecurity protocols as well as calf health. Stewart et al. (2005) demonstrated the extent of potential bacterial contamination of colostrum on farms due to poor hygiene during milking, failure to quickly cool or preserve colostrum for storage, or improper cleaning and sanitation of feeding devices.

Although on-farm pasteurization of waste milk was becoming more widely used, pasteurization of colostrum is more problematic because of its high protein content, which can increase viscosity and coagulation if overheated (Godden et al., 2003). Godden and colleagues at the University of Minnesota defined conditions (60 min at 60°C) for successful batch pasteurization of colostrum (McMartin et al., 2006) that decreased colostral bacteria counts with minimal damage to Ig, vitamins A and E, and β-carotene (Johnson et al., 2007; Donahue et al., 2012). Heat-treated colostrum resulted in increased IgG in calf serum at 24 h after birth and greater apparent absorption efficiency of IgG (Johnson et al., 2007); these findings were confirmed by Penn State University researchers (Elizondo-Salazar and Heinrichs, 2009). Moreover, feeding heat-treated colostrum decreased morbidity (particularly diarrhea) in young calves (Godden et al., 2012; Gelsinger et al., 2015). Malmuthuge et al. (2015) reported that calves fed heat-treated colostrum had greater colonization of bifidobacteria and less *Escherichia coli* than those fed unheated colostrum, suggesting a possible reason for improved gut health.

Another area of active research was development and evaluation of colostrum supplements and colostrum replacers, with Ig derived from either bovine blood fractions (plasma or serum) or dried colostrum. Although earlier experiments with colostrum supplements from dried colostrum showed that IgG absorption was not consistently improved and that apparent absorption efficiency decreased (Hopkins and Quigley, 1997; Morin et al., 1997; Arthington et al., 2000a), subsequent colostrum replacer products derived from dried colostrum resulted in acceptable passive transfer when fed at higher doses (Godden et al., 2009). Products derived from serum or plasma proteins were effective as supplements (Arthington et al., 2000b). Colostrum replacer products derived from spray-dried and concentrated bovine serum were shown to be effective as sources of passive immunity (Quigley et al., 2001; Swan et al., 2007; Priestley et al., 2013).

In addition to the importance of colostrum for activation of the immune system, colostrum contains many hormones and growth factors of the endocrine system, which interact with cells in the gut to program and activate the digestive system and muscle (Ontsouka et al., 2016). Research groups led by J. W. Blum and R. M. Bruckmaier (University of Berne, Switzerland) and H. M. Hammon (Institute of Nutritional Physiology "Oskar Kellner," Leibniz Institute for Farm Animal Biology, Germany) were major contributors to this effort.

Nutrition

The majority of articles published during this period dealt in some way with nutrition. The most prolific research group during this time was that of T. M. Hill and colleagues at Akey (subsequently Provimi and Cargill), who researched a wide variety of topics related to milk replacer composition and feeding rates, starter composition, provision of forage, and other management topics.

Increased Rates of Milk or Milk Replacer Feeding

With the objective of better defining energy requirements for growth of Holstein calves, Diaz et al. (2001) fed calves a milk replacer containing 30% CP (to ensure that protein would not limit growth) and 20% fat in amounts calculated to provide ADG of 500, 900, or 1,400 g/d. Body composition was measured when calves reached 65, 85, and 105 kg of BW. Calves were fed no starter. Actual daily gains from birth to harvest were 560, 973, and 1,100 g/d; protein deposition (140, 204, and 247 g/d) increased linearly, whereas fat deposition (44, 154, and 161 g/d) increased less from the middle to highest feeding rate. Jasper and Weary (2002) compared calves fed milk at 10% of BW with those allowed ad libitum access to milk from a teat along with free-choice access to starter and hay. Ad libitum consumption of milk increased to more than 9 kg/d by d 4 and ranged between 9 and 10 kg/d through d 35. However, consumption of starter and hay was less than that in the restricted-fed calves. Ad libitum-fed calves gained BW at more than twice the rate of controls before weaning (0.78 vs. 0.36 kg/d) and were 10.5 kg heavier at d 35; however, as observed in earlier studies, growth rates were lower during the period of weaning and postweaning.

These reports stimulated several research groups to compare so-called intensified or accelerated milk feeding programs with conventional limit-fed programs. Findings were generally consistent among studies that preweaning growth rates were increased by greater milk feeding rates, but because starter intake was suppressed, the immediate postweaning growth rates were lower than with conventional programs. Research by T. M. Hill and colleagues at the Akey research facility (now Cargill) and A. Bach at IRTA in Barcelona, Spain, evaluated ad libitum or high milk feeding rates versus intermediate or moderate programs. Feeding moderate amounts of milk replacer (~6 L/d or 0.68–0.75 kg/d of solids) resulted in greater growth rates than controls but larger starter intake than calves fed 8 L/d or ≥1

kg/d of milk solids (Hill et al., 2010; Bach et al., 2013). Liang et al. (2016) demonstrated that Jersey calves in the first week of life were well able to digest and utilize protein from large amounts of milk.

Protein Energy Effects on Growth and Body Composition

Following the Cornell study (Diaz et al., 2001), other researchers studied how amounts of protein relative to fat or carbohydrate affected body composition and composition of gain. Research by Tikofsky et al. (2001) demonstrated that greater fat intake from milk replacer was partitioned mainly to body fat deposition. Bascom et al. (2007) reported similar findings in Jersey calves. Bartlett et al. (2006) used 2 feeding levels (1.25 or 1.75% of BW as DM) and 4 CP concentrations (14, 18, 22, or 26%) in milk replacer with calves slaughtered after 5 wk. Greater feeding rate increased growth rate, and increasing CP percentage increased growth rate and lean tissue deposition. Labussiere et al. (2008) reported similar results with veal calves. More recently, Silva et al. (2015) determined the effects of amounts of milk and starter on body composition of Holstein × Gyr calves.

Protein Sources in Milk Replacers

A major change in the milk replacer industry during this period was the near-total replacement of skim milk or CN in "all milk protein" milk replacers with whey proteins from dried whey, whey protein concentrate, delactosed whey, or whey protein isolates (Davis and Drackley, 1998). Penn State University researchers (Terosky et al., 1997; Lammers et al., 1998) found that whey proteins were utilized by the calf at least as well as skim milk proteins.

Researchers continued to examine nonmilk proteins for their ability to replace skim milk or whey proteins in milk replacers. Researchers at L'Institut National de la Recherche Agronomique in France (Montagne et al., 2000) found that intestinal endogenous secretions, including mucin, were greater when calves were fed milk replacers containing soy protein concentrate or partially hydrolyzed soy protein isolate compared with when calves were fed milk replacers containing skim milk proteins. Guilloteau et al. (2011) determined that more pancreatic juice protein or trypsin were needed to achieve maximal nutrient digestibility when calves were fed milk replacer containing soy protein concentrate compared with a skim milk protein control. Drackley et al. (2006) found that supplemental L-glutamine (1% of diet) did not improve growth or health in calves fed a milk replacer with 60% of the milk protein replaced by soy protein concentrate.

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Kanjanapruthipong (1998) found that supplementing a milk replacer containing 43% of the protein as soy with Lys, Met, and Thr significantly improved ADG and N retention compared with the unsupplemented diet. In a series of experiments, Hill et al. (2008) determined that the Lys requirement for 48-kg calves gaining 0.46 kg/d was 17 g/d, with Met:Lys of 0.31 and Thr:Lys of 0.60. Other studies confirmed the importance of supplementing at least Lys and Met when using protein sources other than milk proteins.

Fat and Fatty Acid Profiles

Little research was conducted on different fat sources during this period. However, several groups reported that providing more essential fatty acids, particularly the n-3 fatty acids, improved health and growth. Mills et al. (2010) found that calves fed greater amounts of medium-chain fatty acids from coconut oil deposited more dietary energy and fat than controls during cold stress. A combination of medium-chain and essential fatty acids plus sodium butyrate resulted in improved growth and measures of health (Hill et al., 2011; Esselburn et al., 2013).

An interesting study in veal calves showed that a high dietary concentration of calcium (1.24%) decreased apparent digestibility of fat by 5.6 percentage units and increased fecal bile acid excretion by 90% (Xu et al., 1998). The authors' hypothesis was that high Ca would form insoluble complexes with Mg and P and as a result would bind bile acids and inhibit their reabsorption. As a result, a limitation of bile acids available to form micelles for fat absorption would decrease fat digestibility.

Minerals

Kegley et al. (1997) fed calves milk replacers with no supplemental Cr or 0.4 mg/kg of Cr from CrCL3 or Cr-nicotinic acid complex. Supplementation of Cr had minimal effect on growth or intake but did increase glucose responses to insulin challenge. Depew et al. (1998) compared control calves with those fed milk replacer and starter supplemented with 1 mg/kg of Cr from Cr tripicolinate. Only minor effects on growth and metabolism were noted, and only during the first few weeks of life.

Kincaid et al. (1997) supplemented a basal starter diet (60 mg/kg of Zn) with 150 or 300 mg/kg of Zn in

the form of Zn-Met and Zn-Lys or 300 mg/kg of ZnO to calves from 6 to 12 wk of age. Feed intakes and BW gains were not affected. Absorption and retention of Zn were greater for the Zn-Met and Zn-Lys groups than for the ZnO group, but the extra Zn did not affect immune function. Wright and Spears (2004) supplemented a basal starter diet with 20 mg/kg of Zn from $ZnSO_4$ or Zn proteinate. Gain and intake were not affected by diets. When calves were fed 500 mg/kg of Zn from the same sources, tissue Zn was increased more by Zn proteinate than by $ZnSO_4$. Zinc status in veal calves was impaired in calves fed a combination of skim milk plus soybean protein despite higher dietary Zn, likely due to the presence of phytate in the soy protein source (Xu et al., 1997).

Gengelbach and Spears (1998) fed calves a Cu-deficient milk replacer for 8 wk, and then after weaning calves were fed a control (nonsupplemented) diet, 10 mg/kg of Cu from $CuSO_4$, 5 mg/kg of Mo from Na_2MoO_4, or 5 mg/kg of Cu from $CuSO_4$ and 5 mg/kg of Mo. Calves fed either the Cu- or Mo-only diets gained BW more efficiently than those fed the control or Cu+Mo diets, but calves fed the Mo diet had more serious signs of Cu deficiency.

Vitamins

Although little research was conducted on water-soluble vitamins during this period, several research groups reported on fat-soluble vitamins. Franklin et al. (1998) showed that ratios of vitamins A and E should be considered and that high doses of vitamin A to calves while scouring could be detrimental. Nonnecke et al. (1999) reported a negative correlation between plasma retinol and vitamin E concentrations, suggesting that vitamin A influences the absorption and distribution of vitamin E. Hammell et al. (2000) suggested that 1,700 IU of vitamin A/d was adequate for calves, whereas Swanson et al. (2000) reported evidence that milk replacer should contain 11,000 IU of vitamin A/kg.

Feeding Waste Milk

Butler et al. (2000) and Stabel (2001) showed that pasteurization of waste milk was effective in destroying *Mycoplasma* sp. and *Mycobacterium paratuberculosis*, respectively. Ultraviolet light treatment is being used by some producers to reduce (but not eliminate) bacterial counts in nonsaleable milk fed to calves. In a field study conducted by Gelsinger et al. (2014) on 9 Pennsylvania farms, UV treatment of raw milk intended for feeding to calves resulted in large reductions of bacterial counts. Langford et al. (2003) found that increasing amounts of penicillin residues in waste milk increased antibiotic resistance of gut bacteria in calves, supporting earlier recommendations not to feed milk from antibiotic-treated cows.

Starter Composition and Management

Research continued during the 20-yr period beginning in 1997 to maximize starter intake and optimize calf performance from starters. Lesmeister and Heinrichs (2004) examined different degrees of corn grain processing, including whole, dry rolled, roaster rolled, and steam flaked. Calves fed starter containing roaster-rolled corn grain had greater structural growth and ruminal butyrate concentration than calves in other treatment groups. The physical form of oats (whole vs. ground) did not affect digestive development in calves (Suarez-Mena et al., 2015). When iso-starch (25% of DM) diets were fed to calves, consumption of starter DM and BW gain were greater when starter contained corn than when starter contained barley, oats, or wheat (Khan et al., 2007).

A series of experiments conducted at the University of Guelph and Institució Catalana de Recerca i Estudis Avançats (ICREA) in Spain demonstrated that calves have preferences (presumably based on palatability) for energy- and protein-providing ingredients. For example, Miller-Cushon et al. (2014) found that wheat meal was the highest ranked energy ingredient, whereas rice meal and corn gluten feed were lowest ranked; among protein ingredients, soybean meal was highest ranked and corn gluten meal was lowest ranked. Of interest was that these preferences were still identifiable when ingredients were combined into mixtures.

Some other novel findings were presented during this time. Kim et al. (2012) showed that fermented soybean meal was better utilized than regular soybean meal in just-weaned calves. Beiranvand et al. (2016) found that adding water to dry starter to either 75% or 50% final DM improved starter intake and increased ADG during hot summer weather.

Due to changes in the European Union regulations for veal production, the influence of solid feed consumption by veal calves was a prominent research topic. Researchers explored the effects of amount of solid feed as well as the composition of that feed on nutrient utilization and welfare in veal calves (Suárez et al., 2006; Berends et al., 2012; Webb et al., 2015).

Rations and Feeding Behavior

A new area of investigation during this period evaluated factors related to preference, palatability, and sorting of feed mixtures in young calves (Miller-Cushon et al., 2013a,b; Groen et al., 2015).

Provision of Forage

Research during the 2000s and 2010s again explored the effect of provision of small amounts of forage to preweaned and postweaned calves. Coverdale et al. (2004) found that 5 to 10% chopped forage in starter improved ADG. Khan et al. (2011) reported that provision of hay improved performance in calves consuming higher quantities of milk. Castells et al. (2012) compared intakes and performance of calves offered different forages and showed that alfalfa hay was consumed in the greatest amounts with no benefit to ADG, whereas calves consumed approximately 5% of their total ration as chopped straw but had greater ADG. Suarez-Mena et al. (2016) evaluated different particle sizes of straw included at 5% of starter DM: 0.82 (in pellet), 3.04, 7.10, and 12.7 mm. Size of straw particles did not markedly affect rumen development or organ size. Although research in this area is subject to several potential confounding factors (Kertz, 2007), at present it appears that small amounts (~5%) of chopped straw or other lower nutrient forages may improve overall intake and utilization of starter feed when pelleted for improved growth.

Weaning

When and how to wean calves became a topic of interest again with the advent of the intensified milk programs in which larger amounts of milk or milk replacer delay increases of starter intake. Weaning at 8 wk instead of 6 wk (Eckert et al., 2015) and stepping down milk intake by use of automated feeders (Sweeney et al., 2010) appear to be the best methods for avoiding growth slumps around weaning.

Housing, Bedding, and Automated Feeding Systems

During this period, research showed that calves could be raised in groups without problems if they were fed greater amounts of milk or milk replacer (Costa et al., 2016). Automated milk feeders were shown to be especially useful in making gradual changes in milk intake (Jensen, 2006; Borderas et al., 2009; de Passillé et al., 2011), which makes management of feeding larger amounts of milk much easier.

Behavior and Welfare

The late 1990s saw the emergence of behavioral research as an accepted discipline, led by groups at the University of British Columbia and the Danish Institute of Agricultural Sciences, among others. These groups developed and carefully validated various methods to quantify effects of treatments on animal well-being.

Chua et al. (2002) found that calves raised in pairs grew faster and ate more starter than calves fed individually and displayed more evidence of well-being. Other area that have been explored include bedding comfort (Camiloti et al., 2012), mixing (O'Driscoll et al., 2006), and competition (Jensen et al., 2008).

The effect of various management procedures such as dehorning (Faulkner and Weary, 2000) and castration (Stewart et al., 2010) on pain and calf welfare became an important topic of research during this period. In addition, surveys of public opinion of aspects of dairy production have provided avenues for future management research (Ventura et al., 2013).

Effects of Improved Early Nutrition on Subsequent Milk Production

A research report by Bar-Peled et al. (1997) indicated that heifer calves allowed to suckle their dam during early life tended to produce more milk in later life. This finding created interest in potential effects of early-life nutrition on later milk production. Soberon et al. (2012) used data from 2 herds to reveal a relationship between preweaning growth rate and subsequent milk production, which they attributed to the effect of nutrient intake above maintenance. Preweaning growth rate explained 22% of the variance in first-lactation milk yields. Soberon and Van Amburgh (2013) conducted a meta-analysis of 12 data sets that reported early-life intake and growth rates as well as first-lactation milk production. The model showed a highly significant effect of early nutrient intake on first-lactation milk yield (+435 kg) and an odds ratio of 2.09, indicating that calves fed more milk or milk replacer in early life were 2 times more likely to have a greater milk yield in first lactation. Gelsinger et al. (2015) conducted a meta-analysis of preweaning diet and growth rate on first-lactation milk production. Their analysis also found that calves that grew more rapidly produced more milk. Brown et al. (2005) reported that heifers that were fed to grow more rapidly during the milk feeding period had greater mammary parenchymal development. In contrast, Kiezebrink et al. (2015) conducted a controlled study in which calves were fed either 4 or 8 L of whole milk and managed similarly from there on. No difference was found between the 2 treatments for first-lactation milk production.

Maternal and Epigenetic Effects on Calves

A new area of research in calves that emerged during the period from 1997 on was the effects of maternal

environment and nutrition on calf health, growth, and future productivity. From a mechanistic standpoint, the field of epigenetics seems likely to explain many of these phenomena. For example, differences in maternal intake of trace minerals resulted in different phenotypic and physiologic responses in calves (Jacometo et al., 2015).

A major maternal environmental factor affecting calves is heat stress. Several studies during this period documented various aspects of calf growth and productivity that were negatively affected by maternal heat stress, which were reviewed by Tao and Dahl (2013). A recent series of studies by Monteiro et al. (2016a,b) showed that calves born to heat-stressed dams had lower birth BW, ate less starter, grew more slowly as calves, and produced less milk in first lactation. Genomic selection tools (Weigel et al., 2012) promise to have a major effect on selection of calves with only high genetic merit.

FUTURE PROSPECTS FOR CALF NUTRITION AND MANAGEMENT

As genomics identifies calves of superior genetic merit, nutrition and management will intensify and be more targeted to these calves to optimize their growth and development via the science of epigenetics. Marrying behavior with nutrition and management will further allow calves to be more comfortable and thrive better. Calf comfort will become as important as cow comfort is to cows. Management schemes and housing will increasingly take into account all of these factors and will evolve to allow improvement in calf performance. Feeding and management of calves will be done to incorporate knowledge of how to improve calf immunity. Methods to improve use of nonmilk protein in calf liquid diets will be developed. As herds get larger, calf feeding and management will become more attuned to best feeding and management practices and protocols.

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APPENDIX

Table A1. Milestones in the past 100 yr of calf nutrition and management

Date	Milestone
1919	The first calf article is published in the *Journal of Dairy Science* evaluating factors influencing birth weight of calves.
1919	Nonmilk ingredient sources for liquid diets are evaluated.
1922	Growth chart from calf measurements
1923	Early weaning at 1 mo to conserve whole-milk feeding and utilize starter ration
1934	Water intake is measured with 30 heifers from birth to 24 mo of age.
1937–1956	A total of 77 papers are published on minerals and vitamins, particularly vitamin A.
1942–1952	Various fat sources in milk replacers are evaluated.
1947–1950	Milk is replaced with milk by-products and nonmilk protein sources.
1947–1951	Colostrum composition is measured.
1948–1956	Initial calf rumen development studies, including role of VFA from rumen fermentation, are performed.
1950s	Antibiotic feeding for growth and performance
1958–1960	Antibiotic feeding and performance
1958–1962	Forage sources and levels for calves and feed efficiency
1958–1965	Calf rumen development studies are performed.
1958–1973	Milk replacer ingredients are evaluated.
1962–1973	Calf starters, ingredient evaluations, and protein requirements
1969	Calf immunity review
1970s–mid 1980s	Colostrum collection, feeding, and preservation
1975	Calf feeding, management, and housing review
1977	Standard procedures for conducting calf trials and reporting data
1977 and 1980	Met requirements in milk replacers
1979	Early calf weaning program review
1980s and 1990s	Nonmilk protein sources, abomasal clotting, and antinutritional factors are evaluated.

Continued

Table A1 (Continued). Milestones in the past 100 yr of calf nutrition and management

Date	Milestone
1980–1987	Macromineral requirements
1981	25-yr summary of calf nutrition and management
1984	Effect of water intake on calf performance is established.
1986–1991	Trace mineral requirements and excesses
1987 and 1992	Cold weather requirements and fat supplementation
1987	Vitamin E requirements
1991–1994	Calf starter protein levels are evaluated.
1994	Effect of fat levels in milk replacer and calf starter on performance is indicated.
Late 1990s–early 2000s	Studies on trace minerals and some fat-soluble vitamins show efficacy and interrelationships.
Early 2000s	Milk replacers change from skim and CN to whey proteins and some nonmilk protein sources along with some AA and fatty acid supplementation.
2000–2001	Conditions to heat-treat waste milk for enteric pathogenic organism control
2001–2006	Studies establish growth and body composition when calves are fed more and higher protein milk replacers.
2004 and 2015	Whether or how to process grain in starters
2005–2015	Heat-treated colostrum factors and benefits are established.
2006–2011	Managing automatic milk feeders and weaning
2012–2015	Studies establish the effect of early calf growth and subsequent milk production.
2012–2015	Forage provision for calves fed pelleted starters
2013–2015	Palatability of starter ingredients and feeding behavior
2014–2016	Negative effects of maternal heat stress on subsequent calf growth and milk production