Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

Veterinary supply and demand

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The market forces that impact the economic status of the veterinary profession are complex and diverse. The recent AVMA report on veterinary markets describes the market for veterinary education, market for veterinarians and the market for veterinary services.[1] Each of these markets is impacted by the forces of supply and demand often described as the "law of supply and demand". The interaction of market forces and other factors within the veterinary profession impact the demand for veterinary services, supply of veterinarians and the level of compensation that veterinarians receive. This presentation will explore the economic relationships that are currently impacting the veterinary profession.

Introduction

In simple terms the law of supply and demand is a common sense principle that defines the generally observed relationship between demand, supply and price. The basic concept is that when the price of a good or service increases the consumer demand tends to decrease. Supply considers the relationship between the price and available supply from the perspective of the producer rather than the consumer. Increasing consumer demand for goods and services often drives the price up and producers generally respond to the increase in price by producing more goods and services. The degree to which demand or supply reacts to a change in price is described as elasticity. If a good or service is considered to be essential by the consumer the demand may not be negatively affected by increasing prices and is described as being inelastic. Factors that influence demand price elasticity are the availability of substitutes, consumer disposable income, access to capital and time. Another important economic principle is "utility". Utility is an abstract concept regarding the satisfaction or benefit that an individual gains from consuming or using a good or service. In general, a high level of utility results in an increase in the demand for goods or services and would be considered inelastic. The utility for veterinary services is often high for individuals who have strong emotional attachments to the animals in their care.

Economists assume that there is competition in the marketplace, thus prices change in response to supply and demand. Substitutes can play an important role in the market. If a product gets too expensive, consumers often substitute a less expensive product. If there is only one company producing a product that does not have a substitute, the company is said to have a monopoly on the market.

Understanding market forces

Market forces that affect supply, demand and price come in many forms. Common forces encountered in today's economy that affect the various markets in veterinary medicine include technological developments such as new products and equipment, along with the development of new communication, marketing and sales strategies. Political and governmental policies that influence regulations and tax rates affect how veterinary businesses operate. The position the profession takes on certain moral and ethical issues of our time can influence consumers' view of the veterinary profession and affect consumer spending. Weather condition can impact the profession directly and indirect due to the economic impact that these forces have on the agricultural sector. These markets forces and many others impact the various markets that define veterinary supply and demand.

Price signals are important indicators in a free market economy. Rising prices generally indicate a decrease of supply or an increase in demand. However, if prices are influenced by government policies or other forces then changes in prices may not be a reliable indicator of shortages, surpluses, or consumer preferences. The 2015 AVMA Report of Veterinary Markets points out that there are three principal markets within veterinary medicine and each is influenced by supply, demand, and price.[1] Outside forces such as governmental policies, state licensure, degree of public funding for institutions of higher education, federally backed student loan programs and many other forces can make the price signals difficult to interpret.

Another important concept to understand is the difference between need and demand. Demand is described as a consumer's desire and willingness to pay a particular price for a specific good or service. Demand often changes as the price goes up or down. Need is described as a consumer's desire for a specific good or service, yet they may not be willing to pay the current price for the good or service. If the price goes down the consumer may be willing to pay the lower price and thus in this case need is turned into demand.

The 2006 Foresight Project: Envisioning the Future of Veterinary Medical Education stated that one of the most important principles (needs) for the future of the profession was that veterinary medicine must remain relevant to the changing needs of society.[3] The report suggested that veterinary medical education could only respond to these changing needs by expanding the areas of education and that the number of graduating veterinarians should increase to address population growth and allow the profession to respond to new demands and roles.[3] While many would agree with this vision for the needs of the future, it does point out the importance of understanding the difference between need and demand.

Supply represents how much the market can offer and often refers to the amount of a certain good or service producers are willing to supply at a certain price. An oversupply exists when the supply of a certain good or service exceeds the need. If the supply of a certain good or service exceeds the demand yet fails to meet the need then excess capacity exists. The correction for oversupply is to reduce supply. Corrections for excess capacity include turning need into demand, reducing the price or reducing the supply.

The supply and demand for veterinarians, veterinary services and veterinary education are somewhat independent markets that guide the allocation of resources for veterinary medicine and provide goods and services to owners of animals, the veterinary industry, government, academia and the general public. Each of these markets has a supply and demand that influences the price providing signals to those involved in the markets about the relative supply and demand conditions.[1]

Market for veterinarians

The practice of veterinary medicine in the US is regulated by state statues that require certain standards be met to obtain a license, including the DVM degree or equivalence. Thus the supply of veterinarians is influence by the market for veterinary education. In 2014 the AVMA estimated that 100,137 veterinarians were actively practicing veterinary medicine in the United States.[1] The results of the AVMA survey of graduating seniors between 2010 and 2014 indicated that 16,267 new veterinarians (approximately 3,253 per year) were added to the veterinary workforce. Of those entering private practice, 64.5 % were employed in companion animal practice, 17.9% in mixed animal practice, 7.8% in food animal practice and 3.6% in equine practice.[1]

The demand for veterinarians is influenced by the compensation employers are willing to pay and the level of compensation is linked to the market for veterinary services. As the demand for veterinary services increases, the price for veterinary services will likely increase, resulting in increased revenue for veterinary practices. Thus, veterinary practices have the ability to increase the level of compensation they could pay veterinarians. However, if there is an oversupply or excess capacity they may be able to employ new veterinarians without increasing compensation. If the demand for veterinary services is weak then price changes for veterinary services would tend to remain flat or decrease, negatively impacting practice revenue and reducing the level of compensation the veterinary practice owners can pay new veterinarians who are entering the workforce.

Three indicators for the demand for new veterinarians are level of compensation (starting salary), level of unemployment and level of underemployment. In 2001 the nominal mean salary was approximately $45,000. Starting salaries increased at a steady rate of 7.65% annually from 2001 to 2008 at which time the starting salary was $70,000. From 2008 through 2014 the nominal mean starting salary for new graduates has remained flat at $70,000.[1] The AVMA reported the unemployment for 2014 at 3.9% and a negative underemployment that equates to room in the profession to employ an additional 951 full-time veterinarians.[4]

Market for veterinary education

Veterinary education provides the necessary training for individuals entering the veterinary workforce. The veterinary educational supply chain can be viewed by the number of seats available at accredited veterinary colleges, both domestic and international, and the price (tuition and fees) institutions are charging to provide seats. The number of seats filled at veterinary colleges determines the number of graduates produced each year. The number of graduates from US schools in 1980 was 1746 and since then the rate of increase has been approximately 2 % per year with 2977 new graduate expected in 2015.[2] There are US citizens obtaining their veterinary training from accredited international veterinary schools. The number of US citizens graduating from accredited international veterinary schools was not available until 2012. In 2012 there were 538 graduates and in 2015 the projected number of US citizens graduating by accredited international colleges is expected to be 619. The combined number of veterinary graduates in 2015, foreign and domestic is estimated to total 3596. With two new US schools admitting their first class (fall 2014) the expect number of veterinary graduates is expect to rise to 3226 (US), 644 (international) for a total of 3870 new graduates in 2018.[2] The AVMA projects that number of veterinary graduates will level out moving forward.[1]

The demand for the veterinary education market can be defined as the number of applicants who are willing to pay the current price for a seat. The price for a seat at a veterinary college varies significantly among institutions and whether or not the student pays resident tuition rates or non-resident rates. The median resident tuition for US Colleges of Veterinary Medicine, excluding the University of Pennsylvania, Tufts University and Western University of Health Sciences was reported in the 2014 AAVMC comparative data report as $21,753. The minimum was $16,546 and the maximum was $30,813. The median non-resident tuition was $45,910. The minimum was $25,809 and the maximum was $62,083.[2] Over the last decade the median resident tuition has increase at a rate of 4.3% annually and the rate of increase or non-resident tuition was 2.9%.

Data for the number of applicants that apply for a seat at a veterinary college come from the American Association of Veterinary Medical Colleges (AAVMC). The AAVMC provides an applicant service in which students wishing to apply to a veterinary college can apply using a centralized service, VMCAS. Currently 90.5% of the first year seats at US colleges of veterinary medicine are represented in VMCAS data.[2] The number of applicants per available seat as reported by AAVMC has remained steady at about 2.25 applicants per seat over the past decade.[2] However, if you consider the number of available seats at international colleges, the ratio drops.

By the mid 1980's the student debt to income was becoming an issue. In 1984 AVMA president Dr. Delano L. Proctor indicated that an in-house survey of 1984 graduates showed that 85 percent would have education related debt and the mean was $20,000. The mean starting for graduate was also about $20,000.[5] In 1999 the JAVMA published the KPMG study which stated that increased student debt was a significant issue facing many recent graduates.[6] At the time the mean starting salary for new graduates was about $42,000 and the mean debt had grown to $63,000. Since 2001, the debt of new veterinarians has been growing faster than starting salaries. The debt to income gap in 2001 was approximately $10,000 when adjust to 2014 dollars. The gap had increased to nearly $65,000 in 2014.[1]

Market for veterinary services

The general economic condition of consumers and their willingness to pay for goods and services has significant impact on market conditions for veterinary services. The amount of money that households have available for spending and saving after income taxes is referred to as disposable income.[7] The rate of growth in household disposable income is an important indicator of the money consumers have available to spend on veterinary services. Since 2000 the rate of growth in disposable income has declined as compared to historic growth (1960-2000).[1]

Currently, 66.5 percent of active veterinarians are employed in companion animal practice and would be directly impacted by the level of disposable income for the consumers in their practice area. Six point three percent of the active veterinarians are in food animal practice and would be impacted by the profit margins associated with the various livestock sectors they serve. Three point nine percent are in mixed

practice and would be impacted by the level of disposable income and profit margins in the livestock sectors. Equine practice represents 6.1 percent of active veterinarians and would be impacted by profit margins in the equine sector, along with availability of disposal income.[1]

The AVMA has been collecting aggregate economic data such as total revenue and expenses from US veterinary practices for more than two decades through the biennial economic survey. However, this survey did not contain information on the quantity and prices of specific veterinary services.[1] This information is needed to develop the supply and demand curves for specific veterinary services that can be aggregated to produce market supply and demand curves. Given the lack of data, the AVMA's Veterinary Economic Strategy Committee concluded that the currently available data would not result in a sufficiently robust analysis to provide useful information regarding the supply and demand for veterinary services.[1]

Implications

Socio-economic factors are believed to be directly correlated with lifestyle choices and are linked to patterns of drug use, disease prevalence and rates of mortality. The results from the first mental health survey of US veterinarians show that veterinarians are more likely to suffer from psychiatric disorders, experience bouts of depression and have suicidal thoughts compared with the US adult population.[8] Common predictors of suicidal behavior include hopelessness, stressful life events, substance abuse, depression and axiety.[9] It has been reported that a high percentage of veterinary students have depression levels at or above the clinical cut-off.[10] While I am not aware of any studies that have evaluate the effects of economic stress on veterinarians, it stands to reason that the widening debt to income gap could affect lifestyle choices and lead to an increase of mental health disorders.

As the future unfolds it is extremely important that as a profession we continue to gather good data and develop the necessary skills required to interpret the data and evaluate market signals. We need to develop a better understanding of how the market for veterinarians, market for veterinary education and the market for veterinary service interact and impact each other. While it is difficult to predict the future, having robust discussion regarding the factors that influence the markets of veterinary medicine and how the markets interact will be helpful to plotting the future of the profession.

Questions to consider for ongoing discussions: Can the needs of the profession, be turned into demand? How would this be accomplished? Understanding the elasticity of the many veterinary services offered and making appropriate price adjustments could improve efficiency and profitability. Should there be an adjustment in the supply of veterinarians? If so, how would this accomplished? Will the demand (ratio of applicants to seats) for veterinary education continue at same level? Will the debt to gap income continue to widen and if so at what point will applicant numbers be impacted? What are the socio-economic impacts of the growing debt to income ratio?

In closing, I would like to acknowledge the excellent work of the AVMA, Veterinary Economics Division and AAVMC for their on on-going efforts in collecting and analyzing data. Their reports are vital to understanding markets forces and how they impact the profession.

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A review and update of research on pregnancy associated glycoproteins (PAGs) in cattle

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Key points

- PAGs are a large gene family found in all ruminants.
- The function that PAGs play during gestation remains a mystery. In this review speculation about putative roles for the PAGs will be highlighted.
- The PAGs display a wide range of expression patterns and localization. These proteins may be playing different roles depending on the exact location or time of pregnancy that they arise.
- Understanding the roles that PAGs play in ruminant pregnancy may lead to a better understanding of pregnancy success and failure.

Introduction

The placenta is a multifaceted organ that has a critical role in maintaining and protecting the developing fetus by transferring nutrients and metabolic wastes, acting as a regulator of the maternal immune system, and serving as a major endocrine organ.[1] In the ruminant placenta there is a unique cell type (binucleate cells) that constitutes 15 - 20% of the fetal placental trophoblasts. These cells become visible around d 19-20 of gestation in cattle and have been shown to secret a plethora of hormones and proteins, including placental lactogen (PL) and pregnancy associated glycoproteins (PAGs). Although much of the focus on PAGs has been directed toward PAGs expressed in bincucleate cells, there are PAGs that have been shown to be expressed by mononucleated cells as well. This section will focus on the characterization and functions of PAGs in cattle and attempt to link data presented in the review of "Application of PAGs to manage reproductive efficiency in cattle."

Characterization of pregnancy associated glycoproteins (PAGs)

PAGs were first reported by Butler[2] after isolation of two proteins from fetal membrane extracts, referred to at the time as pregnancy specific proteins A and B (PSPA/PSPB). In the same study it was determined that PSPA was alpha-fetoprotein and that PSPB was specific to the placenta. Sasser[3] developed a PSPB specific radioimmunoassay which they used to measure PSPB in the maternal circulation. In subsequent reports, they demonstrated that measurement of PSPB in females could be used to successfully detect pregnancy in dairy cattle,[4] sheep,[5] and goats.[6] Around the same time, Zoli[7] reported the purification of another pregnancy specific protein that they called 'bovine pregnancy associated glycoprotein' (PAG). Bovine PAG and PSPB had very similar amino acid sequences at the amino-terminus[8] suggesting that these two proteins were similar, if not identical.

Initial immunolocalization studies determined that PAG was synthesized by the trophoblast binucleate cells and stored in large secretory granules prior to delivery into the maternal circulation.[9] The exact function of PAG remained unclear; however, Xie[10] reported that 60% of the nucleotide sequence of PAG was shared with pepsinogens. Furthermore, it was shown that mutations in and around the active site rendered PAG inactive as a proteinase.[10,11]

As time progressed, new members of the PAG family were discovered in cattle and many species within the Ruminantia suborder. Consequently, the original PAG was renamed PAG1 and PAGs discovered afterwards were numbered sequentially. It is now known that PAGs comprise a large diverse gene family belonging to the aspartic proteinase superfamily.[12-14] In cattle alone, there are >20 distinct PAG cDNAs represented in Genbank.[15]

Hughes[16] reported that PAGs can be divided into two distinct groups: 1) *ancient* PAGs which are estimated to have originated about 83 million years ago (around the time the Artiodactyla order is thought to have arisen), and 2) *modern* PAGs which are estimated to have originated approximately 54 million years ago. These two distinct groups of PAGs have been studied extensively and characterized based on

their mRNA expression. Ancient PAG mRNAs are usually expressed throughout gestation in both mononucleate and binucleate trophoblast cells of the cotyledons; whereas, modern PAGs are synthesized primarily in binucleate cells of the trophoblast and their expression seems to change during gestation.[13]

Ancient PAGs

The ancient lineage of PAGs seems to have arisen from duplication of a single pepsinogen F-like gene around 85 million years ago.[16] Kumar[17] also reported the divergence of the even-toed ungulates (Artiodactyla) and odd toed ungulates (Perissodactyla) around this same time period suggesting that these two events may be closely related. In cattle, the ancient PAGs are comprised of a relatively small group of about six genes.[13] These six bovine (b) PAGs are expressed in cotyledons from early placentation to term, in both uninucleate and binucleate trophoblast cells. Following secretion, some of the ancient PAGs accumulate at the microvillar junction between the maternal and fetal interface.[18] Their function is still unknown, however, based on their localization. Wooding[18] suggested that ancient PAGs may be important for the following: 1) adhesion of the uterus and trophoblast cells to maintain appropriate transport, 2) proteolytic processing, 3) activation of growth factors or bioactive molecules, or 4) protection of trophoblast cells from the maternal immune system. There is also speculation that the ancient PAGs and modern PAGs may work together throughout gestation. Collectively, the ancient PAGs were thought to be peptidases, although there had been no solid evidence until Telugu[19] reported that bPAG2, which is the most abundant transcript reported in the PAG family possessed proteolytic activity. A similar PAG, bPAG12, has also been shown to be proteolytically active. The preceding authors concluded that ancient PAGs exhibiting proteolytic activity may function as sheddases to activate latent biomolecules, which could be important for placental development and growth.

Modern PAGs

The burst in gene duplication that led to the lineage of modern PAGs has been linked to the emergence of the synepitheliochorial placenta of the ruminant ungulates.[16,18] The modern family of ruminant PAGs includes a larger number of genes than their ancient counterparts.[13] Wooding[(18)] suggested that the modern PAG family expansion could potentially have evolved to deliver a variety of fetal products and hormones to the mother by bypassing the uterine epithelial barrier. These PAGs are restricted to ruminant species and are expressed primarily in trophoblast binucleate cells from which they are released into the maternal system, with some accumulating in the stromal layer within the maternal caruncles.[18] The authors concluded that the preceding localization pattern could potentially place PAGs in a position to engage in immunological protection, such as blocking lymphocyte or polymorphonuclear leukocyte migration and activation. To date there have been no clear functions related to modern PAGs; however, PAGs have been shown to inhibit different immune cells, in vitro, and may camouflage fetal/placental antigens from the immune system.[20] Alternatively, PAGs have been suggested to have a luteotrophic action based on a report that addition of PSPB/PAG1 to endometrial cells increased the production of the luteal-promoting prostaglandin, prostaglandin E_2 (PGE_2);[21,22)] however, the evidence for a luteotrophic or antiluteolytic action of PAGs is not compelling at this time.

Possible potential function(s) of the PAG

To date the function of PAGs is not clear. However, their expression patterns by the placenta of cattle and related species as well as the proteolytic activity of some PAGs could provide some insight into their roles during pregnancy. For instance; many PAGs such as bPAG-2 and porcine PAG-2 (belong to the ancient PAG group) are found to accumulate at the placental feto-maternal interface. Bovine PAG-2 and procine PAG-2 are known to have a proteolytic activity,[19,23)] which is suggestive of possible roles involving protein turnover or remodeling at the trophoblast-uterine epithelial interface[18] or they could be acting to proteolytically activate bioactive molecules and latent growth factors located at the interface.[24,25)]

There are some PAGs that lack the ability to act as proteinases; these may have another role, such as peptide binding at the uterine- fetal interface. Interestingly, PAGs are able to interact with peptide

ligands via their substrate- binding cleft.[26] Conceivably, binding to other proteins through the substrate-binding cleft could position PAGs to interact with other proteins at the maternal-fetal interface or with transmembrane receptors (e.g. integrins). Furthermore, the carbohydrates displayed on the surface of PAGs could bind to a lectin (carbohydrate-binding protein) at the maternal-fetal interface to sequester them to that location. Pregnancy associated glycoproteins with enzymatic activity typically exhibit proteolysis of substrates at comparatively low pH.[19,23)] The positioning of proteinases at the interface could facilitate release of the cotyledon from the caruncular crypts around parturition when the pH of the interface microenvironment falls and proteolytic activity of these PAGs would be expected to increase.[18]

Pregnancy associated glycoproteins may have an effect on the maternal immunological system in cattle. For example, bPAG-1/PSPB treatment of bovine bone marrow has been shown to cause a drop in bovine hematopoietic cells proliferation.[20] In other experiments, bPAG-1/PSPB treatment of bovine endometrial (BEND) cells induced release of granulocyte chemotactic protein 2 (GCP2).[27] Pregnancy associated glycoproteins have also been shown to associate with the peripheral blood lymphocytes and with endometrial serpin-like proteins in vitro.[28,29]

In cattle and sheep PAG1/PSPB could have an effect on luteolytic activity. For instance, bovine luteal cells progesterone and prostaglandin E2 (PGE_2) production increased in response to PSPB treatment.[30-32] This observation of an increase in bovine luteal cells progesterone production might be due to a luteotrophic effect of PGE_2 but it was not observed consistently.[30-32] It seems to be that PAGs can play multiple roles during pregnancy in regard to placental development and function.[33]

In addition, PAGs may be playing a totally different role than any of the data above suggest. Recent data generated out of Dr. Jon Green's lab at the University of Missouri is potentially pointing to a role of PAGs and uterine remodeling around the time of early placental attachment. These preliminary data found that the addition of purified bovine PAGs to endometrial explants, collected on day 18 from pregnant and nonpregnant heifers increased the expression of members of the matrix metalloproteinase gene family which have a role in tissue remodeling. Furthermore, we have demonstrated that cows likely to undergo late embryonic mortality (between day 28-60 of gestation) have decreased circulating concentrations of PAGs on day 28 compared to cows that will maintain a pregnancy.[34] These results support the idea that cattle pregnant on day 28 of gestation, with decreased circulating concentrations of PAGs, may be undergoing pregnancy loss based on the failure of placental and endometrial crosstalk along with a failure of tissue remodeling that is critical for early placentome formation.

Summary

The bovine PAG family is a large group of related proteins that are encoded by more than 20 genes. The function that PAGs play during gestation have not been elucidated, but preliminary data point to having a role in manipulation of the maternal immunological system, regulation of luteolytic activity, and(or) tissue remodeling. As our understanding of mammalian genomes increases and the advancement of biological tools increase it is likely that the exact function that PAGs play during pregnancy will be discovered.

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Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

Applying ultrasonographic evaluation of antral follicle count to improve reproductive management in heifers

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Abstract

Ultrasonography is a powerful technology that can be used to improve reproductive management in heifers. By counting the number of antral follicles observed on an ultrasound screen the practitioner can gather additional information when reproductive tract scoring, because the number of antral follicles is predictive of the status of the ovarian reserve. The number of antral follicles is also predictive of response to exogenous gonadotropins and can be used as a screening tool to remove poor responders before time and money is squandered trying to recover oocytes or embryos.

Introduction

Palpation of the ovaries and reproductive tract of the cow has been used by veterinarians for decades to evaluate reproductive status. Advantages of this technique are that the reproductive tract is easily accessible, examinations can provide information on ovulatory status and size of the ovaries and the uterus, results are instantaneous allowing management decisions to be made at time of examination, and labor is used efficiently because a relatively large number of heifers can be evaluated in a relatively short period of time. These advantages led to development of the Reproductive Tract Score, a formalized method of evaluating reproductive tract development and ovulatory status in heifers[1] and cows.[2] Especially in the beef industry where access to the heifers can be limited under some management practices, this has provided a method for evaluating replacement heifers that did not require observation for behavioral estrus over several months to determine pubertal status. Nevertheless, adoption by producers has been slow because of need for highly-trained individuals with palpation skills, costs associated with procuring services of such trained individuals, need for suitable working facilities, and need to gather heifers and bring them into the working facilities. From a veterinarian's perspective, strain on the practitioner's arm and shoulder deters enthusiasm for providing such a service. In cases where an extension arm can be used, such as pregnancy diagnosis, ultrasonography can eliminate this strain on the shoulder. Furthermore, ultrasonography can provide additional information not acquired by traditional palpation, such as antral follicle count and blood flow, leading to better decision making about females to retain for breeding purposes.

Ultrasound and its application to reproductive management in bovine females

Ultrasound machines are composed of a primary electronic processing unit and a piezoelectric transducer that emits and detects high frequency sound waves. Based on density of tissue being scanned, the sound waves emitted by the transducer either reflect back to the transducer or continue to penetrate the tissue. Returning sound waves are detected by the transducer and converted to a real-time image displayed on the screen of the processing unit. Fluid filled structures such as the antrum of a follicle or a gravid uterus do not reflect sound waves and appear on a standard screen as a black area.

Transducers may comprise linear or sector (convex) crystal arrays. Linear transducers have crystals arrayed in a single plane and emit waves at a ninety degree angle from the transducer. Sector or convex transducers have a crescent-shaped array of crystals and emit waves across a broader area. In the past, transducers had a fixed frequency and there was a trade-off because a greater frequency gave a better image quality but decreased depth of tissue penetration. In newer machines, frequency for a transducer can be changed and this allows greater flexibility in the information that can be obtained from a single examination.

Compared with traditional palpation, ultrasonography increases the information that can be collected as part of an examination process. Ultrasonography can be useful in detecting multiple

ovulations, size of corpora lutea, number and gender of fetuses, heart beat of fetuses, abnormalities of the genital tract, and other indicators of fertility.[3,4] For example, analysis of gray scale patterns of uterine images may provide some indication of fertility.[5,6] Researchers began using ultrasonography to measure diameters of antral follicles as a method to understand follicular development and ovulation.[7,8] From this research, they discovered that diameter of the ovulatory follicle is related to estradiol production and overall fertility;[9,10] however, follicle diameter measurements must be taken at consistent times during the follicular phase to generate measurements that can be compared across cows and be useful for making decisions. Counting the number of antral follicles that are visible on the ultrasound screen can be used as an indicator of the number of microscopic follicles within an ovary.[11-13] This measurement is less dependent on stage of the estrous cycle because small (< 5 mm) antral follicles comprise the majority of the count and do not fluctuate during follicle wave progression.

Color Doppler ultrasonography makes use of the Doppler effect to determine direction and rate of movement relative to the position of the transducer. It is most commonly used to measure blood flow with the colors (blue and red) simply indicating orientation of the flow to the transducer and having nothing to do with arterial or venous blood flow. Some researchers feel that it has application for early pregnancy diagnosis in cows by focusing on the function of the corpus luteum (CL), because during luteal regression blood flow to the CL decreases. Therefore, by examining blood flow at days 18 to 19 after breeding the technician may be able to identify corpora lutea that are regressing in the absence of pregnancy.[14,15] It is still difficult to time this correctly because bovine estrous cycles with three waves of follicular development will average 24 days in length.[16,17] Color Doppler ultrasonography can also be used to measure blood flow to a gravid uterus and understand the role of blood flow in fetal development.[4] The diagnostic uses of this for reproductive management of cows have yet to be established.

Relative distance from the surface of the transducer to the surface of the tissue being examined along with the frequency of the sound waves being emitted affect the size of structures measured. For pregnancy diagnoses in cattle, curved handles that enclose the probe and connecting cable can be inserted per rectum to decrease strain on the arm and shoulder that are characteristic of palpation. In contrast, the transducer must be held and manipulated by hand to insure consistent measurements of ovaries, diameters of follicles or CL, and cross-sections of uterus. There may be future solutions for this requirement, but the current technology can provide very informative data that helps veterinarians and producers make profitable decisions.

Ultrasound machines have become more compact and mobile with greater computing capabilities. As computing capabilities and image analysis software improve, applications of the machines to improve reproductive management will continue to increase. Ability to store digital video clips of reproductive tract scans of individual animals will create permanent records that are managed, stored, and queried more easily than with the video tapes used in the past. Imaging software will attain a level of sophistication to instantaneously provide pixel densities of the ovaries as a proxy for antral follicle count and blood flow measurements to better evaluate CL and reproductive tract function. This should improve diagnostic capabilities and perhaps decrease the amount of time a diagnostician has to spend with their arm inserted into a rectum. Wireless technologies and adoption of electronic identification will eliminate need for a practitioner to record animal identifications by hand, thereby decreasing the error rate and the time required to complete an examination of an animal.

Antral follicle count as an indicator of fertility

Compared with traditional palpation, ultrasonography allows a practitioner to visualize and count the number of antral follicles present on an ovary. By the early 1990's, human medicine had moved in this direction as a method to assess reproductive status of women with fertility issues. Women that were approaching menopause or that suffered from primary ovarian insufficiency had lower numbers of antral follicles detectable by ultrasonography.[18] Because basic mechanisms controlling numbers of follicles in the ovaries are similar in all mammalian females, we began to investigate the role of antral follicles counts in bovine reproduction, and it has spread as a tool to a number of mammalian species as a way of

addressing reproductive management in endangered species. Antral follicle counts provide practitioners with a non-invasive estimate of the number of follicles in an ovary, because histological studies have demonstrated that there is a positive relationship between the number of microscopic follicles and the number of antral follicles in a bovine ovary.[11-13,19]

Antral follicle counts are associated with a number of important reproductive traits. Heifers that give birth early in their first calving season have greater numbers of antral follicles at pre-breeding ultrasonographic examination than those that give birth later in the first calving season.[20] Similarly, dairy cows with high numbers ($\geq$ 25) of antral follicles required fewer inseminations per conception than those with low numbers ($\leq$ 15) of antral follicles.[21] Because beef heifers that give birth early in their first calving season have greater reproductive longevity,[22] it is tempting to speculate that differences in germ cells numbers may contribute to this increased reproductive longevity. However, there is little information on the mechanisms influencing the rate of depletion of the follicles. If a heifer with high numbers of follicles has a high rate of depletion and a heifer with a low number of follicles has a low rate of depletion, they could still both reach reproductive senescence at the same age. Further research is needed to understand the mechanisms controlling depletion of the ovarian reserve.

Dairy heifers with high numbers of antral follicles have greater serum progesterone concentrations during the luteal phase and greater endometrial area during the first 6 days of the estrous cycle than those with low numbers of antral follicles.[23] Serum follicle stimulating hormone (FSH) concentrations are decreased and follicular fluid estradiol concentrations are increased in cows with high numbers of antral follicles compared to cows with low numbers of antral follicles.[24] Furthermore, ovaries from cows with high numbers of antral follicles are larger, but this is not just due to the increased volume of the antral follicles, because these ovaries have more microscopic follicles on a per gram of tissue basis.[13,19] Thus, there are clearly differences in hormonal milieu and reproductive tract function that contribute to improved conception in cows with high numbers of antral follicles.

Whether there is a difference in oocyte competence between cows with high and low numbers of antral follicles is unclear. The majority of studies have reported increased numbers of blastocysts produced in vitro, but no difference in percentage of blastocysts produced in vitro between cows with high numbers of follicles and cows with low numbers of follicles.[24,25] However, there is one study that reported an increase in percentage of blastocysts produced in vitro from oocytes collected from cows with high numbers of follicles compared to those with low numbers of antral follicles.[26]

Response to exogenous gonadotropins

The use of exogenous gonadotropins either for multiple ovulation embryo transfer or ultrasound-guided oocyte pick up (OPU) is a way to rapidly increase the number of progeny of a genetically superior heifer while decreasing the generation interval. The decision to use a heifer in such a program should not be based solely on genetic merit, but should also be based on reproductive capacity. We demonstrated that the number of microscopic and antral follicles in one ovary was predictive of the response to exogenous FSH in the other ovary.[12] Cows with low numbers of primordial follicles had fewer antral follicles and poor ovulatory response to exogenous gonadotropins while cows with greater numbers of primordial follicles had more antral follicles and a strong ovulatory response to exogenous gonadotropins.

These results led others to investigate the use of ultrasonography to determine antral follicle numbers as a method to predict response to exogenous gonadotropins in cows.[27] One hundred and forty one cows were submitted for ultrasonography and based on total number of follicles $\geq$2 mm that were observed at follicle wave emergence, cows in the top 10% and cows in the bottom 10% were chosen. The cows in the high group had more than twice the number of follicles >5 mm after treatment with FSH for three days than the cows in the low group. The authors concluded that screening of cows prior to the start of treatment with exogenous gonadotropins was a useful way to remove poor responders.

Similar results were reported for OPU,[28] demonstrating that cows with high numbers of follicles had greater numbers of cumulus-oocye-complexes recovered after treatment with exogenous gonadotropin. A follow-up study proposed the need for individual protocols based on follicle numbers, but giving greater doses of FSH in the low follicle number cows did not improve response.[29] This is most

likely because others have demonstrated that cows with low numbers of follicles already have greater circulating concentrations of FSH due to less negative feedback.[24]

Conclusions

Ultrasonography is a powerful tool that can contribute greatly to reproductive management in heifers and cows. The ability to visualize physiological functions that would not be palpable such as heart beat or blood flow and to record these measurements for future diagnostic reference provide advantages beyond traditional palpation. It provides a method for early and accurate pregnancy detection and diagnosis of ovarian cysts. One advantage that it has over traditional palpation for evaluating reproductive capacity is the ability to visualize and count the number of antral follicles. As a predictor of the number of microscopic follicles in the ovaries, antral follicle counts can help to identify replacement females that may not produce enough calves to pay for their development costs or identify poor responders to treatments with exogenous gonadotropins. Continued research on antral follicle counts and the ovarian reserve will identify the mechanisms that contribute to decreased fertility and provide better decision making tools for reproductive management.

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Proceedings of the Society for Theriogenology Annual Conference 2015

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Mechanisms influencing establishment of the ovarian reserve in heifers

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Abstract

Ultrasonography serves as a diagnostic tool for understanding what is occurring at the microscopic level in the bovine ovary. Situations exist, however, in which genetic or nutritional influences can alter the population of microscopic follicles without altering the number of antral follicles observed on the ultrasonically, because correlations between preantral and antral follicle numbers are not 100%. Investigation of the mechanisms controlling establishment of the ovarian reserve and regulation of preantral follicle growth may improve both reproductive management of developing heifers and diagnostic capabilities.

Introduction

Using transrectal ultrasonography to determine the number of antral follicles in the bovine ovary is a powerful tool that can predict changes in the ovarian reserve (e.g., population of microscopic follicles in the ovary) and improve reproductive management. A greater understanding of what is occurring at the microscopic level is necessary to continue to improve development and application of ultrasonography. Genomic tools have enabled identification of genes involved in establishment of ovarian reserves and regulation of preantral follicle activation and growth. Research in this field provides evidence that variation in both gene sequence and gene function are contributing to differences in antral follicle count among cows, and that nutritional programming at specific developmental stages can change the function of a number of ovarian genes, presumably through epigenetic modifications to the genome.

Establishment of the ovarian reserve and follicle development in heifers

In bovine females, primordial germ cells migrate to the germinal ridge by d 30 of gestation.[1] Sexual differentiation of the ovaries occurs by d 40 of gestation, and germ cells proliferate until mid-gestation, reaching a peak of approximately 2.7 million oogonia. During the second half of gestation this number declines. By d 60 to 90 of gestation oogonia begin to form the functional units of the ovarian reserve, primordial follicles, composed of an oocyte surrounded by a single layer of flattened pre-granulosa cells. Primordial follicles are dormant until they enter the growing pool of follicles through an undefined process known as activation. Because of the decline in germ cell number during the second half of gestation, a heifer is born with approximately 100,000 primordial follicles in her ovaries,[2] and as with all mammalian females, it is the slow depletion of those primordial follicles over time that eventually leads to reproductive senescence.[3]

During activation, the flattened pre-granulosa cells become cuboidal granulosa cells and the oocyte enlarges, forming a primary follicle.[4] Activation of primordial follicles is most likely controlled by a combination of stimulatory and inhibitory factors,[4] the most well-described being anti-Müllerian hormone.[5,6] Anti-Müllerian hormone inhibits primordial follicle activation in cows and other mammalian species. Our research indicated that supra-physiological doses of estradiol given to cows over a 60-d period could stimulate primordial follicle activation.[7] This follicular stimulation provides indirect evidence that during normal follicle development, estradiol from the dominant follicle may inhibit ovarian anti-Müllerian hormone production, thereby ameliorating the inhibitory effects of anti-Müllerian hormone and providing a window of opportunity for some primordial follicles to enter the growing pool. However, this potential mechanism does not explain the mechanisms by which primordial follicles of the ovarian reserve are activate during any one of those windows of opportunity.

The cuboidal granulosa cells surrounding the oocyte proliferate forming a secondary follicle consisting of an oocyte surrounded by multiple layers of granulosa cell. Proliferation of granulosa cells is

controlled by a number of local growth factors, many belonging to the transforming growth factor-β super-family.[8] After three layers of granulosa cells are established, theca cells begin to differentiate. Once secondary follicles have attained six layers of granulosa cells, a fluid filled antrum begins to form. Transition from secondary to antral follicle is believed to be controlled mainly by insulin-like growth factor-1 and follicle stimulating hormone, because model systems that remove those two hormones stall progression of follicles at the secondary follicle stage.[9] Once the antral follicle attains 3 mm in diameter, it is distinctly visible via standard real-time ultrasonography.

The number of follicles is repeatable among cows and heifers over time.[10] Although the absolute number of antral follicles differs between heifers and lactating cows, their rank from high to low has a positive relationship[of about 80%. Furthermore, the number of follicles in one ovary is predictive of the number of follicles in the other ovary and the other ovary's response to exogenous gonadotropins.[11]

Genetic selection for numbers of follicles in heifers

Rapid response to genetic selection requires a selection criterion with high predictive value based on repeated measurements obtained at a young age. Conversely, response to genetic selection for reproductive traits in cattle has been limited by their low estimates of heritability, long generation intervals for progeny testing, and sex linkage. In a long-term genetic selection experiment at the U.S. Meat Animal Research Center to increase the frequency of multiple ovulations, ovulation rate was measured by ovarian palpation per rectum in 12- to 18-month-old pubertal heifers for six to ten consecutive estrous cycles.[12-14] Ovulation data were combined with ancestral records and utilized in a repeated-records animal model to estimate an animal's predicted breeding value for ovulation rate.[15] Repeated measurements of ovulation estimated heritability for ovulation rate based on a single observation to be 0.11 in this population; this heritability increased to 0.35 for mean ovulation rate for observations during six consecutive estrous cycles and to 0.43 for the mean ovulation rate for observations during ten consecutive estrous cycles.[16] In addition to an increased frequency of multiple ovulations, ovaries of the selected females contained more preantral and antral follicles compared with control females. The increased frequency indicates that selection for improved ovarian function modified the regulatory system of ovarian folliculogenesis, enabling activation and development of more preantral follicles, maintenance of larger pools of small antral follicles, recruitment of more follicles within the cohort of developing follicles, selection of two or more ovulatory follicles within a follicular wave, and/or a reduced rate of atresia of preantral and antral follicles.[17,18]

The ovarian reserve in *Bos indicus* females

Bos indicus cattle have greater numbers of antral follicles and greater ovarian stroma volume than *Bos taurus* cattle.[19] There is no evidence of increased fertility in *Bos indicus* cattle compared to *Bos taurus* cattle leading investigators to question relationships between follicle count and fertility. *Bos indicus* cows have larger ovaries than *Bos taurus* cows; however, a number of histological studies have reported no difference in the number of primordial, primary, or secondary follicles between age-matched *Bos indicus* and *Bos taurus* cows.[20,21] Taken together, those data indicate that *Bos indicus* cows have fewer primordial follicles per gram of ovarian tissue and appear similar in that regard to *Bos taurus* cows with small antral follicle numbers. Fewer antral follicles further indicates that there is enhanced secondary to antral follicle transition in *Bos* indicus cows, which is hypothesized to be a product of increased serum insulin-like growth factor-1 concentrations in *Bos indicus* cows.[22]

Genome wide association studies for antral follicle count in heifers

Genetic selection was able to increase numbers of follicles in heifers and that *Bos indicus* heifers have greater numbers of antral follicles detectable by ultrasonography; therefore, it was logical to hypothesize that it would be possible to identify chromosomal regions associated with antral follicle numbers. Our hypothesis was that this would aid us in identifying genes involved in establishing the number of primordial follicles, controlling primordial to primary follicle transition, stimulating granulosa

cell proliferation in secondary follicles, controlling secondary to antral follicle transition, and selection of ovulatory follicles.

We used the BovineSNP50 BeadChip to perform a genome wide association study, and identified a number of nominally significant regions of the bovine genome that associated with antral follicle number in yearling beef heifers.[23,24] From our results, the genomic heritability of the trait was 0.44, and subsequently a genetic heritability of 0.25 to 0.31 was reported in dairy heifers and cows.[25] Pathway analysis of genes with a start codon within 50,000 base pairs of a nominally significant polymorphism determined that the top upstream regulator of antral follicle count in heifers was transforming growth factor-β signaling, a result that was in strong agreement with the reported roles of anti-Müllerian hormone, growth differentiation factors, and bone morphogenic proteins in follicle development.

Nutritional programming of the bovine ovary

Variation in gene sequence does not control all of the variation observed in follicle number. Studies have provided evidence that alterations to the diet may regulate the epigenome, thereby changing gene function. This is referred to as nutritional programming. Nutritional programming of the bovine ovary seems to occur at multiple stages during development.

Maternal nutrition during pregnancy can influence follicle numbers and reproductive capacity in daughters. Daughters of dams that were fed 60% of maintenance during the first and second trimester of pregnancy had fewer antral follicles at slaughter at a year of age compared with daughters of dams fed 120% of maintenance.[26] The first half of gestation when oogonia are proliferating seems to be a particularly sensitive time for influencing follicle numbers in daughters. A high protein diet during the first two trimesters resulted in daughters with fewer primordial and antral follicles as yearlings. Thus, it may be inferred that conditions influencing forage quantity or forage quality during the first two trimesters may impact reproductive capacity of the daughters.[27] Additional research is necessary to understand the long-term impacts of such nutritional or environmental conditions on daughter performance.

When nutrient intake was limited to 75% of maintenance during the second and third trimester, no change was detected in daughter antral follicle numbers.[28] Combined with the previous results, lack of a change in antral follicle number indicates that the truly sensitive window of maternal nutritional status is during the first trimester. In this study, cows that were provided a diet containing 125% of maintenance during the third trimester produced daughters that conceived earlier in their first breeding season. Those daughters did not have any change in antral follicle counts before their first breeding season when compared with daughters from the cows fed the control diet (100% of maintenance). Protein supplementation with 0.45 kg/d of a 42% crude protein cake in the third trimester had the same influence on first service conception in daughters.[29] Supplementation with dried distiller's grains with solubles during the third trimester did not change antral follicle numbers in daughters but did improve conception to timed artificial insemination.[30] Taken together, these results indicate that maternal nutrition during the third trimester can improve daughter reproductive performance without altering daughter antral follicle number. It is not clear, however, what impact this nutritional programming might have on daughter preantral follicle numbers.

The peri-pubertal period is another window of development when the ovarian reserve can be influenced by nutrient intake. Caloric restriction between eight and 11 months of age as part of a single phase stair-step development regimen increased the number of primordial follicles, but did not change the number of antral follicles.[31] Similarly, nutrient restriction from weaning through breeding did not change antral follicle numbers in beef heifers.[32] This indicates that nutrient restriction in the peri-pubertal period may have a sparing effect on the ovarian reserve without changing the rate of primordial follicle activation or preantral follicle growth. The mechanism responsible could be a slowing of activation of primordial follicles or a stimulation of formation of primordial follicles. Although primordial follicle formation is reported to cease before birth in heifers, our preliminary data indicates an increase in the mRNA abundance of SLIT/ROBO members, specifically SLIT2, SLIT3, and roundabout, axon guidance receptor, homolog 4 (ROBO4) in the ovarian cortex of the Stair-Step heifers. Those genes are up-

regulated in the ovine fetal ovary during the time of primordial follicle formation.[33] Thus, calorie restriction may be stimulating primordial follicle formation, but further research is warranted. On-going research at the U.S. Meat Animal Research Center also is examining the reproductive longevity of heifers developed using the stair-step diet to determine how such changes in ovarian function impact production efficiency.

Conclusions

Research into mechanisms controlling establishment of the ovarian reserve and preantral follicle growth has demonstrated that gene products are involved in regulating function of the ovarian reserve. Although it is important to understand how variation in DNA sequence contributes to phenotypic differences, understanding how nutritional programming of those genes alters their function in replacement heifers may have the greatest impact on improving reproductive efficiency and the quality of replacement heifers.

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Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

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Bovine temperament impacts immunity, metabolism, and reproduction: a review

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Abstract

Temperament, or excitability, is a behavioral trait that has been shown to impact physiology and performance. Temperament in cattle alters the function of the hypothalamic-pituitary-adrenal axis, thereby influencing circulating concentrations of catecholamines and glucocorticoids. The physiological changes associated with temperament have also been reported to alter immune responsiveness and associated biomarkers. While temperament has been demonstrated to influence metabolism, which can impact growth and carcass performance, the implications of this relationship remains unclear at the present time. With regard to reproductive efficiency and progeny performance, several studies have reported that temperament not only negatively influences the reproductive system efficiency, but also the growth and performance of offspring. Overall, the literature supports the idea that cattle temperament impacts major physiological mechanisms that ultimately impact reproduction, growth, performance and profitability of beef cattle. Therefore, this review is aimed at elucidating the interactions between temperament and immunity, metabolism, performance, and reproduction in cattle.

Keywords: Temperament, reproduction, immunity, metabolism

Introduction

Cattle temperament is known to modify physiological responses with regards to immunity, metabolism, performance and reproduction that ultimately impact production parameters such as milk production, carcass yield and carcass quality. Additionally, temperamental cattle pose a risk to livestock handlers, facilities, and equipment. Temperament may be defined as the fear response or the degree of reactivity to humans or to novel environments. Cattle classified as temperamental have been reported to exhibit reduced weight gain[1] and decreased hot carcass weights.[2] Other production parameters including carcass performance are also hindered in temperamental cattle such as decreases in tenderness[3] and milk yield[4] and increases in carcass bruising.[5] Differences in cattle temperament may become more pronounced during stressful events, and these are detectable by visual observation, as well as physiologically. During illness or an immunological insult, more temperamental cattle do not display as dramatic signs of illness as calmer cattle.[6] Curley et al[7] reported differences in stress hormone concentrations, specifically cortisol, between calm and temperamental cattle. With respect to production parameters and animal health, it is essential that producers minimize the negative physiological impacts of stress to maintain and enhance growth performance and animal health.

Separation of cattle into temperament categories may be accomplished in a variety of ways. However, two of the most common ways are exit velocity (i.e., flight speed) and pen score (Figures 1 and 2). Exit velocity is an objective measurement of temperament that measures the time it takes the animal to traverse a set distance upon release from a squeeze chute.[1] In this manner, an animal with a slower exit velocity is considered more calm than an animal with a faster exit velocity (temperamental). Pen score is a subjective measurement of temperament and is measured by separating small groups of cattle (n = 3 to 5) in a pen and ranking them on a scale of 1 (calm) to 5 (very temperamental) based on their response to a human observer.[8] The pen score and exit velocity of each animal can be averaged to determine a temperament score, comprising elements of both the subjective and objective measures. Schmidt et al.[9] reported that temperament is moderately heritable between generations of cattle. While temperament is not only heritable, epigenetic effects can also play a role in temperament such as the age of the dam, the age of the calf at weaning, environmental events, the herd in which they reside, breed, sex, and other factors such as human acclimation.[10-12]

The effects of cattle temperament can be further elucidated when specific physiological systems in the animal are evaluated. Temperament has been reported to impact immune function in cattle.[13] Cattle temperament has also been reported to influence optimal metabolism and nutrient partitioning.[14] Additionally, temperament may influence cattle reproductive physiology and subsequently their offspring.[15] Therefore, temperament can play a pivotal role in the health and performance of cattle, and this review is aimed at elucidating some of the interactions of temperament associated with immunity, metabolism and performance, and reproduction.

Immunity

The effects of temperament on immunity in cattle have not been fully elucidated. Historically, the prevailing opinion has been that temperament negatively impacts the immune system due to increased basal concentrations of stress hormones exhibited by temperamental cattle. However, more recent research both supports and refutes this idea. In response to stress and activation of the hypothalamic-pituitary-adrenal (HPA) axis, temperamental mice have been reported to over-express phenylethanolamine-n-methyl transferase which coverts norepinephrine to epinephrine, thereby leading to more aggression.[16] Similarly, more temperamental cattle have been found to have greater basal concentrations of circulating cortisol in comparison to calmer cattle.[17-19] Consequently in mice, Cavigelli et al.[20] reported that aggressive mice exhibit increased corticosterone concentrations and decreased tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, thus suggesting that temperament negatively impacts immune function. Curley et al.[18] also reported that there was a positive correlation between temperament (measured via exit velocity) and cortisol concentrations. Furthermore, Fell et al.[17] reported that temperamental cattle entering the feedlot had significantly greater concentrations of circulating cortisol. After transportation, temperamental Brahman bulls exhibited increased cortisol in comparison to their calmer counterparts; however, TNF-α was unaffected by temperament.[13] Generally, temperamental cattle have differential responses to stressors when compared to calmer cattle, and this is evident by increased concentrations of cortisol and catecholamines and further by the decreased stress hormone responses when challenged with corticotropin releasing hormone or adrenocorticotropic hormone.[7,21]

A major factor in determining whether or not to treat sick cattle is their behavioral sickness response, which is a basic visual observation of the general health of the animal. Animals respond to illnesses in different ways and their response is generally correlated with physiological changes within the animal, such as body temperature.[22] In a study by Burdick et al., temperamental animals displayed diminished sickness behavior compared to their calmer cohorts.[6] Though unpublished, this phenomenon has been observed in other studies in which cattle experienced an immunological insult. Thus, since temperamental cattle do not display as dramatic visual signs of illness or sickness behaviors, they may be passed up for treatment when they are ill. Untreated temperamental cattle may represent morbidity and mortality in a herd or pen and may function to further infect the herd.

Temperament may also impact immune function in response to vaccination. Oliphint reported that temperamental calves had a diminished immunological response to Clostridial vaccination.[23] Specifically, Oliphint reported that temperamental Brahman bull calves had decreased lymphocyte proliferation and vaccine-specific IgG concentrations in comparison to calm bull calves. Bauer et al. similarly reported that temperament was negatively correlated with circulating concentrations of IgG and the ability of lymphocytes to produce IgM and proliferate.[24] Therefore, it has been speculated that temperament may negatively influence adaptive immunity. While the inherent differences in temperamental cattle may be partially beneficial with regard to regulation of the innate immune system, these apparent beneficial effects do not necessarily carry over to the adaptive immune response after vaccination.

Temperament, according to the literature, seems to have an overall negative impact on acute stress; however, little is known about the role temperament plays in chronic stress. As mentioned previously, more temperamental cattle have elevated basal circulating stress hormone concentrations. If one attempts to elucidate how temperament, specifically elevated concentrations of cortisol, impacts chronic stress, one may note that induction of chronic stress in mice has resulted in glucocorticoid

resistance[25] which may enhance the ability of an animal to clear bacteria from superficial wounds and heal faster. However, the relationship between hormonal changes associated with temperament, and the subsequent impact on overall cattle immunity and health have not been fully elucidated.[26] Thus, cattle temperament may differentially affect aspects the immune response of cattle, and further investigations are needed in order to reveal and understand the mechanisms of immune modulation within both the innate and adaptive immune systems in concert with temperament.

Metabolism and performance

Temperament has been demonstrated to have effects on metabolism and overall production. Previous work[27] reported that temperament was related to energy requirements; temperamental cattle required more energy to sustain maintenance and growth because of their inefficiency to metabolize and utilize nutrients. Other studies report that temperamental cattle have decreased average daily gains (ADG)[28,29] and calmer cattle exhibited greater body condition scores than more temperamental cattle.[30] One might assume that the activity and/or arousal level of calm versus temperamental cattle could impact their overall energy requirements. This supports the inefficiency data associated with temperamental cattle. An additional aspect associated with efficiency is found in reports that temperamental cows have been observed to produce less milk compared to less temperamental cows.[4,31]

In addition to overall performance, temperament may also play a role in terms of meat quality. Temperament has been reported to decrease overall carcass fat[32] and hot carcass weight,[19] which can decrease carcass value. Furthermore, more temperamental cattle have been observed to produce meat that is less tender in comparison to their less excitable cohorts.[19,33] Voisenet et al.[3] reported that temperamental cattle exhibited darker lean color with greater pH (i.e. dark cutter) which can be detrimental to fresh meat palatability. Off-flavor development (i.e. non-beef like flavor profiles) has also been correlated with temperament when evaluated by taste panelists.[19]

The mechanisms by which temperament influences performance have not been fully elucidated; however, glucocorticoids may be primary factors that impact metabolic changes between these animals.[14] Cortisol, the primary glucocorticoid hormone released by cattle in response to stress during activation of the HPA axis, can influence glycogen release and the production and allocation of glucose within the body. As stated previously, more temperamental cattle have been reported to have greater concentrations of stress hormones such as catecholamines and cortisol.[7,18,19,21] Bradbury[14] reported that temperamental cattle had increased circulating concentrations of insulin and glucose compared to their less excitable counterparts. During a glucose challenge, Bradbury[14] observed a tendency for increased cortisol and insulin concentrations in temperamental cattle. The results of a glucose challenge test suggest that temperamental cattle do not utilize glucose as efficiently as calm cattle, which may thereby alter performance and growth. Bradbury also observed insulin resistance in temperamental heifers which may hinder performance.[14] Temperamental cattle have been reported to maintain greater concentrations of non-esterified fatty acids (NEFAs) in comparison to calmer cohorts, and these differences may impact glucose regulation and utilization.[34,35] In the same studies, Burdick Sanchez et al.[34,35] reported decreased blood urea nitrogen (BUN) and insulin sensitivity in more temperamental steers. These data suggest that temperament may impact nutrient utilization and repartitioning of energy, specifically in relation to what types of tissues are catabolized for energy and maintenance. The endogenous hormonal and physiological differences exhibited by temperamental cattle may contribute to metabolic differences between calm and temperamental cattle. Overall, the effects of temperament on metabolism and performance may be detrimental to feedlot performance, carcass merit, and meat quality, thus decreasing profitability of temperamental cattle in comparison to calmer cattle.

Reproduction

Cattle temperament can impact reproductive performance and may ultimately influence offspring performance either epigenetically or through heritable traits. Reproductive performance and efficiency is a critical component needed to profitably sustain a viable cow-calf operation in the U.S.[15] A study by Cooke et al.[15] reported a negative correlation between temperament and artificial insemination conception

rates of beef cows. Additional studies have also reported decreased conception rates in more nervous cattle.[36-38]

Temperament may impact reproduction via multiple mechanisms. Specifically, temperament may inhibit feed intake and optimal metabolic efficiency.[39-40] Additionally, cortisol and other stress hormones may alter reproductive physiology by altering the mechanisms involved in ovulation and conception through physiological pathways.[41] Cortisol, and its association with stressors, is known to influence reproduction beginning at the level of the hypothalamus.[42] Specifically, elevated cortisol concentrations may inhibit the release of gonadotrophins from the anterior pituitary gland.[43,44] Typically, short-term or acute stress has little impact on reproduction while chronic stress may interfere with various reproductive processes,[42] which may explain why temperamental cattle with chronically elevated basal cortisol have decreased reproductive efficiency. Echternkamp reported that calmer cattle have reduced basal cortisol concentrations and increased concentrations of luteinizing hormone which enhances the onset of puberty and ovulation.[45] In concert with findings from Echternkamp, Cooke et al. reported that calmer heifers reached puberty earlier in life when compared to more temperamental heifers.[15] However, it is important to note that Dobson and Smith[46] reported that stress, regardless of animal temperament, inhibited fertility and oocyte production.

Not only does temperament play a role in conception, but temperament may also impact the offspring of temperamental cows. Temperamental cattle may have offspring with decreased body weights,[1] while Phocas et al.[47] suggested that a calm temperament in dams was correlated with enhanced calf performance. Additionally, milk yield may be increased in calmer dams, which may partially explain one of the mechanisms that contribute to calf performance.[47] While temperament has been reported to be detrimental to reproductive performance, acclimation to human handling may reduce the hormonal stress response in heifers and mitigate some of the negative effects of temperament on reproduction.[45,48,49] It is noteworthy to mention that while some of the effects on reproduction may be slightly mitigated through acclimation, it is important to remember that this acclimation is only applicable to a specific situation/environment, and any alteration in that environment may reduce the positive effects of acclimation.

Conclusion

Temperament has broad effects on cattle physiology ranging from immunity to metabolism to reproduction. All of the factors associated with temperament ultimately impact growth performance, carcass and meat quality, reproductive efficiency, and health, all of which ultimately impact profitability and the sustainability of cattle-based operations from conception to consumption. While most of the aspects associated with cattle temperament imply negative implications with regard to overall production, the stress and metabolic alterations associated with temperament may play a pivotal role in priming the innate immune system. Priming the innate immune system may promote pathogen clearance, which decreases the duration of infection and may have positive implications on performance, health, and overall hardiness. Future research efforts must focus on elucidating the mechanisms associated with these physiological differences between calm and temperamental cattle. Furthermore, research should focus on management strategies to alleviate the negative impacts of temperament on reproduction and growth.

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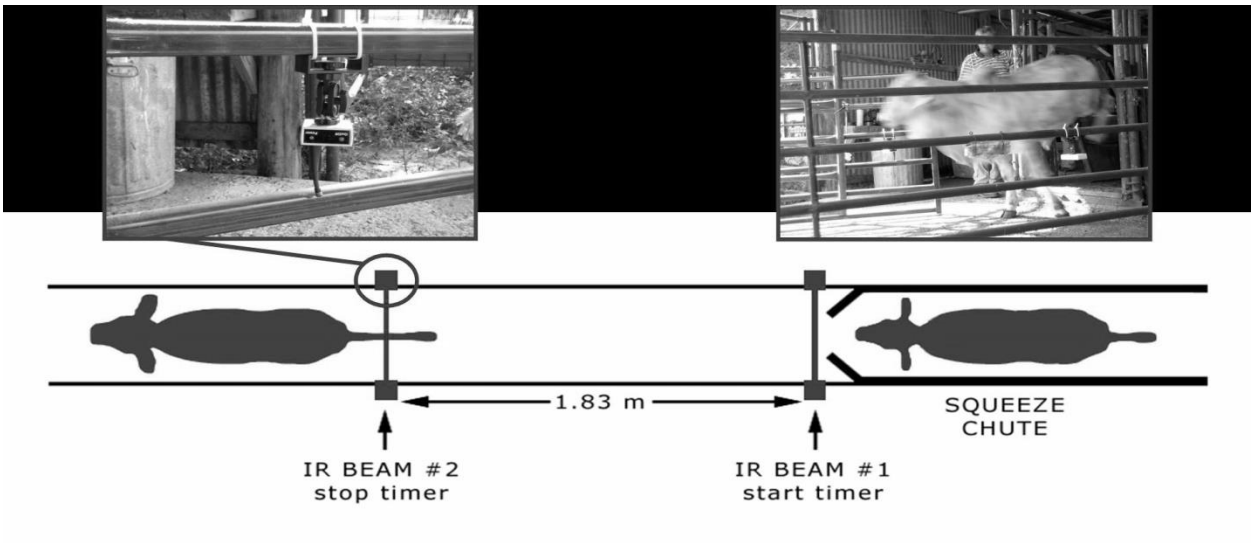
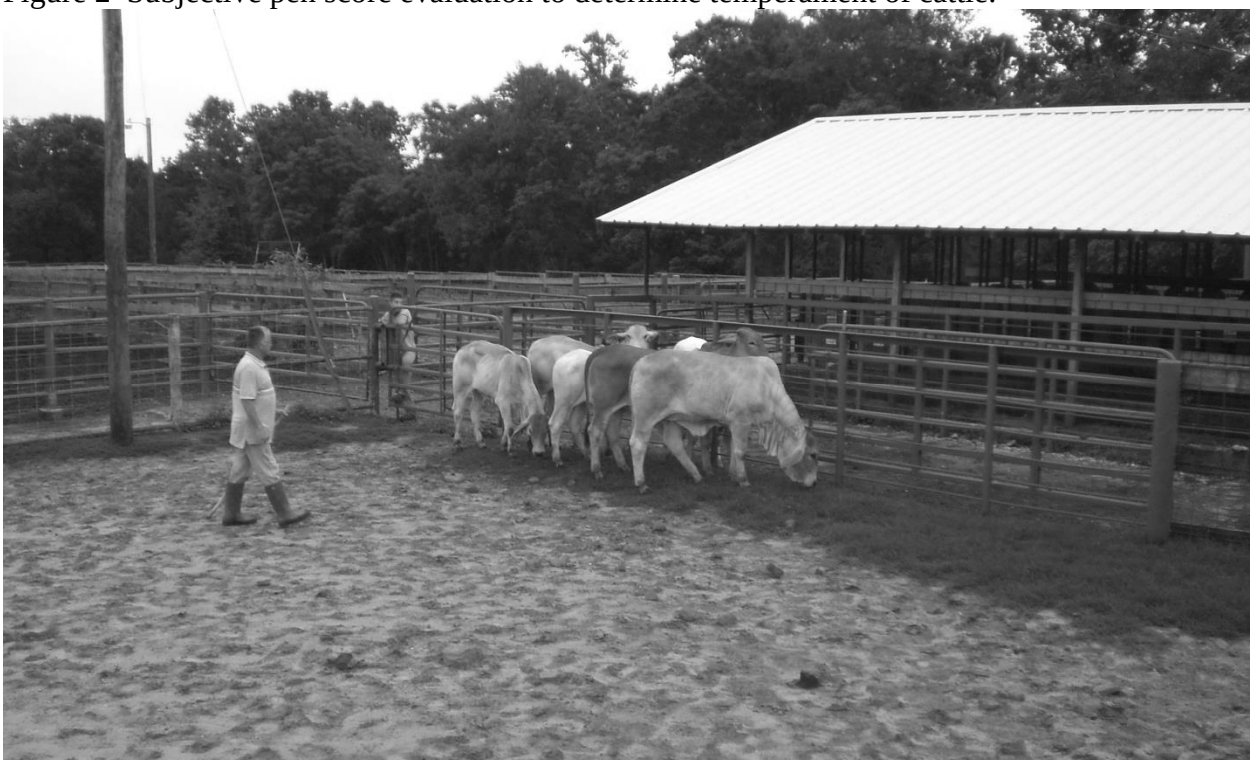
Figure 1. Example of objective (exit velocity) evaluations to measure cattle temperament*



* The exit velocity method of classifying temperament measures the time it takes for cattle to travel a set distance after being released from a working chute or handling equipment.

Figure 2 Subjective pen score evaluation to determine temperament of cattle.†



† The pen scoring method assesses the response of cattle to a human observer.

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Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

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Diagnostic techniques for assessing bull infertility

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Abstract

Investigating causes for bull infertility requires thorough understanding of the anatomy and physiology of erection, coitus and ejaculation. This presentation will review techniques not commonly used during a routine breeding soundness examination for exploring reproductive failure in bulls. Additionally limited therapeutic options will be discussed for management of chronic seminal vesiculitis as well as use of the pudendal nerve block to assist with extension of the penis of the bull.

Keywords: bull, breeding, erection, infertility, cavernosography

Introduction

Erection in the bull occurs when blood flow increases in the deep artery of the penis and into the crus penis and subsequently into the corpus cavernosum penis (CCP) following olfactory or visual sexual stimulation. The CCP in the bull is a closed system in that erectile blood flows into the penis from the crus and leaves this same area during detumescence following erection. The stimulation that causes this reflex dilation of the deep artery of the penis also causes relaxation of the retractor penis muscles which hold the penis in the preputial cavity. As the retractor penis muscles relax, the sigmoid flexure relaxes and the mildly engorged penis protrudes from the sheath. With continued sexual stimulation the ischiocavernosus muscles (ICM) begin rhythmic contraction which raises blood pressure from the normal resting state of 15 mmHg within the CCP. Peak pressure within the CCP may be greater than 14,000 mm Hg. This rapid increase in blood pressure within the CCP causes complete penile extension and erection. Following ejaculation the ICM relax, detumescence occurs as blood pressure within the CCP decreases and the penis is withdrawn back into the preputial cavity.[1-6]

Erection may be induced in the bull with an ejaculator although the optimal method for evaluating erection is with observed test mating. Normal function of the penile nerves is essential for coitus and is most accurately assessed by observed test mating or by semen collection by artificial vagina.[7]

Test mating

Bulls with erectile dysfunction do not achieve sufficient erection pressure to complete coitus.[6-8] Bulls with nerve dysfunction mount the cow but there are no penile searching motions near the vulva and the bull fails to make intromission.[9,10] Usually the penis is placed along the cow's hip or below the vulva in the escutcheon area above the cow's udder.

Semen collection with an artificial vagina

Semen collection with a properly prepared artificial vagina (AV) can confirm that a bull has the sufficient erection and sensation to ejaculate. The temperature within the AV should be 45 to 50°C and sufficiently filled to provide mild pressure on the erect penis. Most reasonably docile beef bulls can safely be collected with an AV as they mount a female in estrus.[9] Failure to ejaculate into the AV could result from in sufficient pressure within the AV or in appropriate liner temperature for that particular bull. More likely failure to ejaculate would be due to a painful condition of the back or hind limbs or to lack of nerve sensitivity of the dorsal nerves of the penis.

Contrast cavernosography for evaluation of the erection failure or penile deviation

Contrast radiography of the corpus cavernosum penis may confirm vascular defects in the penis.[10-12] The procedure is most easily accomplished with the bull restrained on a table in lateral recumbency. Manually extend the penis and place a towel clamp under the dorsal apical ligament

approximately 6-10 cm from the distal end of the penis to aid in manipulation of the penis. Percutaneously place a double strand of heavy suture (0.6 mm) between the retractor penis muscles and the penis to retract the penis away from the abdominal wall in order to enhance visualization of the sigmoid flexure of the penis. The radiographic series will consist of two or three film exposures taken as quickly as practicable progressing from the free portion to the distal bend of the sigmoid flexure.

On the dorsum of the penis near the towel clamp insert a 16-gauge x 3.8 cm needle at a 45^0 angle proximally through the tunica albuginea and into the CCP. After attaching a sterile extension set to the needle for ease of injection and to position the hands away from the radiographic field inject 10 ml sterile saline which should flow into the CCP with ease. Place a radiographic cassette under the penis then rapidly inject 15 ml of water-soluble radiographic contrast medium (Renografin 76, Squibb Diagnostic, New Brunswick, NJ) and expose the film. Slowly inject an additional 15 to 30 ml of medium as the radiographic series is performed. Remove the cassette and quickly place another cassette more proximal under the penis. By using 43 cm-long cassettes the entire penis up to the sigmoid flexure may be radiographed with two or three exposures. Ideally all radiographic exposures should be completed within 60 seconds.

The normal bovine penis has no vascular communications from the CCP to peripenile vasculature.[10-12] Presence of contrast medium outside the CCP is evidence of a vascular shunt as a potential cause for erection failure. Alternatively, failure of complete filling of the vascular spaces within the CCP may indicate fibrosis or failure of proper development of the internal architecture of the penis. These conditions usually result in partial erection failure or deviation of the erect penis.

Erection failure due to corpus cavernosal shunts

Congenital vascular shunts

Occasionally young bulls fail to achieve intromission due to congenital corpus cavernosal vascular shunts. These bulls usually are normal on physical examination but fail to achieve adequate intracorporeal pressure for erection. When observed during erection, either by test mating or with electroejaculation the free portion of the penis becomes noticeably bluish during attempted erection. The bluish discoloration is due to blood from a relatively porous tunica albuginea of the penis exiting the corpus cavernosum penis and being removed by subcutaneous capillaries and veins. These shunts may be confirmed by cavernosography. Typically the shunts are multiple and not considered repairable.

Acquired vascular shunts

The most common cause of acquired corpus cavernosal shunts is penile hematoma due to rupture of the tunica albuginea of the penis on the dorsum of the distal bend of the sigmoid flexure.[8,11] Shunts in this area of the penis may be surgically repaired thereby restoring a bull's ability to achieve erection.

Electro diagnostics for evaluation of penile nerves

Determination of sensory nerve conduction velocity is a well-established modality for evaluating peripheral neuropathy. Injury of the dorsal nerves of the penis may occur during or after rupture of the tunica albuginea of the penis at the distal bend of the sigmoid flexure or due to trauma elsewhere along the course of these nerves along the penis.[15]

The procedure is most easily accomplished with the bull restrained in lateral recumbency. Sedation or anesthesia is not required and most bulls tolerate the procedure very well. Manually extend the penis and place a gauze loop around the glans penis to hold the penis in extension for approximately 15 minutes while the procedure is conducted. Place two spring-type ring electrodes 2.0 cm apart around the middle third of the glans penis. The proximal electrode is the cathode and the distal electrode the anode. Place a 1cm disk electrode as a ground 2.o cm proximal to the stimulating electrodes. Electrode conductivity gel is applied under each electrode.

Recording electrodes are paired needle electrodes placed 1 cm apart at three sites along the penis. Insert the needle electrodes through the skin to the dorsum of the tunica albuginea ensuring that the tip is

near the dorsal nerves of the penis. The distal pair of needles is inserted one half the distance between the urethral orifice and the attachment of the prepuce to the free portion of the penis, the middle site is one half the distance between the attachment of the prepuce to the insertion of the retractor penis muscles at the distal bend of the sigmoid flexure, and the proximal site is just proximal to the distal bend of the sigmoid flexure.

Mean conduction velocity is 55.1 $\pm$ 5.1 m/s for normal bulls.[15] This procedure can confirm loss of innervation of the dorsal penile nerves and also may localize the lesion on the nerves. If the denervation involves the glans or distal few centimeters of the penis the bull will be incapable of intromission.[7] However, if innervation is intact to distal end of the prepuce and proximal few centimeters of the penis the bull should be able to ejaculate into an artificial vagina for semen collection for cryopreservation.[9]

Pudendal nerve block to assist penile extension

The internal pudendal nerve is made up of fibers originating from the ventral branches of the third and fourth sacral and the pelvic splanchnic nerves. Achieve caudal epidural anesthesia then introduce the hand into the rectum to the depth of the wrist and direct the fingers laterally and ventrally to locate the lesser sacrosciatic notch and foramen by rectal palpation. Locate the internal pudendal artery by its pulsations at the cranial angle of the notch and palpate the pudendal nerve approximately 1 cm caudodorsal to the artery. Insert an 18-gauge, 10 cm spinal needle through the skin in the ischiorectal fossa beside the tail and direct the needle forward and slightly ventrally to a depth of 5 to 7 cm. Palpate the tip of the needle through the rectal wall and direct the needle in the direction of the nerve in the foramen. Inject approximately 10 ml of 2% lidocaine hydrochloride along the nerve then withdraw the needle 2 to 3 cm and inject an additional 10 to 15 cm at the cranial border of the foramen to desensitize the muscle branches of the rectal nerve. Repeat the procedure on the opposite side of the pelvis.[16,17]

The advantage of the technique is that the penis and prepuce are easily extended by this procedure and the animal can remain standing. A disadvantage of the technique is that a bull will not be able to retract the penis and prepuce for approximately 30 minutes following the procedure.[17]

Infrared thermography

Infrared thermography provides a non-invasive measure and map of skin surface temperatures.[18] Skin surface temperatures vary according to blood flow regulation to the skin surface and are affected by both internal and external factors. The cutaneous circulation is under sympathetic vasomotor control and peripheral nerve injuries and nerve compression can result in skin surface vascular changes that can be detected by infrared imaging. Inflammation and nerve irritation may result in vasoconstriction, causing cooler thermograms in the afflicted areas. Transection of a nerve and/or nerve damage to the extent that there is a loss of nerve conduction results in a loss in sympathetic tone causing vasodilatation indicated by an increase in the thermogram temperature.

This technology is useful for localizing neuromuscular or vascular pathology and may be particularly helpful when examining a bull for pain in the back or hind limb area. This technology is also useful for graphically depicting issues with scrotal or testicular thermoregulation which may lead to impaired spermatogenesis.[18,19]

Treatment of chronic seminal vesiculitis

The paired seminal vesicles of bulls are 2 to 4 cm wide and 10 to 15 cm long and are located on the pelvic floor lateral to the ampullae and dorsal to the neck of the bladder. The glands are lobulated and secrete a clear fluid, containing nutrients and buffers, which is discharged immediately before and during ejaculation through ducts that open into the urethra adjacent to the colliculus seminalis.[20]

Inflammation or infection of the vesicular glands is fairly common in young bulls housed together on and high energy diets. These peripubertal bulls may spontaneously recover from this condition or respond well to antimicrobial treatment. However, aged or chronically infected bulls rarely recover from seminal vesiculitis Based on clinical and abattoir evaluation of reproductive tracts of bulls the prevalence

of infection of the seminal vesicles is reported to range from less than 1 percent to greater than 9 percent. Peripubertal bulls may spontaneously recover from septic seminal vesiculitis, but aged or chronically infected bulls rarely recover.[21-23]

Bulls with septic seminal vesiculitis are often classified as unsatisfactory potential breeders as their semen may be grossly contaminated with exudate and blood, but often, red blood cells and white blood cells can be detected only microscopically.[2] Abnormal concentrations of polymorphonuclear cells (PMNs), poor sperm motility, low fructose concentrations, and an elevated seminal pH are characteristics of semen of bulls affected with vesiculitis. Semen of bulls with septic seminal vesiculitis freezes poorly, and antibiotics used in extenders often do not significantly diminish the large number of bacteria in the ejaculate. Although chronic, unresponsive, septic seminal vesiculitis does not occur commonly in breeding bulls, the economic impact of this disease is considerable. The greatest economic loss associated with septic seminal vesiculitis occurs in bulls whose genetic value qualifies them for inclusion in an artificial insemination program.

The prognosis for bulls with chronic septic seminal vesiculitis is guarded at best. Prolonged antimicrobial therapy is often unsuccessful and complete surgical removal of affected glands is technically difficult.

The author has successfully treated bulls with chronic seminal vesiculitis by chemical ablation of the glands with 4% formaldehyde.[24] Restrain the bull in a chute and achieve caudal epidural anesthesia and introduce the hand into the rectum and identify the vesicular gland. Approximately 4 to 6 cm ventrolateral to the anus on the side adjacent to the infected vesicular gland introduce an 18 gauge x 30 cm stainless steel needle through the skin parallel to the rectum to a depth of 10 to 12 cm. With the aid of the hand in the rectum advance the needle and guide the tip into the vesicular gland. Inject 10 to 15 ml of sterile saline into the gland and palpate for gland enlargement to verify needle placement. Inject 4% formaldehyde into the gland until swells and its surface is quite firm. Withdraw the needle and repeat the procedure on the opposite vesicular gland if indicated. Immediately administer flunixin meglumine as bulls so treated frequently display signs of abdominal pain following the procedure. Allow sexual rest for 45 to 60 days before examining the ejaculate for evidence of seminal vesiculitis.

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Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

Lameness in breeding bulls

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Abstract

Lameness or structural unsoundness often prevents a bull from being classified as a Satisfactory Potential Breeder. This presentation will review the anatomy and etiology of common causes of conformation flaws and lameness in breeding bulls. Lameness accounts for tremendous production loss in the cattle industry and has been identified as a particular concern in animal welfare. Cattle are relatively stoic animals and often do not show lameness until significant pathology is present. This discussion will explore anatomic and management relationships with common orthopedic conditions of the back, hip, stifle and feet in cattle. Additionally, diagnostic and therapeutic options will be reviewed.

Keywords: Bull, lameness, hoof, claw, stifle, fibroma

Introduction

The Computer Generated Breeding Soundness Evaluation (CG-BSE or eBSE) form was introduced at the Society for Theriogenology Annual Conference.[1] The ability to capture photographs of the bull onto the form is a valuable asset in documenting conformation, body condition and structural soundness of that animal.

In 2014, Dr. Robert Carson reported on trends of bull breeding soundness examinations utilizing records over a twenty year period.[2] For comparison, three time periods were evaluated: 1993-1995, 2006-2008, and 2009-2013. Data from bulls examined between 2009 and 2013 were put into the eBSE. Evaluation of those records between 1993 and 2013 revealed 5.4% to 10.4% of bulls presented to the Auburn University Large Animal Teaching Hospital were classified as unsatisfactory or deferred because of physical conditions. A large portion of these bulls were either lame or had conformational flaws that prevented them being classified as Satisfactory Potential Breeders. This paper will review the most common causes of lameness or conformational flaws in bulls.

Anatomy and conformation

Lameness in cattle is frequently associated with conformation flaws that create abnormal forces on the back, limbs and feet.[3-7] Additional causes of lameness are due to trauma or degenerative changes associated with heavy weight and aging. Conformation describes the dimensions and shape of an animal, and deviation from ideal conformation may lead to unsoundness as animals grow due to abnormal stressors on bones, tendons, ligaments and joints.[4] Posture refers to the manner in which an animal stands, and gait refers to the manner in which an animal moves.

When cattle are viewed from the rear, they should stand with the hind feet approximately as far apart as their hips. It is unusual to see cattle that are base-wide, that is, their feet are farther apart than the width of their hips; however, cattle that are base narrow with their feet closer together than the width of their hips are quite common, especially among beef breeds.[6]

Screw claw

Cattle that are base-narrow in their rear limbs frequently develop screw claw as the animal grows. Due to the conformation and abnormal forces on the hind feet, the tendency is for the hind feet to tend toward supination and the hoof wall of lateral claw (abaxial) hoof wall grows under the hoof displacing the sole dorsomedially.[5,7] Cattle may develop bruises on the sole of that lateral claw which frequently lead to sole ulcers or subsolar abscesses. Likewise, due to abnormal lines of stress on the hoof during weight bearing, vertical cracks frequently develop in the axial hoof wall. Additionally, degenerative arthritis frequently develops prematurely in the coffin, pastern and fetlock joints of affected animals.[7] This condition is common in continental breeds of beef cattle and their crosses and, in the opinion of the author, is becoming more prevalent in other beef breeds as greater selection is being applied to enhance

musculature of the beef carcass. Although the classic twisted or cork screw claw frequently does not develop until the animal is two or more years of age, the conformation leading to this condition is evident at weaning. Animals with this conformation are usually heavily muscled and base narrow when viewed from the rear.

When viewed from the side, there should be obvious but not excessive angulation of the joints of the rear limb. There should be approximately 130^{o} between the femur and tibia and approximately 145^{o} of angulation between the tibia and the metatarsus with the tuber calcis directly below the caudal aspect of the tuber Ischia . Animals with excessively straight rear legs (post legs) are more prone to develop joint, tendon and hoof diseases.[7] In the author's experience, large breed beef cattle with this conformation are more likely to develop cartilage disease in the stifle than more moderate conformation cattle.

Front limb conformation is also correlated with soundness. When viewed from the front the distance between the feet should be slightly less than the width of the shoulders, and the hooves should point straight ahead. Cattle that have toes that point out (laterally) are more likely to develop abnormal hoof growth similar to screw claw in the rear hooves. The adjacent toes on one hoof should be equal length, and the distance from the coronary band to the sole at the heel should also be equal.[7]

Laminitis

Chronic or sub-clinical laminitis is quite common in beef cattle due to the common practice of high concentrate feeding to achieve rapid growth and high yearling weights. Clinical or sub-clinical laminitis in cattle may cause an array of hoof problems and is frequently the predisposing cause of lameness.[8-10] Cattle develop overgrown "slipper" hooves and frequently suffer vertical or horizontal fissures in the hoof wall due to loss of flexibility of the hoof. Subsolar hemorrhage, bruising and ulceration are frequent sequelae to chronic laminitis. Additionally, the affected hooves often grow excessively long which changes the angles of the coffin, pastern and fetlock joints thereby leading to abnormal stresses on these joints and their supporting soft tissue structures. These hoof growth changes lead to white line disease and separation of the hoof wall from the lamina of the hoof. Premature degenerative joint disease is a common occurrence in severely affected cattle.

Interdigital fibroma

Interdigital fibromas, also called interdigital hyperplasia or corns, are proliferative growths of the skin of the interdigital space caused by dermatitis or chronic irritation.[8,10] The condition is more common in bulls than females and more common in heavy versus lighter weight animals. *Bos indicus* crossbred cattle appear to have a higher incidence of interdigital fibroma than *Bos taurus* cattle. Cattle that have a very wide interdigital space and cattle that are extremely narrow in the interdigital space appear more at risk for development of this condition than cattle with normal interdigital conformation. Rarely does the problem develop in cattle less than two years of age and most animals presented for treatment are four to seven years of age. It is troubling that in recent years significantly more two year-old Angus bulls have been presented to our teaching hospital for removal of these growths.

Treatment for interdigital fibroma involves surgical excision of the hyperplastic tissue utilizing local or regional anesthesia.[10] After thoroughly cleansing the area for aseptic surgery, grasp the apex of the interdigital mass with towel forceps. Begin on the dorsal surface and make a longitudinal skin incision along each side of the mass being careful to preserve the axial coronary band. Continue the dissection caudally until the entire hyperplastic tissue is removed. Remove any protruding interdigital fat with blunt dissection and apply topical antibacterial powder on sterile non-adherent surgical gauze over the incision. Wire the toes together to hold the bandage and to prevent separation of the claws. Remove the bandage in approximately five days and continue to confine the animal to a dry area for another two weeks. Systemic antibiotics are rarely indicated unless the interdigital fat pad is infected prior to surgery. Some surgeons routinely remove the interdigital fat pad; although, this technique slightly prolongs postoperative healing.

Stifle injuries

Stifle injuries are common in cattle, and one or more structures may be involved.[11] Rupture of the collateral ligament produces the least degree of lameness, and cattle with this condition are only slightly lame. Cattle with stifle injuries are generally reluctant to kick and are easier to examine than an animal without injury in this joint. The injury is most easily diagnosed by watching them walk away from you to observe instability during the weight bearing portion of the stride. Medial-to-lateral instability will cause the stifle to deviate either medially or laterally toward the affected side when the animal is bearing full weight. While standing behind the restrained animal, place fingers of one hand on the medial aspect of the stifle joint while abducting the lower limb. If the medial collateral ligament is torn there will be excessive joint space while the leg is abducted. Place the fingers of one hand on the lateral aspect of the stifle and adduct the lower limb to examine for excessive motion if the lateral collateral ligament is torn. The torn collateral ligament may be visualized by a skilled ultrasonographer.[12]

Meniscal injuries cause the next most severe degree of lameness in cattle. The most common meniscal injury is similar to other species in that the posterior horn of the medial meniscus is injured more commonly than the lateral meniscus. With acute injury, there may be evidence of joint effusion. Lameness will be evident during weight bearing and because the animal does not advance the limb normally while walking. The injury appears to occur more commonly in heavy muscled beef bulls than in other cattle. There may be an audible or palpable "click" during the weight-bearing portion of the stride. This damaged meniscus may be visualized by a skilled ultrasonographer.

The third common and most severe stifle injury is rupture of the anterior crucial ligament (ACL). This injury causes marked lameness and usually obvious joint effusion. The animal is very reluctant to bear weight on the affected limb, and the mass of the animal usually precludes palpation of the classical anterior drawer sign as may be detected in dogs. However, many beef cattle with this injury will tolerate flexion of the affected limb whereby the veterinarian may be able to detect excessive motion in the stifle joint and perhaps grating of bony surfaces due to loss of articular cartilage.

These stifle injuries are discussed together as they all appreciably shorten the productive life of cattle. Additionally, animals with an initial collateral ligament tear may quickly develop degenerative joint disease due to joint instability and abnormal wear of joint surfaces. Cattle with an initial meniscal tear likewise have the added risk of suffering cruciate ligament tears due to the atrophy of leg muscle that frequently and rapidly accompanies this injury and more severe loss of stability of the stifle joint. Cattle with cruciate ligament tears suffer severe joint instability, rapid muscle atrophy and frequently quickly develop meniscal tearing and loss of articular cartilage.

Therapy for any of the above conditions consists of confining the animal to a stall or small paddock that is level and free of mud for six to eight weeks. Bulls with anterior cruciate ruptures should not be used for breeding for a minimum of six months. Animals with this injury usually do not return to soundness and have permanent muscle atrophy on the injured limb. Analgesics are not recommended during the acute phase of the injury as animals so treated may use the limb excessively and sustain additional trauma to the joint. However, anti-inflammatory agents, joint lavage, polysulfated glycosaminoglycans and other therapies utilized in management of joint injuries in the equine athlete may prove beneficial in conjunction with a few months convalescence to assist a bull through a breeding season.

Shoulder injuries in cattle

Fortunately shoulder injuries are relatively uncommon in breeding cattle. Fractures or bruises of the shoulder are occasionally encountered in lightweight cattle while being worked in a chute which is usually due to excessively wild or excited cattle and/or inadequate footing, maintenance, or design of the working chute.

Fractures of the scapula or humerus occasionally result from bulls fighting. These injuries are readily diagnosed by the degree of lameness and swelling accompanying the injury. The spine of the scapula, humerus and shoulder joint are difficult to palpate in heavily muscled beef cattle, especially beef

bulls. Additionally, quality diagnostic radiographs are difficult to obtain due to the size and conformation of these animals.

Fortunately, fractures of the scapula or humerus often heal with stall rest in beef cattle. Contracture and swelling of the heavy muscles of these animals serve to reasonably splint the injured bones. These animals should be confined to a stall for a minimum of eight weeks followed by at least four months confinement in a flat paddock area. We do not recommend analgesic therapy as freedom from pain may induce the animal to excessively use the injured limb creating additional traumatic injury and potentially further displacing bone fragments.

Spinal injuries or disease

Diseases or injury of the spine are common among large bulls. Discospondylosis or spondylosis is commonly caused by repetitive trauma and increases with age and activity. The resulting, severe proliferative bony and fibrotic arthritis of the intervertebral joints may cause nerve root entrapment, and potentially spinal compression and pain. This condition may be confined to only one intervertebral joint but more often affects several in varying stages of progression. The hind limbs and spine are mainly affected. The proliferative bone growth may form arthritic bridges between vertebrae. These bony bridges may fracture, producing an acute crisis episode of pain or weakness. Probably less likely, fibrocartilagenous emboli may enter the blood stream resulting in stroke-like symptoms of the spinal cord.

Another spinal cord condition which may affect breeding soundness is spastic syndrome which is a latent recessive condition generally developing at two to seven years of age.[13] This syndrome is characterized by spastic contractions of the muscles of the hind limbs and back. These contractions are often mild and are most evident when the animal first rises after lying down. The syndrome usually persists for the lifetime of the animal and contractions are often exacerbated by arthritis or other painful conditions. In some bulls, the contractions tend to progress to more frequent and more severe episodes in the standing animal. Spastic syndrome condition is probably inherited as a single recessive trait with incomplete penetrance.

Conventional radiography or nuclear scintigraphy or bone scan may be useful for identifying or localizing lesions in the vertebral column, hips or limbs of bulls. Bone scan has been used for various applications in horses for many years and currently many private practices and most veterinary schools have gamma cameras. These cameras are used to image an injected radionuclide in the animal. Skeletal scintigraphy is quite sensitive and is well suited for detecting acute abnormalities as radionuclide uptake often precedes radiographic detection. Scintigraphy can also be useful in locating potential areas of abnormal osseous turnover in cattle with chronic or vague lameness.[14]

The authors have used radiography, ultrasonography and nuclear scintigraphy to localize and characterize sources of lameness in cattle. Several of these bulls returned to soundness and had semen collected for cryopreservation following intra-articular corticosteroid injection or steroid epidural therapy delivered through a spinal catheter. Similar to the equine patient, bulls may benefit from systemic or intra-articular glycosaminoglycan therapy and other anti-inflammatory modalities.

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Trichomoniasis in cattle

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Abstract

Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus,* an obligate parasite of the reproductive tract of cattle. Infected bulls are often asymptomatic carriers of *T. foetus*. However, these infected bulls are capable of transmitting the organism to a cow during coitus. Infections in cows cause endometritis, cervicitis, vaginitis which may result in early embryonic death, abortion, pyometra, fetal maceration, or infertility. The major economic losses associated with *T. foetus* are due to: 1) reduced calf crop due to early embryonic loss or abortion, 2) reduced weaning weight due to delayed conception, and 3) culling and replacement of infected cattle . Due to the inability to use efficacious drugs, such as the nitromidazoles, for control and prevention of *T. foetus* infections in food animals, most control efforts have targeted identification and elimination of positive bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd. This paper will review trichomoniasis in cattle and discuss pathogenesis of disease, transmission, consequences of infection, immunity, diagnostic techniques, and control and prevention strategies.

Keywords: *Tritrichomonas foetus*, trichomoniasis, bovine, cow, bull, prevention, control

Introduction

Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus,* an obligate parasite of the reproductive tract of the cow and the folds on the mucosal surfaces of the bull's penis and prepuce. Infected bulls are often asymptomatic carriers of *T. foetus*. However, these infected bulls are capable of transmitting the organism to a cow during coitus.[1] Infections in cows cause endometritis, cervicitis, vaginitis which may result in early embryonic death, abortion, pyometra, fetal maceration, or infertility.[1] The major economic losses associated with *T. foetus* are due to: 1) reduced calf crop due to early embryonic loss or abortion, 2) reduced weaning weight due to delayed conception, and 3) culling and replacement of infected cattle. Due to the inability to use efficacious drugs, such as the nitromidazoles, for control and prevention of *T. foetus* infections in food animals, most control efforts have targeted identification and elimination of positive bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd.

Pathogenesis in the female

Life cycle

The life cycle of *T. foetus* is thought involve two forms 1) a tear-shaped trophozoite form and 2) a pseudocyst form. The trophozoite is 10-25μm long and possesses three posterior flagella, one anterior flagellum and an undulating membrane. Trophozoite multiply asexually through binary fission.[2] Pseudocysts usually appear as a result of unfavorable conditions; although, a small percentage of pseudocysts exist under normal conditions.[3] Pseudocysts occur when *T. foetus* trophozoites round up and internalize their flagella in response to assorted stimuli.[3-5] The pseudocyst form lacks a protective cyst wall and does not represent a true cyst form.[4]

Trophozoites of *T. foetus* are transmitted between cows and bulls during coitus and remain in the genito-urinary tract where they multiply by longitudinal binary fission. Under stressful conditions trophozoites will internalize their flagella and replication of the nuclei and other cellular structures will occur, resulting in a multinucleated pseudocyst form. When conditions become desirable once more, mononucleate trophozoites will bud from the pseudocyst. In bulls, infections are usually chronic and asymptomatic and often persist for the life of the animal. Infected cows will initially experience vaginitis

which may or may not resolve spontaneously. In some cases, endometritis can occur resulting in complete sterility. Tritrichomonas infections may also result in fetal loss during pregnancy.[6]

Studies have revealed that pseudocyst formation and reversal can be rapidly and simply effected by certain cooling and warming patterns.[4] However, the induction of pseudocysts by chemicals, dependent of exposure time and concentration, can lead to an irreversible process that leads to the death of the cells.[7] Historically, there has been some uncertainty about whether pseudocysts represent a normal or infective form rather than a degenerative form. More recent research indicates that *T. foetus* is easily stimulated into the pseudocyst form and that these immotile pseudocysts are able to proceed with the process of adhesion to the vaginal epithelial cells.[5] In addition, it has been demonstrated that the pseudocysts are more cytotoxic when in contact with host cells when compared to trophozoites.[8]

Transmission

Cows become infected with *T. foetus* primarily through coital exposure with an infected bull. Subsequently, a mild vaginitis occurs that may go undetected. The organism gains entry into the uterine lumen via the cervix during estrus. Colonization of the entire reproductive tract with *T. foetus* occurs within one to two weeks.[9] Although, contaminated semen or contaminated insemination equipment may also be minor sources of infection.[1] Penetration of the vagina is seemingly necessary because swabbing the vulvar area with high numbers of organisms does not result in vaginal or uterine infection.[10] Infected cows conceive but infection causes endometritis, cervicitis, or vaginitis which results in death of the conceptus within the first half of gestation, abortion, pyometra, fetal maceration, or infertility.[1] These infected cows usually remain infertile for a period of two to six months. In heifers, the duration of infection is reported to be as short as 95 days[11] or as long as 22 months.[12] *Tritrichomonas foetus* has been detected in the reproductive tract for 13 to 28 weeks after experimental infection in heifers.[13]

Consequences of infection

T. foetus organisms arrive in the female reproductive tract concurrently with spermatozoa. However in most cases, fertilization occurs in spite of the presence of the pathogen. In vitro studies have demonstrated that fertilization and early embryonic development to the hatching stage (8-10 days) are not significantly affected by simultaneous culture with *T. foetus*.[14] Conceptus deaths most commonly occur between 50-70 days of gestation. Therefore, the majority of pregnancy loss is during the fetal period (>42 days of gestation). Although unusual, occasional abortions can occur of fetuses greater than four months of gestation.

Most producers do not recognize a problem in the early breeding season as conception occurs normally. The conceptus in most infected cows typically survives long enough to release sufficient interferon tau to prevent the prostaglandin $F_{2\alpha}$-mediated lysis of the corpus luteum. Fetal death in infected cows occurs between seven to ten weeks of gestation. Death of the conceptus during the early stages of pregnancy results in a prolonged interestrous interval.[9,15] Due to abortions and subsequent immunity, the distribution of pregnancies is unusually skewed with a higher proportion of pregnancies conceived towards the end of the breeding season. Although in many progressively managed herds with a limited breeding season, the bulls may no longer be available by the time the cow aborts and clears the infection. Therefore, *T. foetus* infection in a herd may go unnoticed until the time of pregnancy diagnosis when a high percentage of females are diagnosed not pregnant. Pyometra, along with abortions, may be the first physical signs of *T. foetus* infection in a herd, but are thought to occur in less than 5% of infected cows.[16] Pyometra results as the corpus luteum of pregnancy is maintained with a large purulent response which may cause damage to the uterine endometrium.[17]

Most infected cows will clear the organism and develop short-lived immunity of six months to one year. However, carrier cows do occur and are capable of spreading the protozoa. In the case of carrier cows, a very small percentage of cows (<1%) in infected herds have been shown to remain infected throughout pregnancy and into the following breeding season. Thus, the carrier cow has the potential to be quite devastating to control efforts and emphasizes that control programs must focus on the entire herd, not just the bull.[9]

Pathologic changes have been reported in several late-term, *T. foetus* aborted fetuses.[18] The placentas had focal or diffuse invasion of the chorionic stroma by *T. foetus* as seen on hematoxylin and eosin (HE) stained sections of placentas. There was also evidence of a moderate inflammatory cell infiltrate comprised mostly of mononuclear cells. Six of eleven fetuses that were examined had bronchopneumonia with identifiable trichomonads in the airways. Another examination of late term abortions associated with *T. foetus* described a necrotizing enteritis and pyogranulamatous bronchopneumonia with tissue invasion by trichomonads. The exact mechanism that leads to the death of the conceptus is not fully understood. Although, cytotoxic and hemolytic effects by *T. foetus* on mammalian cells have been described.[19]

The preputial cavity of the bull provides an ideal environment for *T. foetus* as the organism localizes in the preputial smegma of the epithelium of the bull's penis and prepuce. The organism does not penetrate the epithelium and does not cause any observable gross pathology or affect semen quality or libido. Histological changes are subtle at first with an increase in the number of neutrophils in the nonkeratinized, stratified squamous epithelium of the glans penis and preputial epithelium followed by an infiltration of lymphocytes and plasma cells penetrating into the intraepithelial area which coalesce in the subepithelium to form lymphoid nodules.[20]

The duration of infection with *T. foetus* for bulls is not clearly understood. There are two theories regarding this debate: 1) transient infection and 2) chronic carrier state. Bulls with the chronic carrier infection of *T.foetus* rarely clear the infection regardless of time. The pathophysiology of infection regarding the carrier state in mature bulls in not fully understood. *T. foetus* infections in bulls less than three to four years of age are more likely to have a transient infection. Younger bulls may not efficiently transmit the organism to a noninfected cow unless the sexual contact occurs within minutes to days of breeding an infected cow. Thus, transmission of *t. foetus* by a young bull is thought to be more passive, mechanical transmission as compared to transmission in older, chronically infected bulls. Nonetheless, any bull exposed to a *T. foetus* infected cow as a result of natural breeding is capable of becoming chronically infected, regardless of age.

Immunity

In the female, *T. foetus* induces inflammation of the mucosa of the vagina, the cervix, the endometrium and the oviductal mucosa. In the first one to two weeks after infection, neutrophils and eosinophils predominate; however, this is followed by a moderate to severe mononuclear infiltration of lymphocytes and plasma cells. Subepithelial and periglandular lymphoid nodules resembling lymphoid follicles begin to develop at almost six weeks post infection. In addition, there is also an apparent degranulation of mast cells between six to nine weeks after infection.[20]

T. foetus specific IgA and IgG_1 antibodies are detectable in uterine and vaginal secretions by the fifth to sixth week after infection. The IgA antibodies do not kill the organisms but may be responsible for immobilization and agglutination of parasites as well as preventing adhesion of the organisms to the mucosal surfaces. The IgG_1 antibodies are presumed to facilitate complement mediated lysis of the parasites as well as opsonization and enhanced phagocytic killing by neutrophils or macrophages. Immunity following natural infection and clearance of *T. foetus* is short-lived with females becoming susceptible within a year, in time for the following breeding season. Because *T. foetus* is an extracellular pathogen, the immune response from the host is predominately humoral and the result of the short-lived immunity. The uterine mucosal inflammation that is seen with infection may allow systemically derived IgG and complement to gain access to the lumen of the uterus and, thus, clear the organism. A relative lack of IgG from the vagina or possibly blocking of IgG effects by vaginal IgA binding of organisms may help explain the carrier state that can be seen in infected herds.[20]

Although specific immunoglobulins have been detected in small amounts in preputial smegma by some researchers, there seems to be no effective acquired immunity to *T. foetus* in the mature bull.

Diagnosis

The comparison of diagnostic assays for detection of *T. foetus* infections has primarily focused on the bull. Collection of *T. foetus* samples from bulls involves recovering the organism from the preputial cavity of the bull. Several techniques have been described for collection of diagnostic specimens in the bull and include a dry pipette technique, a wet pipette technique a douche technique and a swab technique. While the douche method is preferred in Europe, the dry pipette technique is most commonly used in the United States. Regardless of which technique is used, it is generally recommended that bulls be given two weeks of sexual rest prior to sample collection in order to allow accumulation of the organism on the bull's penis and prepuce and a greater chance of recovery.

Isolation of *T. foetus* from the female is reported to be less sensitive when compared with techniques used for bulls.[21,22] In one study, the InPouch™ TF system (BioMed Diagnostics, Inc; White City, OR) was more effective than Diamond's medium (88% versus 68% in detecting heifers that had been experimentally infected with *T. foetus*.[23] The accuracy of prevalence in the cow most likely depends on the timing of sampling relative to exposure. The immune response in females begins to eliminate the infection within eight to ten weeks after exposure in unvaccinated females.[13] Therefore, cultures from females are best performed before the infection is possibly eliminated by the immune response.[23]

Sample handling is also crucial for accurate detection of *T. foetus*. When evaluating temperature and media type it has been found that when laboratory of field isolates were cultured in Diamond's medium or InPouch™ TF, all cultures were positive for *T. foetus* when maintained for up to four days at either 22° or 37°C. However, samples maintained at 4°C or less resulted in inconsistent sensitivity.[24] It is important to remember that time, temperature, type of isolate, and type of medium all have an effect on the sensitivity of *T. foetus* culture.

Microscopic evaluation of cultured organisms is not sufficient to differentiate *T. foetus* from nonpathogenic intestinal or coprophilic trichomonads (*Pentatrichomonas hominis*, *Simplicimonas moskowitzi*, *Tetratrichomonas* spp., etc).[25] Therefore, several conventional and real-time polymerase chain reaction (PCR) assays have been developed for the definitive diagnosis of trichomoniasis, and this methodology has demonstrated some advantages over culture.[25] However, accurate PCR results are directly related to the quality of the sample, which can be affected by transport condition parameters such as temperature and time of transport to the laboratory. There have been a number of issues that have limited the sensitivity of various conventional PCR assays for the detection of *T. foetus*. These problems include DNA degradation, accumulation of inhibitory compounds, sample contamination, and unexpected amplification products.[26] One study demonstrated a decrease in sensitivity of PCR testing with samples that were stored for five days or more. However, PCR was in 100% agreement with culture as long as the PCR was performed within 24 hours of the sample being submitted.[26]

A more recent study evaluated the effect of different simulated transport conditions on samples containing *T. foetus* for the diagnosis of trichomoniasis using culture and quantitative PCR (qPCR).[25] This study demonstrated that transport temperatures of 4-20°C for one to three days before culture reduced or temporarily inhibited parasite replication but maintained viability. Samples tested by either culture or qPCR would have been expected to give positive results. However, diagnosis of trichomonads by both methods was negatively affected when specimens were maintained at transport temperatures of 42°C for 24 hours or more. This study emphasizes the importance of ensuring that clinical samples arrive to the diagnostic laboratory within 24-48 hours and of avoiding temperature transport conditions above 37°C in order to achieve an accurate diagnosis of *T. foetus*. The effects of high incubation temperatures on culture and real-time PCR for *T.* foetus have also been evaluated following inoculation into the InPouch™ TF system.[27] This study showed that *T. foetus* was detectable at microscopically in inoculated pouches incubated at 37°C regardless of exposure time (1, 3, 6 and 24 hours), whereas those samples incubated at 46.1 °C detected *T. foetus* only after one and three hours of incubation. *T. foetus* was detected in samples incubated at 54.4°C after only one hour. Testing using real-time PCR for all inoculated medium samples (37°C, 46.1°C, and 54.4°C at 1, 3, 6 and 24 hours) produced positive results for all inoculated medium samples. This study suggests that samples collected for culture alone should be protected from high temperatures.

Prevention and control

One complicating factor with bovine trichomoniasis in the United States is the lack of effective treatments with Food and Drug Administration approval. Historically, the most successful treatment for bulls with trichomoniasis was systemic treatment with nitromidazole derivatives.[28] Currently, the use of nitromidazole derivatives is illegal in food-producing animals in the U.S., and no effective alternative treatments are available. The lack of effective, approved therapies for bovine trichomoniasis emphasizes the need for appropriate preventive and control measures. Prevention of trichomoniasis includes the following recommendations: 1) avoid movement of animals (co-grazing, leasing of bulls, good fences); 2) utilize artificial insemination, if possible; 3) use a defined breeding season and cull all non-pregnant females after the breeding season; 4) purchase virgin bulls and heifers as replacements; 5) test all bulls for *T. foetus* prior to introduction into the herd and maintain a young population of bulls; and 6) breed purchased cows and heifers in a separate herd.[9]

Once *T. foetus* has been confirmed in a herd, there are additional measures that should be considered in order to "clean up" the herd. These measures include 1) testing and culling all infected bulls and purchasing *T. foetus* negative bulls; 2) intense management of bulls so that smaller breeding units are used and bulls are bred to the same cattle until trichomoniasis is under control; 3) create high and low risk herds; and 4) vaccinate all herd females with an approved *T. foetus* vaccine.[9] Vaccination is an important aspect of any control program as it has been shown to reduce pregnancy wastage associated with *T. foetus* infection in cattle herds. Currently, TrichGuard® (Boehringer Ingelheim Vetmedica, Inc.) is the only commercially available vaccine licensed by the USDA for the control of trichomoniasis in the United States. TrichGuard® is a proprietary vaccine that is a Freund adjuvant killed *T.* foetus-derived vaccine that requires two doses subcutaneous injections administered two to four weeks apart with the last injection to be given four weeks prior to the breeding season.[9] One study compared pregnancy and calving rates between beef heifers vaccinated with TrichGuard® and control heifers after heifers were exposed to *T. foetus* infected bulls and intravaginally inoculated with a large number (10 million) of *T. foetus* organisms.[29] At calving twice as many vaccinated heifers calved when compared to control heifers (61% versus 31%). Thus, the vaccine appeared to offer at least some protection against *T. foetus*.

Conclusion

Trichomoniasis can be an economically devastating infection in cattle herd with losses due to reduced calf crop due to early embryonic loss or abortion, reduced weaning weight due to delayed conception, and culling and replacement of infected cattle. Carrier females and concerns with diagnostic sampling and testing have made the control of trichomoniasis in cattle even more complex. Control and prevention of *T. foetus* infections in cattle must focus on identification and elimination of positive cows and bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd.

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Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

Semen evaluation and overview of common sperm abnormalities

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Abstract
A veterinary breeding soundness examination as directed by Society for Theriogenology (SFT) standards provides information that is vital for the cowman and in turn the beef cattle industry. An incomplete exam, specifically the omission of a microscopic evaluation of sperm morphology compromises the results and thus its value. This is also the case with a poorly performed morphology exam whether due to sample handling, equipment (microscope) quality or competency of the veterinarian.

From the standpoint of specific sperm abnormalities, when identified in significant numbers, there is utility in assigning prognosis if possible. Likewise, a suspected etiology can also differentiate bulls that might be held for re-testing versus immediate culling. Thus the category of abnormality, suspected etiology, and age of the bull represent information useful in providing both a prognosis and the scheduling of a date for re-test.

Keywords: Bull, breeding soundness, morphology, sperm abnormality

Introduction

The economic justification for a veterinary breeding soundness examination (VBSE) is based on the identification and the subsequent culling of individuals that will potentially have a negative effect on cowherd productivity. Basically, this effect can be expressed both by decreases in pregnancy percentage and sale weight, with secondary resulting effects being increased culling of open cows and those that appear to be less productive, and also the insidious propagation of low fertility offspring. While percent pregnant has an obvious and easily observed impact on profitability, decreased sale weights due to calves being conceived late in the breeding season might be less readily identified by a producer, as is the decrease in fertility of retained females . Specifically, low pregnancy rates and/or a wide distribution of calvings are the hallmarks of a subfertile bull and as the completely sterile bull is rare, it is the identification of this individual (subfertile bull), which in the general population can represent 20-40% of all bulls,[1] that is the focus of our efforts. For a variety of reasons, evaluation of sperm morphology is the single best tool for identifying these bulls. Morphology and indeed every aspect of the VBSE must be performed conscientiously, consistently and competently in order for the results to be valid. The purpose of the following narrative to provide guidance toward a consistent approach with regard to semen evaluation.

Sample collection, handling and slide preparation

A detailed review of semen collection is beyond both the scope and mission of this manuscript, but needless to say a suitable sample is most efficiently obtained via electroejaculation, following rectal palpation in which the urethralis muscle is stimulated and the rectum fatigued. Additionally, a good quality, uncontaminated (absence of dirt/hair/debris) sample is more easily obtained when the penis is extended and gross observation indicates a color change of the ejaculatory fluid from clear to milky and preferably creamy white. As sperm motility must be assessed, careful handling from collection to microscopic evaluation is crucial and can be accomplished by utilizing a disposable, Styrofoam™ coffee cup as a receptacle followed by examination within 3-5 minutes with a pre-warmed slide. The process described works fairly well as long as the environmental temperature is greater than 40° F and duration of time to evaluation is short. When ambient temperatures are below 40° F utilization of a warmed collection vial is recommended.

The evaluation of semen begins with a gross examination, following this visual assessment, motility is evaluated microscopically, and following preparation of a suitably stained semen smear morphology is performed. Because of both the importance of the morphology examination and the need,

when evaluating large numbers of bulls, to proceed quickly, the microscopic evaluation of morphology can be postponed until a later time.

Evaluation of sperm morphology is facilitated by the use of a good semen smear. It is crucial to start with a clean, warm slide. Some "new" slides may have detergent coatings that interfere with staining. India ink is an acceptable stain but does not actually stain the sperm cells and is considered a "background" stain. Eosin-nigrosin (E-N) is a vital stain and is the currently recommended (SFT) stain. It is easy to use and has consistent staining properties. The eosin portion will penetrate dead sperm cells, staining them pink (red is dead) while leaving live cells unstained (white) against a dark background provided by the nigrosin component. A smear stained with Diff Quik™ is also adequate for visualizing sperm morphology and provides the advantage of allowing easy visualization of white blood cells. Another useful staining procedure is the Feulgen staining method. This technique begins with preparing a smear and then allowing it to dry for an hour. Next place the slide in 5 N HCl for 30 minutes. Wash the slide by running water into the corner of a staining dish containing the slide for two minutes. Then place the slide in Schiff's reagent for 30 minutes. Wash again as before and air dry. The Feulgen technique is superior for identifying the nuclear vacuole (crater) defect and because the process removes fat globules, it is an excellent staining technique for smears from extended semen.

My approach utilizing the SFT E-N stain is to first place a small droplet of stain on the slide, then add a drop of semen and mix. Placing the semen drop first followed by the stain can result in contamination of the stain solution if the tip of the bottle or dropper inadvertently touches the semen. Once the stain and semen is mixed a second slide is used to push (spread) the semen across the slide in the same manner a blood smear is prepared. Because the feathered edge is the best area for evaluation, an alternative method is to create several thickness gradients by stopping and starting as the mixture is spread. It is also often a good idea to make a second slide at the same time, as it is faster to make two, than to come back later and make another slide on the occasion that the first slide was not of diagnostic quality.

Equipment

The type and quality of microscope utilized is likely to be one of the causes of the inconsistency in results cited as a problem with VBSE's and indeed there seems to exist among theriogenologists a strong preference for the use of phase contrast microscopy.[2] A study compared the evaluation of sperm morphology with a wet mount (fixed with isotonic formol saline) sample utilizing differential interference phase contrast (DIC) or an E-N stained dry-mount bright field (BF) microscopy provides insight on this issue.[3]. In this study the sample slides were examined at 1000X. The DIC was superior in identifying morphologic abnormalities of the sperm head such as the presence of vacuoles but the percentage normal was the same with both types of microscopy. Since the percent normal did not differ, clinical results should not be affected. So based on these findings, for the routine VBSE the use of BF microscopy and the E-N stain is adequate. Another study comparing the use of the E-N stain with BF microscopy, Feulgen-stained with BF microscopy, or phase contrast microscopy revealed that the Feulgen identified more head defects and the phase contrast revealed more distal cytoplasmic droplets.[4] Again, the authors felt that the differences were not enough to be clinically important.

With respect to microscope quality if maintenance and cleaning are assumed and the oil immersion objective utilized to evaluate a properly prepared sample a simple guideline follows: if you can readily identify the diadem defect in a spermogram, the microscope you are using is of adequate quality. If this defect is never observed, you should likely upgrade your microscope.

Gross evaluation of semen

After collection the semen should first be observed grossly. A rough estimation of the concentration can be made based on the opacity or lack of and the color of the semen. Very concentrated samples look like heavy cream while very dilute samples have the appearance of watered down skim milk. Yellow tinted semen can result from urine contamination and this can be substantiated by smell or the use of a blood urea nitrogen test strip. Additionally, semen contaminated with urine will not be motile

or at least will display rapidly declining motility when examined microscopically. Conversely a gold appearance is also associated with very highly concentrated semen and the presence of riboflavin.[5] This is a common finding in many Jersey and some Angus bulls. Red or brown colored semen indicates the presence of blood or blood pigments and the source should be determined.

Evaluation of sperm motility

A small "standing" drop is placed on a pre-warmed slide and evaluated under low power microscopy (40X-200X) for gross motility. Thick, dark, rapidly oscillating swirls are indicative of excellent motility (defined as a high velocity or high speed motility), a high percentage of sperm that are progressively motile, and a sample of high concentration. That sample would typically be classified as Very Good. A sample that displays slower moving swirls is classified as Good. A Fair sample displays no swirls, but significant individual sperm movement. A Poor sample has no or very little movement/oscillation. Because the concentration of a sample impacts the gross motility designation, individual motility should be assessed if there is any question about the validity of a motility rating based on gross motility. Individual motility can be assessed utilizing 200X-400X microscopy and depending on the concentration either a cover slip over either the previously examined droplet or a diluted droplet (dilute w/ warmed sodium citrate solution). Individual motility greater than 70% is Very Good, 50-69% is Good, 30-49% is Fair, and if less than 30% the sample is categorized as Poor.[6]

Based on our current standards (SFT), bulls must have a minimum of Fair sperm motility based on either individual or gross assessment.[7] While this may seem to be low, it is a minimum threshold and while there is a positive correlation between motility and fertility with the use of artificial insemination, this is not reported to be the case with natural service bull fertility.[8]

Evaluation of sperm morphology (the spermogram)

There is no aspect of the VBSE in which there are greater concerns with respect to the delivery of accurate and repeatable results than the portion dealing with sperm morphology. Specifically, inconsistency among veterinarians due to differences in their ability to evaluate a spermogram[9,10] and this does not take into account veterinarians or others that perform VBSEs or the euphemistic "semen check", fertility examination, etc. but do not include the evaluation of sperm morphology. This underscores the need for adequate training for veterinarians who wish to provide this service and the protection of the public (cattlemen) from those that pass off a substandard service as a VBSE.

Current SFT- VBSE standards set a morphology threshold of 70% normal with no distinction in regard to classification of abnormalities.[7] The 70% normal metric can be justified by a study[11] in which cows and heifers exposed to bulls with either 70% or 80% morphologically normal sperm had similar pregnancy rates and these pregnancy rates were statistically higher than cows and heifers exposed to untested bulls. An additional argument can be made due to discrepancies among the various classification systems of sperm abnormalities- Primary vs Secondary, Major vs Minor, Compensable vs Uncompensable; and the realization that as more becomes known about a specific abnormality it's classification status may change.

For comparison sake, the standards set forth by the Western Canadian Association of Bovine Practitioners sets a minimum morphology standard as no more than 30% total sperm defects **OR** 20% nuclear (head) defects.[12] This approach provides, at least to some degree, a safeguard with respect to the accounting of those defects believed to be more significant in their impact on fertility, specifically by the fact that their occurrence in an ejaculate is predictive of the presence of defective cohort sperm that appear morphologically normal.[13-15] Therefore, it appears to me that for those of us utilizing the SFT standards, we might at the very least more closely scrutinize those morphology smears that display greater than 20% head abnormalities and take that into consideration when evaluating the marginal bull.

Generally speaking, the important thing to remember when approaching the evaluation of a spermogram is that the presence of a specific sperm abnormality in significant numbers represents the clinical manifestation of a problem occurring during either spermatogenesis or sperm transport. This "problem" could be the pathological response to a transient insult as is seen with stress or exposure to

environmental temperature extremes of short duration. It can also represent permanent or at least semi-permanent pathology such as found with testicular degeneration. Insult to the seminiferous epithelium resulting in abnormal spermatogenesis and in turn the presence of abnormal sperm also results in the production of sperm that while morphologically normal in appearance undoubtedly have a compromised ability to fertilize an ovum. Thus an ejaculate with 30% abnormal sperm present does not mean that we can say with certainty that there are 70% completely normal sperm, but instead is representative of a threshold by which we can reasonably assure the fertility of that bull to a level that meets the standard described in the introduction.

Commonly encountered sperm abnormalities

Detached head

The normal detached head, which is found in small numbers in virtually all ejaculates, is often present in high numbers in bulls following sexual rest ("rusty load" scenario), in peripubertal bulls, and from bulls that have experienced a recent stress with or without a high fever. It is also found in young bulls with testicular hypoplasia, but these should have already been excluded from further testing based on an inadequate scrotal circumference measurement. It is categorized as both a secondary and minor abnormality and is considered to be compensable due to the obvious lack of motility . Detached heads that are abnormal are categorized based on that abnormality.

If this defect is found in threshold levels, the bull can often be re-collected immediately and the number will be dramatically decreased. When placed in the deferred category, bulls with a history of recent stress etc. can be re-tested as early as two weeks, because it is an abnormality of epididymal origin and epididymal transit is around 11 days. Bulls believed to be peripubertal may benefit from a longer wait time before re-testing.

A very rarely encountered version of the detached head that has a genetic basis and in which affected bulls are sterile is the presence of separated and motile tails. This is a different, distinct abnormality in which 80-100% of sperm in an ejaculate will be affected.[16]

Distal midpiece reflex

The distal midpiece reflex (DMR) is the most common abnormality of the sperm tail.[17]. It is considered to be epididymal in origin and therefore a secondary defect. It is also categorized as a minor defect and compensable. This defect is compensable due to the lack of forward, progressive motility. Evidence for its origin is based on its rapid appearance in the ejaculate of bulls within a few days of a thermal insult. This defect appears as a sharp hairpin bend at the distal midpiece[18] with a cytoplasmic droplet within the bend. If a droplet is not observed to be present, it is likely that the "bend" is due to contact with a hypotonic solution, presumably the stain that was used. This defect can often be identified during the evaluation of motility as the affected sperm will appear to be swimming backwards.

The etiology is believed to be due to a negative effect on epididymal function due to depressed testosterone levels which can in turn be caused by stress, thermal stress (either high or low), exogenous estrogen, or induced hypothyroidism; although normal, fertile bulls can have up to 25% of this defect in an ejaculate[17] due presumably to its compensable nature. However, I have observed that when this defect is present at a level of 20-25% in the ejaculate of a bull that meets standards (>70% normal) when tested at a time of moderate weather and absence of stress, the same bull when re-evaluated during times of environmental temperature extremes will have an increased percentage of this defect in his ejaculate. I now closely scrutinize these bulls and discuss this issue with the owner with respect to the time of year that the bull will be placed into service. Additionally, that this defect could very well have a genetic etiology or at least predisposition in some of the beef breeds is something that should be considered.[19] It has definitely been shown to be heritable in Jersey bulls, some of which would have up to 100% DMR defective sperm in an ejaculate.[20]

Cytoplasmic droplets

The distally located cytoplasmic droplet is thought to be epididymal in origin and its significance or rather status as an abnormality is debatable. As there is no correlation between a high incidence of this sperm type and infertility and also due to the fact that these sperm will often shed their droplets during even short periods of incubation,[21] these should be re-categorized as a variation of normal.

Proximally located cytoplasmic droplets are a result of abnormal spermiogenesis and are categorized as uncompensable.[17,20] However, the placement of the proximal droplet defect in the uncompensable category might be problematic as a study that used ejaculates of either high or low numbers of sperm with that defect for in vitro fertilization revealed that while fertilization was decreased as the percentage of proximal droplets in an ejaculate was increased (this meets the definition of uncompensable), of the ova that were fertilized, cleavage rates were similar.[22] Indeed the presence of abnormal sperm (proximal droplets) did not impact the fertilizing ability of the normal cohort sperm insinuating that increasing the number of normal sperm could "compensate" for the presence of the defect. This reiterates the problem of becoming overly concerned with the categorization of certain sperm abnormalities instead of focusing on the likely etiology and in turn prognosis. Specifically, in the case of this defect, as previously stated the cause is undoubtedly due to abnormal spermiogenesis with the potentially underlying etiology either immaturity or conversely testicular degeneration. So in the case of the young peripubertal bull we can place that individual in a deferred status with the reasonable assurance that with age (maturity) his spermiogram will improve and in the case of the older bull we can safely assume testicular degeneration and therefore less chance for a return to fertility.

Abnormal midpiece

I will include in this category the "pseudodroplet" defect and the various mitochondrial sheath defects as well as "Dag like" defects. Additionally, the midpiece may appear swollen, "corkscrew", bent, or asymmetric. These defects are all designated as compensable because of the obvious impact on motility and all are classified as a primary defects in the SFT system. Since the development of this sperm region occurs almost completely during spermiogenesis the specific origin for most of these defects is undoubtedly testicular. It has been shown that some forms of this group of defects can be caused by increased levels of gossypol,[23] a compound found in the cotton plant and specifically cottonseed, in the diet of bulls. Bulls fed diets high in gossypol appear to be especially sensitive to this compound during puberty.[24] The etiology of defects caused by gossypol appears to result from damage to sperm structure during spermiogenesis with further damage occurring during epididymal transit.[23] From a practical standpoint, simply limiting the intake of whole cottonseed to less than five pounds per day for bulls of an age presumed to coincide with the attainment of puberty should be sufficient to avoid this problem. Also, this seems to be more common in Brahman bulls and indeed in the Chenoweth report[24] the bulls described were Brahman. This or at least a similar defect can be created in rats fed gossypol and also rats deprived of selenium.[17]

The specific abnormality referred to as a pseudodroplet is actually not common and may have a genetic component.[17] It is best described as local thickening at and slight thickening of the midpiece.

Pyriform head

This is the most common defect of the sperm head[17] and is usually found in low numbers even in the ejaculates of fertile bulls.[18] Because there are bulls of normal fertility that have narrowed sperm and there appear to be variations in the range of "taperedness", it can be hard to distinguish at what point a designation is made between normal and pyriform.[17] For example, in the human, sperm formerly categorized as pyriform or pear-shaped sperm are no longer considered as abnormal.[25] However, there was not a clearly defined distinction with regard to degree of taperedness. In veterinary literature this is a defect and categorized as both a primary and major defect. The evidence for whether or not this abnormality is compensable is equivocal. In general, sperm with misshaped heads do not transverse the reproductive tract, but sperm with this defect apparently do,[26] although that in fact, appears to be dependent on the level of deformity.[27] The level of deformity also apparently impacts fertilization rates as

trials evaluating this defect reveal decreased levels of zona penetration, fertilization, and cleavage rates.[27,28] For example the previously cited work revealed that semen containing this defect at high percentages (85% pyriform heads) had zona penetration at about half the rate of control (90% normal) semen. Considering that semen containing a high percentage of pyriform head defects still resulted in some, albeit much lower, fertilization, these authors came to the conclusion that this could be due to the presence of a small number of normal sperm as well as a percentage of less affected pyriform sperm that may be capable of successful fertilization suggesting that this abnormality could be partially compensable.[28]

With respect to etiology this defect is seen following environmental heat stress, validated by scrotal insulation studies,[13] and also from bulls with testicular hypoplasia.[17] In addition to environmental causes of heat stress, the scrotal insulation effects of fat deposition around the scrotum that results from heavy feeding during gain tests has the same deleterious effect. Bulls examined after recently coming off a gain test or going through adverse environmental extremes that have this abnormality in numbers that contribute to not meeting the metric for percent normal sperm should be deferred. In the case of bulls with testicular hypoplasia, they typically do not meet VBSE standards for scrotal circumference anyway and older, mature bulls that do not have a history of a transient insult that would provide a reason for a disruption in spermatogenesis carry a poor prognosis.[17] But remember, slight degrees of taperedness may be normal. Also those young, over-fitted bulls that are deferred, might need more than 60 days to recover and meet standards.

Terminally coiled tail (coiled principal piece)

The terminally coiled tail defect also termed a coiled principal piece has been described to not be as commonly found,[17] but we seem to encounter bulls with significant numbers of this defect especially following environmental temperature stress. It will be seen with other heat stress related defects and this has been documented by a scrotal insulation trial.[13] It is also a defect that is increased proportionally following gossypol toxicity [29]. Due to poor motility it is compensable.

Less commonly encountered sperm abnormalities that are important due to their significance

Knobbed acrosome

The knobbed acrosome defect can be identified as an apical swelling that may protrude from or fold over the head[30] but appears most often as a flattening or indention of the apex.[17] This defect was identified as having a genetic etiology, specifically being an autosomal sex-linked recessive trait in the Friesian breed.[16,30] A genetic etiology should be considered when this defect is prominent in the ejaculate over time, but when identified with several other defects an environmental (temperature related)[13,14,17] or other transient cause is likely. Thus when this defect is encountered with other head defects there is a better prognosis for recovery[31] and it would be prudent to defer and recheck the bull in 60-90 days. It is considered to be both a major and primary defect; and the best current evidence is that it is uncompensable.[14] The uncompensable nature of this defect is not straightforward as it actually appears to be compensable based on the fact that sperm with this defect don't transverse the reproductive tract of cows efficiently[26] and those that do are unable to penetrate the zona pellucid.[14] However, this defect is the perfect example of a defect the presence of which denotes the occurrence of normal appearing, but defective cohorts.[13-15] These defective, but morphologically normal cells although able to penetrate ova, had lower rates of fertilization and reduced cleavage by zygotes.[14,15] Therefore from a practical perspective we should scrutinize more closely those bulls whose ejaculate displays this defect predominately in large numbers (>20%) as we know it can have a genetic basis and that it expresses infertility at levels higher than its occurrence within an ejaculate.

Nuclear vacuole

The nuclear vacuole defect is also termed as a crater defect and includes the diadem defect, which is a string or line of vacuoles around the acrosome-nuclear cap junction.[32] While small numbers (<15%)

of this defect in an ejaculate can be compatible with fertility, larger numbers suggest a disturbance in spermatogenesis and in fact most instances in which this defect is present at 10% or greater it is accompanied by other defects that reduce semen quality.[33]

The etiology of this defect is undoubtedly environmental stress as the appearance of this sperm abnormality follows within days of the administration of dexamethasone or the application of scrotal insulation[34] and the possibility of a genetic etiology has been ruled out.[35] The prognosis for recovery is good if the inciting cause is eliminated.[34,36]

Dag

The Dag defect named for the Jersey bull from which it was identified has a genetic etiology. Because up to 100% of the sperm in an ejaculate can be affected[21] it has proven to be largely self-limiting. "Dag like" defects seem to be an etiologically distinct abnormality and were grouped with the midpiece defects.

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Circulating microRNAs and associated gene regulation in puerperal metritis in dairy cows

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Dairy cows that calved recently are prone to uterine and metabolic disorders due to suppressed immune function and negative energy balance. Metritis is one of the most common uterine diseases of dairy cows diagnosed during puerperal period (first 10 days in milk). It is defined as presence of fetid reddish-brown watery vaginal discharge, systemic signs of illness with fever (rectal temperature of 103°F or greater). Cows that suffer from metritis associated with poor reproductive performance, including irregular estrous cycles, lower conception rates and greater intervals from calving to pregnancy. Although uterine infection occurs most commonly after calving complicated by dystocia, retained fetal membranes, twins or stillbirth, cows with poor immune function are most likely to develop metritis. MicroRNAs (miRNAs) are small non-coding molecules that are partially complementary to one or more messenger RNA (mRNA). Their main function is to down-regulate gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation. The objective was to compare circulating miRNAs and their integrated genes in cows suffering from metritis and normal cows. Dairy cows diagnosed with (n=4) or without (n=4) metritis from a single farm were included in this study. Blood samples were collected via coccygeal venipuncture at the time of diagnosis. In individual serum samples, we investigated 84 prioritized cow-specific miRNAs using RT-PCR method. Total RNA, including miRNAs, was isolated from frozen-thawed serum, complementary DNA was synthesized and mature miRNA expression profiling was performed using real time PCR. MiRNA-specific forward primer and universal reverse primer were used to amplify mature miRNAs. *Caenorhabditis elegans* miRNA, cel-miR-39-3p was used as endogenous control to normalize target miRNA expression. Data were analyzed using the ΔΔCT method of relative quantification using the computational software at http://pcrdataanalysis.sabiosciences.com/mirna. Circulating miRNAs (n=34) were identified in differential abundance in cows with metritis compared to normal cows. Of those 34 miRNA, 18 were observed in abundance and 16 were scarce among cows with metritis compared to normal cows. Specifically several miRNA families were scarce, including bta-let-7f (-31.3), bta-miR-10a (-20.1), bta-miR-127 (-4.2) and bta-miR-148b-3p (-61.8); and several families were found to be in abundance, including bta-let-7a-5p (25.6), bta-miR-101 (88.2), bta-miR-142-3p (77.5), bta-miR-150 (16.4), bta-miR-16b (27.18), bta-miR-181a (4.2), bta-miR-191 (21.9), bta-miR-192 (8.2), bta-miR-21-5p (3.1), bta-miR-24-3p (2.8), bta-miR-25 (3.1), bta-miR-26b (169.3), bta-miR-30d (2.5) and bta-miR-30e-5p (4.0) in cows with metritis compared to normal cows ($P<0.01$). A considerable number of miRNAs were predicted to inhibit the expression of genes associated to proinflammatory and immune-related responses, angiogenesis, cell-cycle progression, and adhesion molecules. In most of the cases, the levels of these miRNAs were abundant in cows with metritis compared to those without metritis. In conclusion, the presence of distinct miRNA profiles between cows with metritis and normal cows indicates that miRNA may have a role in the pathophysiology of metritis. It is possible that these miRNA could be targeted for treatment using inhibitors and/or mimics.

Keywords: Dairy cows, postpartum, metritis; miRNA, genes

Proceedings of the Society for Theriogenology Annual Conference 2015

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Evaluation of novel sampling technique for bovine trichomoniasis (*Tritrichomonas foetus*)

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Bovine trichomoniasis is of great concern to producers and veterinarians in the beef industry. A standard breeding soundness examination (BSE) does not usually include evaluation for venereal diseases, such as *Tritrichomonas foetus (T. foetus)*, and typically goes undiagnosed and is commonly suspected during pregnancy diagnosis which reveals poor conception rates, early embryonic deaths, and extended calving seasons. Trichomoniasis can be adequately diagnosed via preputial/penile scraping; however research has exposed detection rate limitations including: sample collection methods and techniques, sample numbers, media selection and sample processing, and diagnostic laboratory modalities. The aim of the study was to identify a potential technique that increased sensitivity and could easily be used at a BSE or during reproductive investigations. Commercial and purebred bulls (n=76, age = 1 to 5 years, 8 different locations), of unknown infection, were sampled for detection of *T. foetus* using two different collection methods: 1) traditional preputial/penile scraping (TPS) and 2) preputial/penile swabbing (PPS). All (n=76) bulls were subjected to both procedures on the same day or within one week. Traditional preputial/penile scraping samples were taken by vigorously scraping preputial/penile mucosa using a rigid insemination pipette while applying negative pressure with a syringe. Preputial/penile swabbing samples were obtained by briskly swabbing the preputial/penile mucosa with gauze (4x4) during full extension of the penis either by manual stimulation or by use of electrostimulation. All samples were processed using InPouch™ TF (Biomed, White City, OR) media and submitted under similar conditions for polymerase chain reaction (PCR) (Vetmax™ Gold Trich Detection Kit, Life Technologies, Grand Island, NY) testing at Iowa State University Veterinary Diagnostic Laboratory (Ames, IA). Samples were analyzed based on laboratory specifications for positive and negative cycle cutoff points. Positive PCR results were observed in 25/76 (33%) bulls using TPS technique, however 28/76 (37%) were positive using PPS technique. Comparatively, 25/28 (89%) bulls were confirmed positive on TPS technique versus PPS. Fundamentally, PPS technique was 11% more sensitive compared to TPS. Data were analyzed using McNemar's Exact Test and significance was defined as $P \leq 0.05$. There was not a significant difference between the two methods, even though there was a numerical difference. This newer technique alone reduces false negative rates (numerically), which can increase proper diagnosis using one-sample testing. These data indicate that the new PPS technique is a viable alternative to the TPS method which allows practitioners to choose between the collection methods. Further studies with a larger sample size are required to prove the significant difference between the two methods.

Keywords: Bovine, trichomoniasis, venereal, polymerase chain reaction (PCR)

Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

Using color flow Doppler ultrasonography to estimate progesterone concentrations at embryo transfer and during early pregnancy in recipient mares

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Color-flow Doppler sonography (CF) has been described as a means of rapidly assessing corpus luteum (CL) function in cycling mares because luteal blood flow correlates with circulating progesterone (P_4) concentrations. The hypothesis was that CL size and blood flow would provide an indication of luteal function as represented by circulating levels of P_4, in mares at and following embryo transfer (ET). Day 8 equine embryos (n=48) were transferred into available recipient mares (ovulated 1 day before to 3 days after donor) as part of a commercial program. B-mode and CF sonography were performed using a MyLab™Five (Esaote, Maastricht, The Netherlands) immediately prior to ET (ET+0), and blood was collected from the jugular vein to measure plasma [P_4] (Coat-A-Count TKPG; Siemens Healthcare Diagnostic BV, Los Angeles, CA). For every detectable CL, fixed settings of 10 cm depth in B-mode (frequency 7.5MHz, maximum gain) and color mode (frequency 5.0MHz, 70% gain, pulse repetition frequency 1.0kHz) were used to examine size and vascularity. Three cross-sectional images at the position of maximal size and area of blood vessels of the respective CL were captured and stored. Measurements were repeated at day four after ET for all mares, and days 11, 18 and 25 in pregnant mares. The cross sectional area (corrected for presence of lacunae) and area of color pixels within the cross-section were analyzed using ImageJ software (National Health Institutes, Bathesda, MD), and statistical analysis was performed using SAS® (Version 9.4, SAS Inst., Cary, NC). None of CL area, area of color pixels or [P_4] at the time of ET were predictive of pregnancy outcome when analyzed with binary logistic regression. The total area of color pixels in the CL correlated significantly ($r = 0.35$ to 0.45), if only moderately, with [P_4] at all-time points except day 18 after ET (Spearman's rank-order correlation). A significant correlation between CL area and [P_4] was evident until day 11 ($r = 0.37$ to 0.60). Corpus luteum vascularity (area of blood vessels) decreased significantly after day 18, whereas CL area had already decreased from day four (Wilcoxon signed rank test). These findings confirm that area of color pixels in the CL cross-section is a reasonable index of circulating [P_4] at the time of ET and during early pregnancy and can be used to indicate luteal insufficiency.

Keywords: Equine, mare, color flow Doppler, embryo transfer, progesterone