Kisspeptin and bovine follicular cysts

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Dairy cows, Follicular cyst, Kisspeptin.

Summary
The aim of this study was to investigate kisspeptin (Kp) and sexual hormone blood concentrations in healthy dairy cows (H) and in cows diagnosed with ovarian follicular cysts (FC). Forty dairy cows were enrolled in this study and divided in 2 groups of 20 subjects each. All cows underwent blood collection on the day of diagnosis and on that of heat detection, respectively. Kisspeptin 10, estradiol, and progesterone blood concentrations were assessed. All the parameters were higher in Group FC than in Group H. These results suggest that the higher steroid levels found may have determined the increase in Kp secretion in the cystic cows. This, in turn, may have directly or indirectly stimulated the sustained Luteinizing Hormone (LH) release, known to occur in dairy cystic disease.

Kisspeptina e cisti follicolari nella bovina

Parole chiave
Bovina da latte, Cisti follicolari, Kisspeptina.

Riassunto
Lo scopo di questo studio è stato quello di valutare le concentrazioni di kisspeptina (Kp) e degli ormoni sessuali (Estrogeni e Progesterone) in bovine clinicamente sane, al momento del calore (H), ed in bovine con diagnosi di cisti follicolari (FC). Quaranta bovine da latte sono state divise in due gruppi di 20 soggetti ciascuno. Su tutte le bovine sono stati eseguiti dei prelievi di sangue il giorno della conferma di diagnosi di cisti follicolari, nel gruppo FC e al momento del calore, nel gruppo H. Su tali campioni sono state dosate le concentrazioni di Kisspeptina 10, β-estradiolo e progesterone. Tutti i parametri sono risultati superiori nel gruppo FC rispetto al gruppo H. Tali risultati suggeriscono che i più alti livelli di ormoni steroidei potrebbero aver determinato un incremento della secrezione di Kp-10, nelle bovine con cisti follicolari. A sua volta, essa potrebbe aver stimolato, direttamente o indirettamente, il rilascio sostenuto di LH, evento presente nella patologia delle cisti follicolari della bovina.

Recently, a new family of molecules, implicated in the control of the reproductive axis, has been discovered: kisspeptins (Kp). These hypothalamic peptides derive from the cleavage of their common 145 aminoacid precursor encoded by *Kiss 1* gene. Among these products, kisspeptin 10 (Kp-10) plays a pivotal role in the control of gonadotropin secretion and ovulation (Tena-Sempere 2006). Kp-10 directly stimulates GnRH neurons to secrete GnRH both at the onset of puberty (Pineda *et al.* 2010) and before the preovulatory surge of LH, during reproductive cycles (Clarkson *et al.* 2010).

Brown and colleagues (Brown *et al.* 2012) demonstrated that an atypical kiss1 expression in the brain may contribute to the multiple ovarian abnormalities observed in women with polycystic ovary syndrome (PCOS). Based on these data, the aim of this study was to investigate Kp and sexual hormone blood concentrations in healthy dairy cows and in cows diagnosed with ovarian follicular cysts.

The study was performed on a commercial dairy farm, consisting of 180 Holstein Friesian cows kept in a semi-intensive regimen, 80 ± 15 days post partum

and between the 3° and the 4° lactation (27 kg/die) (mean body weight 550 kg).

The cows were chosen through 2 clinical examinations performed 10 days apart, to diagnose cystic ovarian follicles, keeping into account the criteria indicated by Mutinati and colleagues (Mutinati *et al.* 2013), namely the mean diameter of at least 17 mm, wall thickness < 3 mm, blood progesterone < 1 ng/mL, persistence for more than 6 days.

Twenty cows diagnosed with follicular cysts (Group FC) and 20 healthy cows on heat (Group H) were enrolled in the study. Concerning oestrus detection, oedema of the vulva, clear mucosal vaginal discharge, standing to be mounted were observed and oestrus was then confirmed by the detection of a preovulatory follicle on transrectal palpation and ultrasonography.

Blood samples were taken from the coccygeal vein, in vacutainer serum tubes, at the following times:

- on the second examination in which ovarian follicular cysts were confirmed (Group FC);
- on the day of heat (Group H).

On serum, Kp-10, estrogen (estradiol 17β) (E2), and progesterone (P4) concentrations were assessed.

Kisspeptin 10 was dosed with a human radioimmunoassay method (RIA) (Phoenix Pharmaceuticals, Inc. Burlingame, CA, USA; human range: 10-1280 pg/mL; specificity: 100%), since no analogue kit exists for the bovine species and given that human and bovine Kp-10 differ for only 1 amino-acid (Figure 1) (Oakley *et al.* 2009 modified).

This aminoacid is Phenylalanine (F) substituted by Tyrosine (Y); F and Y differ only for an oxydrile group (-OH), which does not affect the ligand binding affinity to the specific antibody (Voet and Voet 1997).

Estradiol 17β high sensitivity ELISA kit (sensitivity 14 pg/mL; specificity 100%) and progesterone ELISA kit (sensitivity 8.57 pg/mL; specificity 100%) (Enzo® Life Sciences, Postfach, Switzerland) were used for dosing E2 and P4.

All data obtained were expressed as Mean ± S.D. Student's T test for independent variables was used for intergroup comparison and a value of $P < 0.05$ was set as significance level. Statistical analysis was conducted using IBM SPSS Statistics 19 (IBM Corporation Software group, Somers, NY, USA).

Kisspeptin 1	
Human (NP_002247)	**Y N W N S F G L R** F
Cattle (XF_872566)	**Y N W N S F G L R Y**

Figure 1. *Kisspeptin-10 sequences in human and cattle.* Conserved amino acid residues are in bold (Oakley *et al.* 2009 modified).

Serum concentrations of Kp-10, E2 and P4 are illustrated in Table I.

The data obtained show that Kp-10 blood concentrations are statistically higher in Group FC than in Group H. This is in agreement with the study conducted by Jeon and colleagues (Jeon *et al.* 2013), in which the authors observed that Kp levels were significantly higher in the PCOS group than in the control one.

As for the present study, the reason for the hyperproduction of Kp-10 in FC cows is to be found in the paradox of the endocrine characteristics of this pathology. In fact, cows which form cysts notoriously display higher LH blood concentrations, but they do not show an LH surge (Vanholder *et al.* 2006). This high LH level seems to be an essential requisite for cyst maintainance (Calder *et al.* 2001).

It is also well known that follicles that then turn into cysts secrete higher estradiol concentrations than follicles that undergo ovulation, possibly due to their exposure to higher and sustained LH levels (Vanholder *et al.* 2006). This is in accordance with the findings of the present study, in which higher E2 concentrations were observed in FC than in H group. High E2 circulating levels are paradoxically known to impair the positive feedback, as they physiologically exert on the hypothalamus (Kaneko *et al.* 2002). Moreover, in case of follicular cysts, high E2 and P4 levels synergize and lead both to a negative feedback and desensitization in GnRH hypothalamic secreting nuclei (Mutinati *et al.* 2013, Sciorsci *et al.* 2003).

In this study, the higher Kp-10 blood concentrations detected in cows with FC compared to healthy cows suggest that the hypothalamic nuclei implicated in Kp secretion may be stimulated by higher steroid levels.

The higher Kp levels, found in this study, the sustained and high circulating LH concentrations, and the lack of the preovulatory GnRH/LH surge typical of a cystic condition open the way to 3 hypothesis: Kp could directly act on gonadotropic cells stimulating persisting and sustained LH secretion (Oakley *et al.* 2009) or it could indirectly trigger LH synthesis through stimulation only of

Table I. *Blood Kisspeptin 10, estradiol 17 β and progesterone (mean ± S.D.) concentrations in cows with Follicular Cysts (Group FC) and in healthy cows on heat (Group H).*

	Group FC	**Group H**
Kisspeptin 10 (pg/mL)	125.06 ± 34.47^{a}	97.72 ± 21.34^{b}
Estradiol 17 β (pg/mL)	12.07 ± 2.9^{c}	6.16 ± 1.25^{d}
Progesterone (ng/mL)	0.52 ± 0.16^{c}	0.15 ± 0.03^{d}

In the row a, b: $P < 0.05$; c, d: $P < 0.001$.

the tonic hypothalamic centre, or both. The second hypothesis is strengthened by the knowledge that the hypothalamic neurons implicated in both Kp and tonic GnRH secretion are the same, being mainly located in the arcuate nucleus (Senger 2006).

In conclusion the altered gonadotropin secretion characterizing FC may reflect altered patterns of Kp secretion and suggest novel therapeutic approaches to face this common gynaecological disease in dairy cows, such as Kp antagonists.

References

Brown R.E., Wilkinson D.A., Imran S.A., Caraty A. & Wilkinson M. 2012. Hypothalamic kiss1 mRNA and kisspeptin immunoreactivity are reduced in a rat model of polycystic ovary syndrome (PCOS). *Brain Res*, **1467**, 1-9.

Calder M.D., Manikkam M., Salfen B.E., Youngquist R.S., Lubahn D.B., Lamberson W.R. & Garverick H.A. 2001. Dominant bovine ovarian follicular cysts express increased levels of messenger RNAs for luteinizing hormone receptor and 3β-hydroxysteroid dehydrogenase 4, 5 isomerase compared to normal dominant follicles. *Biol Reprod*, **65**, 471-476.

Clarkson J., Han S.K., Liu X., Lee K. & Herbison A.E. 2010. Neurobiological mechanisms underlying kisspeptin activation of gonadotropin-releasing hormone (GnRH) neurons at puberty. *Mol Cell Endocrinol*, **324**, 45-50.

Jeon Y.E., Lee K.E., Jung J.A., Yim S.Y., Kim H., Seo S.K., Cho S., Choi Y.S. & Lee B.S. 2013. Kisspeptin, leptin, and retinol-binding protein 4 in women with polycystic ovary syndrome. *Gynecol Obstet Invest*, **75** (4), 268-274.

Kaneko H., Todoroki J., Noguchi J., Kikuchi K., Mizoshita K., Kubota C. & Yamakuchi H. 2002. Perturbation of estradiol-feedback control of luteinizing hormone secretion by immunoneutralization induces development of follicular cysts in cattle. *Biol Reprod*, **67** (6), 1840-1845.

Mutinati M., Rizzo A. & Sciorsci R.L. 2013. Cystic ovarian follicles and thyroid activity in the dairy cow. *Anim Reprod Sci*, **138**, 150-154.

Oakley A.E., Clifton D.K. & Steiner R.A. 2009. Kisspeptin signaling in the brain. *Endocr Rev*, **30** (6), 713-743.

Pineda R., Garcia-Galiano D., Roseweir A., Romero M., Sanchez-Garrido M.A., Ruiz-Pino F., Morgan K., Pinilla L., Millar R.P. & Tena-Sempere M. 2010. Critical roles of kisspeptins in female puberty and preovulatory gonadotropin surges as revealed by a novel antagonist. *Endocrinology*, **151**, 722-730.

Sciorsci R.L., Rizzo A., Robbe D. & Minoia P. 2003. Le cisti follicolari nella bovina. *In* Proceedings of the 1st Congress of the Italian Society of Animal Reproduction (S.I.R.A.), Vico Equense, Napoli, Italy, 98-107.

Senger P.L. 2006. Pathways to pregnancy and parturition. 2nd ed. Current Conceptions Inc, Pullman Washington.

Tena-Sempere M. 2006. GPR54 and kisspeptin in reproduction. *Hum Reprod Update*, **12**, 631-639.

Vanholder T., Opsomer G. & De Kruif A. 2006. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. *Reprod Nut Dev*, **46** (2), 105-119.

Voet A. & Voet J.G. 1997. Biochimica. Ed. Zanichelli. Bologna, 793-796.

Witchel S.F. & Tena-Sempere M. 2013. The Kiss1 system and polycystic ovary syndrome: lessons from physiology and putative pathophysiologic implications. *Fertil Steril*, **100**, 12-22.