UTERINE HEALTH

Proceedings from a European seminar
in Uppsala, Sweden, November 7, 2013

Renée Båge, Bodil Ström Holst & Patrice Humblot (editors)

Uppsala 2013

CRU Report 29

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Foreword

CRU (Centre for Reproductive Biology in Uppsala) is a multidisciplinary network of almost 100 scientists with different academic backgrounds at SLU, Swedish University of Agricultural Sciences, and UU, Uppsala University.

During two days in 2013, November 7-8, CRU hosted a seminar and research discussions on the topic “Uterine health” in Uppsala. The meeting was initiated with the aim to bring together scientists, clinicians and research students from both human and veterinary medicine to create a European network of specialists in the field of uterine health.

In all animal species and in women, uterine health is crucial for successful conception, implantation and for the development of a healthy pregnancy. Uterine diseases are important causes of infertility. They affect health and welfare and in the case of production animals they also affect economy.

In order to illustrate the wide spectrum of physiological and pathological events affecting uterine health, renowned scientists were invited to present their research. The seminar program covered immune processes involved in uterine function, interactions between gametes or embryos and the maternal uterus, uterus-pathogen interactions and genetic and epigenetic aspects on endometrial receptivity and disturbances.

Understanding phenotypic, genetic and environmental factors associated with uterine health will guide disease diagnosis, treatment and prevention, and it will have implications for animal breeding.

On behalf of CRU and the organizing committee we wish to express our gratitude to all participating delegates representing a number of European research institutes and universities. We also wish to thank the Swedish Institute, CRU and the Research School in Translational and Comparative Medicine at the Faculty of Veterinary Medicine and Animal Science, SLU, for making the seminar and the research discussions possible.

We hope that our joined forces will lead to further exchange of information, new research ideas and future cooperation across Europe.

Uppsala, December 2013

Renée Båge, Bodil Ström Holst and Patrice Humblot
(editors)

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Program “Uterine Health” Seminar
7 November 2013 – SLU, Ultuna campus, Room KC2

8h30 – 8h35 Welcome, Introduction, Patrice Humblot (SLU, Clinical Sciences)

8h35 - 8h40, SI/LEARN, Christine Jakobsson (SLU)

Session 1: Immune processes involved in uterine function:

8h45 – 9h30 Alireza Fazeli, Sheffield University, UK: *Innate immunity and uterine health, the missing links*

9h30 – 10h15 Olivier Sandra, INRA BDR, France: *Mechanisms involved in the immune response of the uterus at implantation in mammals*

10h15 – 10h30 Annabel Bergmann, Federal Research Institute for Animal Health, Germany: *Sperm binding to porcine uterine epithelia cells: an immunological process or something more sweet?*

10h30 -10h45 Coffee break

Session 2: Endometrial receptivity and disturbances

10h45 – 11h15 Nathalie Beaujean, INRA BDR, France: *Endometrial epigenetic status and disturbances*

11h15 – 12h00 Marina Suhorutshenko, Tartu University, Estonia: *Gene regulation of endometrial receptivity by steroid hormone receptors*

12h00 – 12h30 Daniel Vaiman, Institut Cochin, France: *The epigenetics of endometriosis*

12h30 -13h15 Lunch (sandwiches)

13h15 – 13h30 Paola Roncada, Università degli Studi di Milano, Italy : *Perspective of proteomics for uterine health*

13h30 - 13h45 Enrique Gomez, SERIDA, Spain: *Monitoring the embryo by its environment*

Session 3: Infection and inflammation in the female genital tract

13h45 – 14h30 Martin Sheldon, Swansea University, UK: *Bacterial infection and innate immunity in the female genital tract*

14h30 – 14h45 Trudee Fair, University College Dublin, Ireland : *Immune cells and inflammatory markers in the ruminant endometrium*

14h45 - 15h15 Coffee break

15h15 - 16h00 Gaetano Donofrio, Universita' degli Studi di Parma, Italy: *Involvement of BoHV-4 in bovine uterine diseases*

16h00 - 16h15 Andres Waldmann, Estonian University of Life Sciences, Estonia : *Cytological evaluation of uterine health status in the cow*

16h15 - 16h30 Claire Wathes, Royal Veterinary College, UK : *Antimicrobial peptides in bovine endometrium*

16h30 - 16h45 Short break

16h45 – 17h00 Rita Payan Carreira, Universidade de Trás-os-Montes e Alto Douro Portugal: *Uterine health in dogs: the participation of local factors*

17h00 – 17h15 Ragnvi Hagman, SLU, Sweden: *Pyometra in dogs*

17h15 – 17h30 Patrice Humblot, SLU, Sweden: *Use of Ecoli LPS as a model to approach inflammation in the endometrium, preliminary results*

17h30 - 17h45 General Discussion and concluding remarks

18h Departure for First Hotel Linné

19h Dinner

ABSTRACTS

Innate immunity and uterine health: the missing links

Alireza Fazeli

Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, United Kingdom

The innate immune system is probably the most ancient form of immune mechanism. This system follows nearly the same principals in many species: protecting individuals from pathogenic threats by helping them to recognise and differentiate the self from the non-self entities.

In humans and other mammals various organs and systems have developed a special form of collaboration to function in harmony with the innate immune system. They ensure optimum conditions for the development and health of the individual. For example the digestive tract is in constant contact with the outside environment. The ingestion and utilization of food by the organism is accomplished by this tract in close collaboration and cooperation with the innate immune system. While this immune system recognises and repels harmful entities it allows friendly microorganisms useful for food digestion to thrive.

A close relationship exists between the innate immune system and the reproductive tract. In the past we regarded the reproductive tract, in particular the female reproductive tract, as an immune-privileged area which allows special reproductive events taking place in this organ. Today we suspect an intimate collaboration between the innate immune system and the reproductive tract that allows successful sperm transport, placental invasion, embryo implantation and development.

Understanding the interaction between the innate immune system and the maternal tract will help us unravel the mechanisms responsible for maintaining the health of this organ and how the immune system works in general. It will also provide an opportunity for us to introduce new clinical therapies for infertility, auto-immune diseases and the growing incidences of allergies.

Mechanisms involved in the immune response of the uterus at implantation in mammals

Olivier Sandra

INRA, UMR1198 Biologie du Développement et Reproduction, Jouy-en-Josas, France

Implantation is a critical process whose progression conditions the development of the foetal-placental unit, the issue of pregnancy and the health of the progeny. Successful implantation has been shown to integrate a controlled inflammation process, which involves the regulation of cytokines and chemokines followed by the recruitment of immune cells from the blood system to the tissue. These cells secrete different cytokines that will induce tissue remodelling by stimulating cell proliferation and differentiation.

Based on cattle, mouse and human studies, the current talk will illustrate the global inflammation reaction that takes place in the endometrium during oestrous cycle and early pregnancy. In addition, the evolution of the inflammatory reaction in the endometrium will be presented based on human and bovine experimental models (e. g. using embryos displaying distinct rates of successful pregnancy up to term; females that display spontaneous fertility troubles such as implantation failures or recurrent pregnancy loss). Eventually, the positive impact of a stimulated inflammation on pregnancy rate in bovine, murine and human species will be presented and discussed.

Playing with inflammation-related factors could offer new means to improve endometrium receptivity and quality in order to increase term pregnancy rates as long as harmful consequences on offspring health have not been reported.

Sperm binding to porcine uterine epithelia cells: an immunological process or something more sweet?

Annabel Bergmann, Ulrike Taylor, Sonja Junge-Krämer, Detlef Rath

Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute (FLI), Neustadt-Mariensee, Germany

In swine reproduction it is current practice to inseminate 1-3 x109 spermatozoa trans-cervically to gain fertility rates of >90%. These very high sperm numbers derate ejaculate efficiency to an average of 10-30 portions per boar and ejaculate. Smaller numbers can only be applied successfully when deposited closer to the site of fertilisation in the caudal isthmus of the oviduct. However, such invasive techniques are not practical under field conditions.

The question arises why a reduction in sperm numbers is thus possible and what happens to the sperm cells on route to fertilisation. It is known that a large volume of semen is expelled from the sow's tract by retrograde efflux. Further sperm losses are assigned to phagocytosis by the influx of immune cells into the uterine lumen and the passing of sperm cells past the infundibulum into the peritoneal lumen. However, neither a single nor the sum of these processes allow for such a drastic loss of so many sperm cells. We thus proposed that porcine sperm undergo a transient binding with the endometrium before proceeding towards the oviductal reservoir

In vivo experiments showed that inseminations with sperm extended in seminal plasma shifted the endometrial gene expression whereas volume control treatments (extender only inseminations) did not show significant changes. Interestingly, no up-regulation of immune relevant genes was to be seen, but down regulation suggesting suppression of an immune reaction to the sperm cells. These findings nourish the idea of a sperm-endometrium interaction. We thus developed a primary cell culture model from porcine epithelial cells (UEC) to study possible sperm-endometrium interactions in vitro.

Fresh and extended boar semen was released to the confluent UEC monolayer and incubated for 10 min. Sperm binding was observed with a phase contrast microscope. Sperm bound within few minutes and via the apical head membrane and formed clusters. Control incubations with porcine foetal fibroblasts showed that sperm bound, too but in significantly less dense patterns than to UEC.

These findings underline that porcine sperm undergo evitable binding with uterine epithelial cells post AI, modulating gene expression in the endometrium and possibly influencing further reproductive events such as fertilisation and nidation.

Endometrial epigenetic status and disturbance

Nathalie Beaujean

INRA, UMR1198 Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France

It is widely thought that epigenetics play key role in cellular identity and lineage determination. Epigenetics refers to heritable processes regulating gene expression without alteration of gene sequences. Epigenetic control is mainly achieved by chemical modifications, which can be propagated through mitosis, and in some cases through meiosis (Bonasio et al., 2010). It involves several mechanisms such as DNA methylation, histone post-translational modifications (PTMs) as well as chromatin structure and nuclear architecture (Schneider and Grosschedl, 2007; Bernstein et al., 2007). Epigenetics has also been recently extended to small non coding RNAs which mainly downregulate gene expression but may also activate gene expression (Bourc'his and Voinnet, 2010).

The epigenome is the set of epigenetic marks present in a given genome, at a given time and in a given cell type. Interestingly, many studies on epigenetic regulation have shown that environment can play an important role in this respect and that, during critical periods of development and differentiation, environmental changes may alter developmental programming (Keenen and De La Serna, 2009; Roper and Hemberger, 2009; Bernstein et al., 2007).

Methylation of DNA for example could play a significant role in regulating the endometrial changes associated with ovulation induction. Indeed, DNA methylation and the corresponding enzymes: the DNA methyltransferases (DNMTs) have been clearly demonstrated to be involved in endometrial receptivity as well as in decidualization (Rahnama F et al., 2009; Vincent ZL, et al. 2011; Yamagata Y et al., 2009; Ding YB et al., 2012).

In one study, mice treated with a DNA demethylating agent, the nucleoside analog 5-aza-20-deoxycytidine, had a dose-dependent decrease in the number of implantation sites, associated with altered expression of endometrial DNA methyltransferases and genes controlling endometrial changes (Ding YB et al., 2012).

Similarly, a recent study on diethylstilbestrol (DES) showed that neonatal exposure to DES significantly reduced expression of several chromatin-modifying enzymes in the uterus and altered several epigenetic marks at the *Six1* locus (Jefferson WN et al., 2013).

All of these findings provide support for a potential role of epigenetic marks in endometrial changes during embryo implantation after natural and/or chemical environmental changes. Further experiments are needed to clarify the respective roles of embryonic or endometrial epigenetic disturbances in implantation failures.

Gene regulation of endometrial receptivity by steroid hormone receptors

Marina Suhorutshenko[1,2]

[1]Competence Centre of Reproductive Medicine and Biology; [2]University of Tallinn, Estonia

Successful pregnancy can be determined by the presence of qualitative embryo, receptive endometrium and a molecular dialogue between them. Uterine lining becomes receptive to blastocyst on days 20-24 of menstrual cycle, which is assured by certain levels of steroid hormones (estrogen, progesterone), which activate their nuclear receptors and regulate the expression of genes, responsible for endometrial growth and preparation for embryo implantation. On the other hand, hormonal disorders may cause infertility by budging the implantation window. A high rate of non-successful IVF cycles brought about the need for molecular markers to predict whether endometrium is ready to welcome an embryo. During past decade quite a few studies implementing microarray and high-throughput sequencing techniques had been performed, resulting in a set of consensus marker genes for prediction of endometrial receptivity.

Our studies highlight the importance of steroid hormone receptor target genes as specific markers for prediction of endometrial receptivity and hormonal treatment outcome on endometrium. A set of ERα, ERβ, PRA and PRB target genes were identified in several endometrial cell lines using ChiP-qPCR and RNA sequencing with respect to endometrial receptivity and most abundant reproductive system diseases. Our recently identified steroid hormone receptor target genes, regulated by tamoxifen and mifepristone, reflect the effect of these two widely used steroid hormone receptor modulators on human endometrium.

The epigenetics of endometriosis

Bruno Borghese, Charles Chapron, Daniel Vaiman

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Endometriosis is a very frequent gynaecological disease, affecting roughly 10% of women. It is characterized by pain and infertility especially during the menses. Histologically speaking this still mysterious disease has been associated with 'implants' located on the peritoneum. It affects various organs, often the ovary (ovarian endometrioma), but also the utero-sacral ligaments, the vagina, rectum, bladder and even sometimes organs of the upper part of the body such as lungs, and even exceptionally the brain. The implants reproduce grossly the structure of the endometrium, with stroma and glands, and most importantly, reproduce the uterine physiological response to estrogen and progesterone; at each cycle, the implant will bleed, and probably be a major cause of pain, many implants being richly innerved.

High throughput technologies are recently been used systematically to characterize three similar but not identical tissues: normal endometrium, endometrium from endometriosis-affected women and endometriosis lesions from endometriosis-affected women. Transcriptomic analysis reveals thousands of transcriptionally-modified genes between eutopic and ectopic endometrium, (see for instance Broghese et al, Mol Endocrinol, 2008). Some congruent elements suggest that endometriosis could trigger epigenetic modifications such as alterations of the histone code, abnormal DNA methylation and alteration of the mi-RNA expression profile. In particular, several studies refer to such anomalies (see for instance Fassbender et al, Fertil Steril, 2013 for a recent review). The methylation profile has been the subject of various studies focused on individual genes and also on genome wide methylation profiling (Calicchio et al, Curr Pharm Design, 2013 for review, Borghese et al, Mol Endocrinol, 2010; Yamagata et al, Plos ONE, 2013 in press). In the study of Borghese (2010), a bias of methylation towards chromosome ends was found and led us to explore the DNA sequence of DNMT3L in patients and control, since rare variants of this enzyme were previously associated with abnormal methylation of chromosome ends (El-Maarri et al, Hum Mol Genet, 2009). We could indeed associate several haplotypes with increased sensitivity or increased resistance to endometriosis (Borghese et al, Am J Pathol, 2012), which may lead to prediction and have possibly a useful clinical application.

Further research on DNA methylation in endometriosis may rely on new tools enabling to approach genome methylation thoroughly and on a comprehensive basis in normal uterus, as well as uterus and lesions of patients. Such a preliminary study that we carried out allowed to identify over 400 CpG-encompassing regions that are abnormally methylated in the lesions. Their analysis in normal and abnormal uterine tissue, in parallel in stromal and epithelial cells may result in a better understanding of endometriosis physiopathology and reflect some pathways where intervention is possible to reach uterine health in broad sense.

Perspective of proteomics for uterine health

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Proteomics, than means the protein expression of a genome in a precise moment of life can be a valid approach to elucidate mechanism of reproduction and to improve cattle fertility. Proteomics tools can be used to identify molecules of relevance in reproductive physiology and pathologies. Proteomics in reproductive biology is still in its infancy and up to date a discrete number of papers about proteomics in human uterine health have been published. But for what it concern farm animals, and in particular dairy cattle fertility, there is still a lack of knowledge.

There are two major approaches for proteomics studies that are complementary. The top down approach, that in general uses separation techniques, starts from two dimensional gel electrophoresis and ends with the characterization of proteins in term of microsequencing or mass spectrometry/western blotting validation. It is also able to identify post translational modifications. The bottom up approach, or shotgun proteomics, is useful to run expression proteomics in terms of semiquantitative analysis, with no information about post translational modification, but it is high throughput and highly automatized.

Proteomics can produce valid responses in comparative studies during embryo development, efficiency of embryo transfer, composition and relative differences of follicle/uterine fluids. Moreover, the very promising field of proteomics in uterine health, is the study of response to bacterial/virus infection to endometrium. The recent progress of metagenomic/metaproteomic approach represents the major challenge in this field.

Monitoring the cattle embryo by its environment

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The analysis of the environment that surrounds an early embryo informs of relevant embryonic traits, both in vivo and in vitro. Thus, during oviductal and early uterine development, the embryo triggers different endometrial reactions depending on its potential of to develop. This dialogue may affect the outcome of the pregnancy and have consequences in adulthood. In monotocus species the presence of tens of early embryos in the uterus showed to overcome the low signal-to-noise ratio inherent to a single embryo. Thus, specific maternal responses in uterine fluid (UF) were detected by DIGE proteomic analysis that characterized both the presence of embryos (Muñoz et al, J Proteome Res (2012), 11:751-66) and the embryonic sex (Gómez et al, J Proteome Res (2013), 12:1199-210). On Day-8, embryos overcome native pro-inflammatory conditions by NFκB down-regulation, and proteins involved showed to increase embryonic development in vitro. Regulation of uterine embryonic development could be asymmetric, as protein abundance differed between left and right uterine horns (Trigal et al, Reprod Fert Dev (2013); in press). The proteasome / immunoproteasome system was involved in uterine recognition of male and female embryos, with UF proteins of male embryos in the uterus showing increased embryotrophic properties in vitro.

The in vitro culture medium (CM) can also inform of sex (Muñoz et al, Metabolomics (2014), in press) and viability (Muñoz et al, submitted) of embryos cultured individually for 24h prior to embryo transfer (ET); recipient blood plasma also informs of embryonic viability. The metabolomic fingerprinting, a spectral dataset directly dependent of metabolites present, was obtained from CM and plasma by Fourier Transform Infrared Spectroscopy (FTIR) analysis and subsequent application of appropriate algorithms. Sensitivity and specificity of male female (embryonic sex) and pregnant / non-pregnant (viability) were obtained at very good to excellent detection rates. Generally, recipient plasma showed higher predictive ability than CM.

Bacterial infection and innate immunity in the female genital tract

Iain Martin Sheldon

Institute of Life Science, College of Medicine, Swansea University, Swansea, UK

One of the most common endemic diseases of cattle is bacterial infection of the uterus after parturition. These infections damage the endometrium lining the uterus, reduce the production of milk, and cause infertility. Uterine disease is caused by *Escherichia coli, Trueperella pyogenes*, anaerobic bacteria and viruses. Epithelial and stromal cells are the first line of defence against microbes in the endometrium, and they have key roles in innate immunity. Endometrial cells possess Toll-like Receptors to detect pathogen-associated molecular patterns, leading to the secretion of chemokines and cytokines, which attract and activate macrophages and neutrophils. Uterine disease also compromises the function of ovarian follicles and the corpus luteum. Granulosa cells from ovarian follicles express Toll-like Receptors, and pathogen-associated molecular patterns perturb their endocrine function, stimulate the secretion of inflammatory mediators, and damage oocytes.

In conclusion, the inflammatory responses in the endometrium and the ovary following postpartum bacterial infections of the uterus are important mechanisms underlying infertility in cattle.

Maternal immune response to the early implanting embryo in cattle

Trudee Fair

School of Agriculture and Food Science, University College Dublin, Ireland

It is widely accepted that the maternal immune system must be modulated to prevent rejection of the embryonic semiallograft in order to establish and maintain mammalian pregnancy. In ruminants, the main maternal recognition factor of pregnancy, IFNT, appears to be the key regulator of the maternal immune response.

Our recent studies implicate the actions of IFNT in the initial maternal response to the presence of the elongating embryo, manifested by expansion of macrophage ($CD14^+$) and dendritic ($CD172a\text{-}CD11c^+$) -cell populations. Furthermore, IFNT appears to regulate the gene expression profile of these cells, as endometrial mRNA expression of *IL12B, MCP1, MCP2, PTX3, RSAD2*, and *TNFA* was dramatically increased on Day 16 of pregnancy and also in response to IFNT supplementation *in vitro*. The macrophage and dendritic cell populations show continued expansion as pregnancy progresses, implicating these cells in endometrial and conceptus modeling during implantation. Although there is strong evidence from studies in humans and mice linking successful pregnancy with an imbalance toward a Th2 immune response type, $CD4^+$, $CD8^+$ and $\gamma\delta TCR^+$ T- lymphocyte populations are not regulated temporally during oestrus or early pregnancy in cattle. However, the mRNA expression profile of Th1 immune factors *IFNA, LIF, IL1B, IL8* and *IL12A* are down regulated during the oestrus cycle, suggesting that the phenotypes of Th cells are modulated during the oestrous cycle in anticipation of pregnancy. In addition, unlike events in human and mouse models, the bovine endometrium population of $CD335^+$ NK cell population is not expanded as an immediate response to maternal recognition of pregnancy.

In conclusion, monocytes/macrophages and dendritic cells play central roles in the bovine maternal immune response to the semiallogenic embryo and are also key drivers of endometrial and conceptual modeling for implantation.

Role of BoHV-4 in bovine uterine infections

Gaetano Donofrio, Sarah Jacca, Valentina Franceschi

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Bovine uterine infections are the most important cause of economic losses in cattle industry. Although the etiology of uterine diseases is mainly ascribed to bacterial infection, they can also be associated with viral infection, such as bovine herpesvirus 4 (BoHV-4), which is often a secondary agent following bacteria. Bovine herpesvirus 4 (BoHV-4) has been most consistently associated with uterine disease in postpartum cattle. The first isolation of BoHV-4 from a case of bovine metritis was reported in 1973. Postpartum metritis has also been associated with BoHV-4 in the USA, Spain and Serbia. Several other isolates were associated with reproductive disorders and BoHV-4 seroprevalence was associated with postpartum metritis and chronic infertility in cattle. Like other herpesviruses, BoHV-4 can establish persistent infections in cattle, particularly in macrophages, and viral infection is often identified concurrently with bacteria that cause uterine diseases. It was suggested that there may be a vicious circle composed of bacterial endometritis, leading to secretion of prostaglandin E2 (PGE2) and then stimulation of viral replication by PGE2 and lipopolysaccharide (LPS), which causes further endometrial tissue damage and inflammation.

In the present study, the interaction between BoHV-4 infected bovine endometrial stromal cells and tumor necrosis factor alpha (TNF-a) was investigated. Bovine herpesvirus 4 possess a special tropism toward endometrial stromal cells, for this reason a simian virus 40 (SV40) immortalized endometrial stromal cell line (SV40BESC) was established and proven to be stable and express Toll-Like Receptors (TLRs) (from 1 to 10), TNF-α Receptors I and II and to be responsive to exogenous TNF-α. Further, an increase of BoHV-4 replication and cytopathic effect was observed in BoHV-4 infected and TNF-α treated SV40BESCs. This increase of viral replication was associated with BoHV-4 Immediate Early 2 (*IE2*) gene promoter trans-activation through the interaction of the nuclear factor KB (NFKB) with the putative NFKB responsive elements found within BoHV-4 *IE2* gene promoter and this interaction was abolished when NFKB responsive elements were deleted. To summarize, a rather complex role for BoHV-4 as a cofactor for the development of bovine post-partum metritis may be hypothesized: in BoHV-4 persistently infected animals, BoHV-4 infection resides within the macrophages. During parturition, infection of the uterus can take place from environmental bacteria. However such infection in normoergic animals is cleared within 3 weeks whereas, in BoHV-4 persistently infected animals, the inflamed uterus attracts BoHV-4 persistently infected macrophages from the periphery to the site of inflammation. Inflammatory molecules produced by the inflamed endometrium and proliferating bacteria, such as PGE2 and LPS, induce the replication of BoHV-4 in persistently infected macrophages and endometrial stromal cells can become infected with newly replicating virus. Furthermore, TNF-α produced by LPS induced macrophages bind TNF-αR1 on the surface of BoHV-4 infected endometrial stromal cells, inducing BoHV-4 *IE2* gene expression and enhanced BoHV-4 replication. The *IE2* gene product ORF50/*Rta*, induces not only BoHV-4 replication but also IL-8 production, thus shifting the inflammation from a transitory and acute status (metritis) toward a chronic status (endometritis).

Cytological evaluation of uterine health status in the cow

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The objective of the study was to assess early metabolic and endocrine risk factors for cytological endometritis (CE). Uterine cytology samples were collected by using the brush technique at 40 ± 2 d postpartum (PP) from 119 multiparous Estonian Holstein cows from a single, 1,200 cow, free-stall dairy herd with previous lactation 305 d milk yield > 8500 kg. The cytological criterion was set at > 8 % of neutrophils as the threshold indicator of CE. Blood was collected at 7d PP. Prepartum body condition was scored (1 to 5) and daily milk yield was recorded. Plasma insulin, insulin-like growth factor-1 (IGF1) and serum amyloid A (SAA) were analysed by EIA. All other plasma variables were analysed by an autoanalyzer. Concentrations of the plasma variables were dichotomized using reciever operating characteristic (ROC) curve analysis. The optimal threshold for each parameter was based on the highest sum of sensitivity and specificity for predicting the occurrence of CE. The prevalence of CE was 30.3%. The median days open for CE positive cows was 197 d and was higher than that of CE negative cows (97 d) ($P < 0.0001$; Log-rank test). Univariate logistic regression analysis showed that the odds of developing CE increased by a factor of 5.96 ($P < 0.001$) when IGF1 was < 13.24 ng/ml, by a factor of 6.39 ($P < 0.001$) when haptoglobin (Hp) was > 0.81 g/l, by a factor of 9.13 ($P < 0.001$) when SAA was > 128 µg/ml, by a factor of 2.96 ($P = 0.009$) when albumin was < 36.45 g/l, and by a factor of 2.33 ($P = 0.044$) when cholesterol was < 2.15 mmol/l. Insulin, β-hydroxybutyrate and nonesterified fatty acid concentrations were not associated with CE. When the plasma variables were submitted to a multivariate logistic regression model, only Hp and IGF1 remained significant predictors for CE (odds ratios = 4.37; 95% CI 1.74-11.00; $P = 0.002$ and 3.57; 95% CI 1.44-8.89; $P = 0.007$, respectively). Hp and IGF1 also remained significant predictors for CE (odds ratios = 4.76; 95% CI 1.60-14.20; $P = 0.006$ and 3.67; 95% CI 1.20-11.23; $P = 0.024$, respectively) after including prepartum body condition score and first 45 d milk yield as covariates.

In conclusion, CE decreased the reproductive performance and was associated with inflammatory and metabolic status. Measurement of Hp and IGF1 at 7d PP can be useful in predicting of CE risk.

Uterine health in dogs: the participation of local factors

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The mammalian endometrium is a highly dynamic and complex tissue, whose main purpose is to guarantee embryo implantation and the success of pregnancy. Receptors for ovarian sex steroid are the main drivers of endometrial fitness, acting through diverse molecular markers, secreted by a variety of cell types within the endometrium. These changes, called the endometrial cycle, occur at species-specific intervals and involve a strikingly coordinate interplay of numerous autocrine and paracrine factors. Dog oestrous cycle present unique characteristics among the domestic species, which might influence the pattern of expression for most molecules identified as participants in the endometrial cycle. Such molecules are the key for a successful implantation/pregnancy and also, if following a normal pattern of expression, a guarantee for uterine health.

In this presentation we will review some data on the pattern of expression of the endometrial markers in dogs during the cycle or in pregnancy, such as CD10, adhesion molecules (Cadherin E and β-catenin; integrin αv-β3 and osteopontin), interleukins (TNF, TGFβ and IL18), Cox-2 and active caspase 3 and the oxidative stress enzymes. Further, it will also be analysed the changes in the cyclic pattern in comparison to the early implantation and the cystic endometrial hyperplasia-pyometra complex.

A long walk is preview to master the knowledge on the canine endometrial cycle. Still, increased understanding of the molecular cues that might be involved in reproductive success and in the lost of endometrial fitness would greatly improve the efficiency of diagnostic tools in canine medicine.

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Pyometra in dogs

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Pyometra is considered as one of the most important diseases of female dogs. It is characterized by uterine bacterial infection and inflammation with pus accumulating in the uterus in combination with potentially life-threatening systemic illness. The disease is mainly diagnosed in metoestrus (dioestrus) and is caused by an ascending bacterial infection of a progesterone-primed uterus. In Sweden, in average 20% of all female dogs are diagnosed with pyometra before 10 years of age. In certain high-risk breeds this proportion exceeds 50%. Age- and breed-related differences in the occurrence show that some breeds are more prone to develop the disease and at a younger age compared with others. It is therefore plausible that a genetic predisposition for pyometra exists in certain dog breeds and families.

Bacterial infection and endotoxins are potent inducers of an inflammatory response, which is apparent in the inflamed uterine tissue observed during surgical treatment and at subsequent histopathological examination. Sepsis is present in the majority of dogs with pyometra. The inflammatory response in infected uterine tissue has also been more closely explored. In one study of the molecular patterns involved in the uterus in pyometra, numerous (<800) genes were found upregulated. Many of these genes are associated with chemokines, cytokines, inflammatory cell extravasation, anti-bacterial action, the complement system and innate immune responses. Increased transcription of genes encoding Toll-like receptors 2 and 4, lipopolysaccharide ligands, prostaglandin synthesis enzymes and matrix metalloproteinases (MMPs) have also been demonstrated in pyometra uteri. Down-regulated genes include zinc-fingers and homeobox genes. More knowledge of the complex local and systemic inflammatory response may allow identification of new disease biomarkers or future targets for treatment in pyometra. Dogs are often used in experimental studies for endotoxemia and sepsis in humans, because of similarities in their inflammatory response. The possibility of using pyometra as a natural disease model for serious bacterial infection in humans is to be considered. A genetic predisposition for the disease might, if identified, provide the possibility of implementing breeding programs aimed at reducing the occurrence in high-risk breeds

E. coli lipopolysaccharide stimulates proliferation of bovine uterine epithelial cells

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The general objective of this work is to find markers of "reproductive robustness" in cows exposed to different stressors /diseases. E coli is one of the most frequent bacteria involved in clinical uterine disease**.** Lipopolysaccharide (LPS) is a component of outer membrane of Gram-negative bacteria involved in the pathogenic processes leading to post-partum metritis and endometritis in cattle. It also causes inflammation of the endometrium and implantation failures in many animal species. Increase of cell proliferation by LPS has been reported in human epithelial and immune cells (1) but not from endometrial cells.

The aim of this study was to characterize the proliferative response of bovine endometrial cells following exposure to E. coli-LPS. *In vitro* culture of bovine endometrial epithelial cells (EEC) and fibroblast were performed (2). On passages 4 to 6, EEC were challenged with 2, 4, 8, 12, 16 or 24µg/ml LPS. At time of challenge and 72 hrs later, numbers of attached cells were counted. The variation of cells number over time was analyzed by ANOVA (SAS 9.1, proc GLM). The effect of passage number, initial number of cells at time of challenge, treatment group and corresponding interactions were included in the model. A significant increase in cell number was observed for cells treated with 2, 4, 8 and 12µg/ml LPS ($P \leq 0.001$), whereas non significant effects were observed for 16 and 24µg/ml LPS. Effects of initial number of cells at time of challenge was significant ($p<0,01$) whereas passage number and interactions between all factors were non significant. For fibroblast, preliminary results suggest that the response to LPS is much less important than for EEC. These findings indicate that E. coli LPS stimulates proliferation of bovine endometrial epithelial cells, and that effect of LPS is dose-dependent but not linear under the range of concentrations tested. The cellular mechanisms involved in this response are under study.

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