Institute of Animal Reproduction and Food Research
Polish Academy of Science in Olsztyn
&
Faculty of Veterinary Medicine,
University of Warmia and Mazury

"ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS"

11-12 September 2017
Olsztyn, Poland



Welcome to the Conference

Dear Colleagues,

We are pleased to welcome everyone to Olsztyn for the International Conference “ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS”

The Conference is especially intended to encourage interaction between researchers, university lecturers and Vet practitioners.

The aim of the Conference is to provide current knowledge about uterine biology and morphology, as well etiology and pathogenesis of endometritis, clinical and subclinical endometritis, new diagnostic methods and new treatment strategies. Moreover, the impact of endometritis on reproductive health and animal productivity will be also discussed.

We want to thank all of our invited speakers that have been accepted our invitation to come to Olsztyn. We are very pleased to have them with us in our meeting and look forward to hearing about the interesting studies that they do.

Finally we want to thank all of participants. We are grateful to you for your support and discussion. We wish all of you many fruitful discussion, meeting old friends, making new partnerships and having a pleasant stay in Olsztyn.

Local organizing and scientific committee

Dariusz J. Skarżyński, Karolina Łukasik, Paweł Kordowitzki, Beenu Moza Jalali (Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research Polish Academy of Science in Olsztyn, Poland)

Tomasz Janowski (Department of Animal Reprodcution, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland)

Konsorcjum Naukowe “Zdrowe Zwierzę - Bezpieczna Żywność”
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POLSKA AKADEMIA NAUK

Sunday, September 10th		
18:00 – 20:00	**Welcome reception, registration** Department of Animal Reprodcution UWM, Oczapowskiego 14	
Monday, September 11th		
8:30 - 9:00	Registration	
9:00 - 9:15	Opening ceremony **Dariusz J. SKARŻYŃSKI** *(IARFR PAS, Olsztyn, Poland)* **Tomasz JANOWSKI** *(UWM, Olsztyn, Poland)*	
9:15 - 10:00	**Plenary lecture I** **Hans -Joachim SCHUBERTH** *(Tierarztliche Hochschule Hannover, Immunology Unit, Hannover, Germany)* **Bovine Fertility and the immune system: from insemination to calving**	
10:00 - 10:30	Coffee break	
10:30 - 13:30	**Session I: Immune-endocrine function of uterus** Moderators: **Anna CHEŁMOŃSKA-SOYTA** *(UP, Wroclaw, Polska)* "Local and peripherial preimplantation pregnancy recognition- insight from mouse model" **Luana RICCI PAULESU** *(Università di Siena, Italy)* "The cytokine MIF in physiology and pathology of human reproduction" **Selected abstracts, each 15min + 5min discussion** • **Westerkamp et al.** "Detection of Lactobacillales spp. in the bovine seminal plasma and their influence on endometrial epithelial cells *in vitro*." • **Brewer et al.** "Defining the inflammatory gene signature that precedes the development of uterine disease in postpartum cattle" • **Korzekwa et al.** "Effect of LPS from Escherichia coli on PPAR expression profile during experimentally induced endometritis in the bovine endometrium explants" • **Socha et al.** "Escherichia coli affects PPAR expression profile in the bovine endometrium during experimentally induced endometritis - *in vivo* studies."	
13:30 - 15:00	**Lunch**	**Poster session**

15:00–18:00	**Session II: Endometritis in mares** Moderators: **Mats HT TROEDSSON** *(University of Kentucky, USA)* "Equine Endometrits" **Graca FERREIRA – DIAS** *(UTL, Lisbon, Portugal)* "The intricacies of mare endometrosis- how far have we gone?" **Selected abstracts, each 15min + 5min discussion** • **Christoffersen et al.** "Infectious endometritis is associated with endometrial expression of pro-fibrotic markers and integrin ITGAV." • **Monteiro de Barros et al.** "Characterisation of transcriptome profiles of fresh or cultured ex vivo equine endometrial explants at different time points." • **Amaral et al.** "Cathepsin inhibition affects mare endometrium prostaglandin secretion during the estrous cycle." **Poster flash talk each 5min** • **Rebordao et al.** "Are mares physiologically protected against endometrial fibrosis induced by NETs proteases?" • **Domino et al.** "Endometritis Coexisting with Carcinoma Metastases to the Uterus in Mare."
20:00 - 24:00	**Gala Dinner** Browar Warmia, Feliksa Nowowiejskiego 15
Tuesday, September 12th	
9:15 - 10:00	**Plenary lecture I** **Martin SHELDON** *(Swansea University, United Kingdom)* **"Mechanisms linking metabolism with endometritis"**
10:00 - 10:30	Coffee break
10:30 - 13:30	**Session III: Endometritis in cows** Moderators: **Geert OPSOMER** *(Universiteit Gent, Belgium)*

	"State of the art and some innovative ideas about (endo)metritis in cattle" **Christoph GABLER** *(Freie Universitat Berlin, Germany)* "Response of bovine endometrial epithelial cells depends on pathogenicity of bacterial strains" **Selected abstracts, each 15min+ 5min discussion** • **Meyerholz et al.** "Genetically selected heifers differ in incidence of metritis" • **Raz et al.** "Reproductive performance, energy balance, ovarian function, and incidence of subclinical endometritis in cows diagnosed with metritis in early lactation" • **Kelly et al.** "Inflammasome-dependent IL-1β production by bovine endometrial stromal cells and polarized epithelial cells." **Poster flash talk, each 5min** • **Ryan et al.** "Serum alpha 1-acid glycoprotein concentration on day 7 postpartum as a potential biomarker for the development of purulent vaginal discharge in dairy cattle" • **Rojas Canadas et al.** "Association between body condition score, uterine health status and ovarian cyclicity on early lactation dairy cows." • **Pedersen et al.** "Presence of bacteria in the endometrium and placentomes of pregnant cows"
13:30 - 13:45	Closing Remark & Review **Dariusz J. SKARŻYŃSKI** *(IARFR PAS, Olsztyn, Poland)* **Tomasz JANOWSKI** *(UWM, Olsztyn, Poland)*
13:45 - 15:00	Lunch
Summer School of KNOW Place: Faculty of Veterinary Medicine	
15.00 – 15.45	Discovering links between animal disease, infertility, and immunity **Martin Sheldon** (Cardiff, GB)
16:00 – 16.45	How to manipulate the immune system: Immunomodulation versus vaccination **Hans-Joachim Schuberth** (Hanover, DE)

17:00 – 17:45	"Role of pro-inflammatory mediators during endometritis in cattle" **Christoph Gabler** (Berlin, Germany)
Wednesday, September 13th - Summer School of KNOW	
8:30 – 9:10	How to diagnose mare endometritis and endometrosis Graca Ferreira -Dias (Lisbon, PT)
09:20 – 10:00	Sperm transport and elimination in the mare reproductive tract Mats Troedsson (Lexington, KY, USA)
10:10 – 10:30	Specific localization and biological activity of dietary flavonoid quercetin in brain. Yoshichika Kawai (Tokushima, Japan)
10:40 – 11:20	Endocrine disrupting chemicals: an hazard in the prenatal life Luana Ricci Paulesu (Siena, Italy)

VENUE PLACE: University Library of the University of Warmia and Mazury

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Plenary lecture I

Bovine Fertility and the immune system: from insemination to calving

Hans-Joachim Schuberth

Immunology Unit, University of Veterinary Medicine, Hannover, Germany

Every aspect of bovine fertility is guided, controlled, or accompanied by immunoregulatory events. Since high-yielding milk cows show up with a in general reduced fertility it seems worth to dissect the various aspects of reproduction immunology to identify possible future areas of intensified research. Before insemination takes place, a competent oocyte must mature within the follicle. Based on recent research, the maturation of a follicle includes the coordinated immigration of various immune cells in a tightly regulated sequence into the theca and the granulosa-cell layer. The interplay between hormones, cellular activity, and mediator release ensures that both a competent oocyte and a functional corpus luteum can develop. Insemination-induced immunomodulation, in contrast to other species, seem to play no significant role in the cow. Central for the successful early and late pregnancy seems to be the progesterone-induced polarization of immune response types. Although the TH1/Th2 concept of immune polarization may be an oversimplification, there is high evidence that progesterone favors a local endometrial Th2 environment in anticipation of pregnancy and that a Th2-dominated immunity is characteristic for the pregnant cow. The local immune priming seems to be relevant for the recognition of the embryonal signaling via prostaglandins and Interferon-tau. The high percentage of reported early pregnancy failures may be due to a dysregulated embryo-maternal communication. Studies analyzing the expression of INF-tau receptors and the role of enhanced systemic expression of INF-tau-stimulated genes are still lacking in the cow. The major causes for such putative dysregulations seem to arise from peripartal infectious or inflammatory diseases, no matter where they arise (udder, endometrium, elsewhere). Thus, major efforts to enhance fertility in cows center now on the question how to prevent and/or to modulate the course of diseases like mastitis, metritis, and endometritis in the postpartum.

Session I: Immune-endocrine function of uterus

Local and peripheral preimplantation pregnancy recognition-insight from mouse model

A. Chełmońska-Soyta, D. Lorek, A. Kędzierska, K. Buska, B. Szponar

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The maternal recognition of an embryo around an implantation is decisive for establishment and maintenance of pregnancy. During this period, critical decisions are being made by a pregnant female: a continuation of a gestation or its rejection to avoid unwanted costs of energy loss due to impaired pregnancy. A high rate loss of embryos before implantation suggests that embryo selection process takes part mostly in preimplantation period of pregnancy. Under in vitro conditions embryos are able to develop till blastocyst stage outside maternal cavity and their transplantation into receptive uterus allows proper implantation and a fetus development. It indicates that embryo-maternal interaction shortly before implantation is not necessary for pregnancy maintenance. However, in normal pregnancy the dialogue between mother and embryo is established very early after conception. Embryos signalize their presence locally in the uterus by changing gene expression and protein secretion, which in turn not only support the preparation of the uterus for implantation but also initiate a selection of developmental impaired embryos. On the other hand, the presence of an embryo during the preimplantation period also modulates the phenotype of immune cells at the periphery.

In our previous study we have shown that antigen presenting cells (APC) in a preimplantation stage of pregnancy upregulated expression of costimulatory molecules (CD80, CD86, CD40) and MHC class II molecule (Slawek et al., 2013). These observations indicated that APC's costimulatory molecules are aware of the presence of paternal antigens even before implantation. However, the mechanism of paternal antigen recognition at this period of pregnancy is still unknown. On the other hand, an increased expression of costimulatory molecules on splenic APCs and transportation of semen antigens to the spleen (Zenclussen et al., 2010) may suggest participation of unspecific receptors in a process of paternal antigens recognition. Our latest experiments indicate that in abortion prone matings (♀ CBAxDBA/2J♂) female's splenic B cells (CD19+) significantly upregulated expression of TLR9 gene compared to normal pregnancy (♀ CBAxBALB/c♂) ones what is accompanied by elevated level of MHC class II gene expression. Neither TLR4 gene nor TLR2 gene significantly changed their expression between the aforementioned groups of mice. Moreover, we did not observe significant changes in an intestinal microbial metabolic profile (short-chain fatty acids and 3-OH fatty acids analysis) between examined groups what may influence TLR expression. This results suggest that immunophenotype of APC cells may serve as a sensor of pregnancy maintenance. On the other hand, mice lymphocytes may be a sensors of embryo quality. Our other studies have shown that the proteome of splenic T CD4+ lymphocytes is different in female recipients of normal embryos in

comparison to recipients of TNFα-treated embryos. Among proteins with altered expression induced by TNFα-treated embryos (TNFαE) were proteins involved in regulation of cytoskeleton stabilization and lymphocyte motility, proteins with immunomodulatory function and proteins influenced by cell stress. In the latest group, increased expression of Heat shock cognate 71kDa and Peroxiredoxin – 2 proteins in T lymphocytes of female mice from TNFαE group may indicate that cells beyond of reproductive tract received stress-inducing signaling from impaired embryos at preimplantation stage of pregnancy. It may suggest that signals derived from biologically impaired embryos are recognized not only locally, but also at periphery, what may further implicate existence very early danger signals of impaired pregnancy at systemic level.

The cytokine MIF in physiology and pathology of human reproduction

F. Ietta, R. Romagnoli, C. Mannell., L. Paulesu

Department of Life Sciences, University of Siena (Italy)

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine promoting cell growth and proliferation, angiogenesis and apoptosis inhibition. Consistent with its role in inflammation, MIF is involved in the pathogenesis of infective, inflammatory, autoimmune and neoplastic diseases. Higher levels of MIF in serum, peritoneal fluid and ectopic endometrial cells, characterize endometriosis. We demonstrated that treatment of endometrial cells with Bisphenol A (BPA) an estrogen-like chemical, induced MIF, suggesting the potential association between BPA exposure and endometriosis.

However, MIF is expressed in many organs and tissues and plays fundamental roles in physiological processes including those that occur in human pregnancy. Evidence showed that MIF is a key molecule in the earlier and the later phases of pregnancy when pro-inflammatory-like events occur. Specifically, blastocyst implantation and placental development occur in the first weeks of gestation and recruitment of immune cells that infiltrate the myometrium, placenta and fetal membranes occur near the term of pregnancy in preparation of labor. Based on our's and literature studies we will show how altered levels of MIF can result in pathological events: early miscarriage, recurrent abortion, pre-term labor and pre-eclampsia.

Detection of Lactobacillales spp. in the bovine seminal plasma and their influence on endometrial epithelial cells *in vitro*

F. Westerkamp[1], M. Ibrahim[1], D. Scheibner[1], J.-H. Osmers[2], C. Gabler[1]

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2RBB Rinderproduktion Berlin-Brandenburg, Groß Kreutz, Germany

Bacterial contamination of bovine seminal plasma is a known problem in reproduction of dairy cattle. Pathogenic bacterial strains in seminal plasma from bulls have been investigated intensively, especially the influence on uterine diseases. However, knowledge about commensal bacteria in seminal plasma is rare. Therefore, the aim of the present study was to evaluate the presence of commensal Lactobacillales species in bovine seminal plasma and to investigate their effect on endometrial epithelial cells in vitro. 57 seminal plasma samples obtained from 40 healthy Holstein-Frisian bulls were incubated on selective agar plates to reveal the presence of Lactobacillales spp. Obtained colonies were enriched and further characterized by sequencing of a part of the 16S rRNA. Endometrial epithelial cells were obtained from healthy cows (n=3) and were incubated in passage 2 in vitro with bacteria at different multiplicities of infection (MOI 1, 5, and 10, respectively). Non-treated cells served as control groups. Cell viability staining with trypan blue was performed every 24 hours for 3 days. In addition, endometrial epithelial cells were incubated with selected bacterial strains at a MOI 1, 5 and 10 for a short time (2, 4, and 6 hours), respectively. Total RNA was extracted and used for reverse-transcription-real-time PCR to analyze the mRNA expression of selected pro-inflammatory factors. 29 seminal plasma samples did not contain any cultivable bacteria. 25 bacterial colonies could be characterized, such as Staphylococcus spp., Enterococcus spp., Plasmodium sp. and Bacillus sp., which can cause endometrial lesions. Five Lactobacillales strains, which were obtained from three different bulls, were identified as L. mucosae (LM1-4) and Leuconostoc mesenteroides (LeucM). Trypan blue exclusion staining showed that LM1 caused death of all cells after 24 hours. LM2 caused death in approximately 30% of the cells starting after 24 hours up to 72 hours at a MOI 10. Viability of the co-cultured epithelial cells was not affected up to 72 hours in presence of LM3, LM4 and LeucM compared with the control group. Real-time PCR revealed a significant increase of the mRNA expression of interleukin 1A (IL1A), IL1B, IL8 and chemokine-ligand-1/2 (CXCL-1/2) in presence of LM2. LM3 caused a slight increase of pro-inflammatory mRNA expression compared with controls. In contrast, LM 4 did not obviously influence the mRNA expression of the selected genes. In conclusion, few Lactobacillales strains could be identified in bovine seminal plasma. The immunological response of uterine epithelial cells to these Lactobacillales isolates varies in a strain specific manner. This study was supported by the Förderverein Bioökonomieforschung e.V. (FBF).

Defining the inflammatory gene signature that precedes the development of uterine disease in postpartum cattle

A. Brewer[1,3], P. Cormican[1], J. J. Lim[2], A. Chapwanya[2], C. O'Farrelly[3] and K. G. Meade[1]

1 Animal & Bioscience Research Department, Teagasc, Grange, Co. Meath, Ireland. 2 School of Veterinary Medicine, Department of Clinical Sciences, Ross University, Basseterre, St Kitts and Nevis. 3 Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College Dublin, Dublin , Ireland.

Following calving, inflammation of the uterus is common during the first week postpartum. However, whilst most cows resolve this inflammation a significant proportion fail to do so. Our previous work identified transcriptomic differences between cattle that resolve and those that develop uterine disease as early as 7 days postpartum (DPP). Here, we hypothesise that over-production of endometrial inflammatory molecules in some animals contributes to development of disease. Endometrial epithelial cells were collected using cytobrushes from cattle classified as healthy or with uterine disease (cytological endometritis; CE and purulent vaginal discharge; PVD) on the basis of vaginal mucus score and >18% polymorphonuclear (PMN) cell infiltrate into the endometrium at 21 DPP. RNA-seq analysis of 30 samples was performed using EdgeR to identify differentially expressed genes. P values were adjusted for multiple testing using the Benjamin-Hochberg test. Clear differences in transcriptomic profile at 7 DPP were observed between cows that subsequently resolved inflammation and those that developed disease. Differential expression of 376 genes was observed between healthy and CE cows, and 1654 between healthy and CE+PVD cows ($P<0.05$). Pathway over-representation analysis identified significant changes in immune-related pathways. The cytokine-cytokine receptor interaction pathway, NOD-like receptor signalling pathway and Toll-like receptor signalling pathway were up-regulated in cattle with both CE and CE+PVD, suggesting a core inflammatory signature exists early postpartum associated with the onset of uterine disease, including up-regulation of IL1A, IL1B, NLRP3, IL8, TNF, IL17, TLR2 and TLR4. This study indicates that by Day 7 postpartum differential inflammatory pathway activation predicts the development of pathology.

Effect of LPS from Escherichia coli on PPAR expression profile during experimentally induced endometritis in the bovine endometrium explants

B.M. Socha, A.J. Korzekwa

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Olsztyn

The main infectious agent in bovine endometritis is Escherichia coli. Lipopolysaccharide (LPS) is characteristic component of the cell wall of Gram-negative bacteria. LPS and its lipid A moiety stimulate cells of the innate immune system by the Toll-like receptor protein family. Peroxisome proliferator-activated receptors (PPAR) are a family of nuclear receptors composing three isoforms: PPARα, PPARβ/δ and PPARγ. They are involved e.g. in the regulation of reproductive and inflammatory processes. The activity of PPARs can be modified by a number of endogenous compounds, including arachidonic acid (AA), its eicosanoid metabolites and synthetic ligands. Therefore, we hypothesized that PPAR mRNA expression profile may change in the bovine endometrium under the influence of LPS from E. coli.

The aim of the study was to determine the mRNA expression of PPARα, PPARβ/δ and PPARγ in the bovine endometrium explants during experimentally induced endometritis. Endometrial explants were obtained post mortem from heifers (n=8) and incubated without (control group) or with LPS (1 μg/mL) for 12, 24, 48, 72 and 96 h. The mRNA expression was evaluated by real-time PCR. Concentration of AA metabolites in medium was determined using ELISA tests. Inflammation efficacy was confirmed by mRNA expression in explants for TLR4-MD3-CD14 and IL1β, IL6 and TNFα. Differences between groups were tested with the Student´s t test.

Increase of mRNA expression for PPARα after LPS treatment compared to the control group was observed from 48 to 96 h, for PPARβ/δ after 24 h and from 48 to 96 h, whereas for PPARγ from 12 to 96 h ($P<0.05$). Concentration of PGF2α was the highest at 72 and 96 h ($P<0.001$), whereas LTC4 was elevated at 24, 72 and 96 h ($P<0.05$).

The overall results indicate changes in the mRNA PPARs expression profile in the bovine endometrium under the influence of LPS from E. coli. Received in vitro results concerning PPAR expression during endometritis should be continued by in vivo studies.

This research was supported by the National Science Centre, grant OPUS 6 (2013/11/B/NZ4/04516) and is a part of a PhD thesis conducted by B.M. Socha.

Escherichia coli affects PPAR expression profile in the bovine endometrium during experimentally induced endometritis - in vivo studies.

B.M. Socha, A.J. Korzekwa

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Olsztyn

Endometritis is a common problem in dairy cows. The main infectious agent is Escherichia coli. Peroxisome proliferator-activated receptors (PPAR) are transcription factors composing three subtypes: α, β/δ and γ. They are involved e.g. in the regulation of reproductive and inflammatory processes. The activity of PPARs can be modified by endogenous compounds, including arachidonic acid (AA) and its metabolites. Therefore, we hypothesized that PPAR mRNA expression profile may change in the bovine endometrium under the influence of E. coli.

The aim of the study was to determine the mRNA expression of PPARα, PPARβ/δ and PPARγ in the bovine endometrium during experimentally induced endometritis – in vivo. Heifers were intrauterine infused with the E. coli suspension (n=8) or NaCl 0.9 % (control group, n=6). Endometrial biopsies were performed before (0 h) and 12, 24, 48, 72, 96 h after the infusion. Blood was collected from the tail vein. The mRNA expression was evaluated by real-time PCR. Concentration of AA metabolites in plasma was determined using ELISA. The efficacy of induced endometritis was confirmed in biopsies by mRNA expression for TLR4-MD3-CD14 and IL1β, IL6 and TNFα. The results were statistically analysed by two-way ANOVA followed by a Bonferroni test.

Increase of mRNA expression for PPARα after E.coli infusion compared to the control group was observed at 48 and 72 h, for PPARβ/δ from 48 to 96 h, whereas for PPARγ at 48 and 96 h ($P<0.05$). PGFM concentration was the highest at 72 and 96 h ($P<0.001$), whereas LTC4 was elevated at 24, 72 and 96 h ($P<0.01$).

The overall results indicate that E. coli has effects on the PPARs mRNA expression profile in the bovine endometrium and on plasma concentration of AA metabolites, depending on the exposure time in vivo.

This research was supported by the National Science Centre, grant OPUS 6 (2013/11/B/NZ4/04516) and is a part of a PhD thesis conducted by B.M. Socha.

Session II: Endometritis in mares

Equine Endometritis

Mats H.T. Troedsson[1,2]

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2Equine Veterinary Medical Center, Al Shaqab/Qatar Foundation, Doha, Qatar

Equine endometritis is a major cause of infertility associated with major economic losses to the equine industry. Decades of research has highlighted multiple levels of failure of the uterine defense mechanisms in horses classified as susceptible to persistent endometritis. We also know that endometritis can be caused by either an infection or breeding. The two causative forms of endometritis require different treatment approaches. While local and/or systemic antibiotics based on microbial sensitivity may be the most effective treatment for infectious endometritis, this therapeutic approach is less likely to resolve an inflammation in mares with persistent breeding-induced endometritis. The increased understanding of the pathophysiology of equine endometritis has resulted in new therapeutic approaches to the problem. While many treatment regimens have been, and are currently used in veterinary practice, there is limited data to support efficacy as well as safety of these treatment options. The objective of this presentation is to review aspect of new diagnostic approaches and commonly used options for equine endometritis. Although a variety of treatment options are available to the clinician, only a few have been tested under controlled conditions and proven beneficial. The clinician should at a minimum have sufficient information on safety of the treatment and its effect on the endometrium before implementing a treatment regimen. We should also keep in mind that semen is the best treatment of infertility, and only 10-15% of brood mares need help to assist the uterus to provide a healthy environment after breeding.

The intricacies of mare endometrosis - how far have we gone?

G Ferreira-Dias [1]., M. R. Rebordão[1,2], A.Z. Szóstek - Mioduchowska [3], A. Amaral[1], S. Morazzo[1], P. Pinto-Bravo[1,2],C. Fernandes[1], A. Galvão[3], D. J. Skarzynski[3]

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Paramount fibrosis (collagen) development in mare endometrium characterizes endometrosis. At mating or in the presence of pathogens, neutrophils arrive at mare uterus. There, in response to bacteria they release neutrophil extracellular traps (NETs). While NETs engulf bacteria to fight endometritis, they may also provoke endometrial fibrosis. Mare endometria challenged in vitro with NETs constituents (mainly elastase) increased collagen type I (COL1) depending on estrous cycle and/or endometrial category. Thus, in mares with chronic endometritis NETs may mediate endometrial fibrogenesis. While COL3 predominates in active endometrosis, it appears to be replaced by COL1 in inactive endometrosis. Besides NETs, fibrogenic cytokines, Transforming Growth Factor-Beta (TGF-β) superfamily members, such as Nodal and TGFβ-1, are also involved in mare endometrosis. Higher gene and protein expression of TGF-β1, TGFRI and TGFRII was found in endometrosis. Nodal and its receptors ALK-4 and ALK-7 were shown in superficial and glandular epithelium, with lower staining of ALK-7, regardless of estrous phase and endometrium category. Also, Nodal in culture enhanced TGFRI and TGFRII mRNA endometrium expression, and altered prostaglandins pathways. It inhibited PGE2 production in follicular phase and gene transcription of its EP2 and EP4 receptors in category I/IIA endometria, both conditions associated with impaired anti-fibrotic PGE2 action in other tissues. Besides, Nodal stimulated PGF2α production in mid-luteal phase endometrium, which is related to a pro-fibrotic effect. In conclusion, NETs and pro-fibrotic cytokines contribute for mare endometrosis progression, which might be mediated by prostaglandins.

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Infectious endometritis is associated with endometrial expression of pro-fibrotic markers and integrin ITGAV

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Susceptibility to persistent endometritis is associated with poor endometrial quality (Woodward, et al., 2012) and studies have demonstrated that periglandular fibrosis is correlated to fibronectin (eqFN) deposition (Walter, et al., 2001) and increased concentrations of transforming growth factor-β1 (TGF-β1) (Ganjam and Evans, 2006). Bacterial interaction with eukaryotic host cells involves engagement of integrin receptors on cell surfaces, and bacterial invasion of tonsillary epithelium has been shown to critically depend on TGF-β promoted the presence of alpha-5 beta-1 integrin (ITGAV) (Nitsche-Schmidtz and Rohde, 2007). In the present study, it was hypothesized that endometrial gene expression of pro-fibrotic markers (TGF-β1, connective tissue growth factor (CTGF), eqFN1) and ITGAV was correlated to infectious endometritis and the presence of endometrial fibrosis in mares.
Endometrial biopsies were obtained from brood mares at a Danish AI-center during the 2014 breeding season by the use of a guarded approach as described by Nielsen (Nielsen, 2005). Mares with clinical signs of endometritis and/or a history of previous un-successful breeding were selected for the study. Two biopsies were obtained from each mare. One biopsy was used for bacterial culture, cytology and histology (H&E) and one biopsy was used for RNA extraction. Relative gene-expression analyses were performed by quantitative reverse transcriptase PCR (qRT-PCR) using validated primers and SYBR green detection. Infectious endometritis was diagnosed in 49% of the mares (29/59) with S. zooepidemicus isolated most frequently (45%). Expression of TGF-β1, ITGAV and CTGF was significantly increased in mares with infectious endometritis. The degree of endometrial fibrosis was not associated with increased expression of selected genes of interest.
The results indicate that TGF-β1, CTGF and ITGAV play a role in the pathogenesis of persistent infectious endometrits. Further studies are however needed to investigate this hypothesis in relation to persistent infectious endometritis and the presence of endometrial fibrosis in the mare.

Characterisation of transcriptome profiles of fresh or cultured ex vivo equine endometrial explants at different time points

M. Monteiro de Barros[1], *M. Davies-Morel*[1], *C. Creevey*[2], *V. Lenis*[2], *D. Nash*[1]

1 Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales
2 Department of Computer Science, Aberystwyth University, Wales

An *ex vivo* equine endometrial explant system measured uterine inflammation using biomarkers of secretion such as Prostaglandin F2α (Nash *et al*, 2008). However, it has not been determined if the transcriptome from explants remain stable in culture. This study aims to determine whether the transcriptomes of *ex vivo* cultured endometrial explants collected from native pony mares are representative for the transcriptome of a live mare (*ex vivo* uncultured 0h endometrial tissue) in the pre-breeding, non-inflammatory state.

Endometrium from eight native pony mares at the follicular stage of the oestrous cycle were sampled at 0h (representing the live mare) and tissue explants were cultured for 24h, 48h and 72h. Tissues were stored in RNA-Later, total RNA was extracted, quality assessed by agarose gel electrophoresis and Qubit quantification. RNA sequencing was performed on the Illumina HiSeq 2500 platform. A previously described data analysis workflow utilising Cuffdiff (Trapnell *et al*, 2012) was used to determine differentially expressed genes, statistical significance was set at $P<0.05$.

From a total of 13,212 genes, none was differentially expressed ($P<0.05$) at the four different time points (0h, 24h, 48h and 72h).

This study showed that no significant transcriptomic changes occurred when comparing the endometrial transcriptome representing the live mare (*ex* vivo 0h) with the transcriptome of autologous tissues cultured for up to 72h. In conclusion, this tissue culture model has potential application for studying the mechanisms underlying uterine inflammation.

References

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Trapnell, C., Roberts, A., Goff, L., Pertea, G., et al. 2012. Nature Protocols 7(3),562-578.

Cathepsin inhibition affects mare endometrium prostaglandin secretion during the estrous cycle

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Endometriosis is a major cause of subfertility/infertility in mares. Bacteria induce neutrophil extracellular traps (NETs) release, which besides killing pathogens in the uterus, may also contribute to endometrial fibrosis. Since cathepsin G (CAT) is a NETs component that stimulates mare endometrial fibrosis, mediated by prostaglandins (PG), the aim of this study was to evaluate if by inhibiting CAT, PG secretion would change, depending on estrous cycle phase. Equine endometrium explants from mid luteal – LP (n=3) and follicular - FP (n=6) phases were cultured, for 24 and 48h, with medium alone (Control), TNFα (10ng/mL; positive Control), CAT (0.1µg/mL, 1µg/mL), cathepsin G Inhibitor I (IN, 1µg/mL), or CAT (0.1µg/mL, 1µg/mL)+IN (1µg/mL) added. Endometrium PGE2 and PGF2α were assessed by EIA. In FP, TNFα increased PGE2 comparing to control (24h; $p<0.05$; 48h, $p<0.01$). In LP, at 24h, CAT (1 µg/mL)+IN increased PGE2 versus control, CAT (1 µg/mL) or IN ($p<0.001$). In FP, at 24h, PGF2α was augmented by CAT (1 µg/mL) with respect to control ($p<0.01$), and CAT (1 µg/mL)+IN ($p<0.05$). In FP (48h), CAT (0.1 µg/mL; $p<0.05$; 1 µg/mL; $p<0.01$) and TNF ($p<0.05$) increased PGF2α relative to control. At 48h, CAT (0.1 µg/mL) +IN increased PGE2 comparing to IN alone ($p<0.05$). In LP, at 48h, PGF2α lowered with CAT (0.1 µg/mL)+IN, regarding control ($p<0.05$). The data were analyzed by one-way ANOVA. Inhibition of CAT during the estrous cycle might reduce the establishment of mare endometrial fibrosis by stimulating the production of anti-fibrotic PGE2, and inhibiting pro-fibrotic PGF2α.

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Are mares physiologically protected against endometrial fibrosis induced by NETs proteases?

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Multiple mechanisms are involved in the complex pathogenesis of mare endometrial fibrosis. In follicular phase (FLP), bacteria or semen within the uterus recruit neutrophils that induce PGF2α release and uterine contractions. Although this is crucial for uterine physiological clearance, increased PGF2α may induce fibrogenesis. In mid-luteal phase (MLP), increased PGE2 receptor 2 (EP2) transcripts in mare endometrium, may be a physiological mechanism against fibrosis, as increased EP2 or PGE2 have anti-fibrotic actions. Mares´ endometrium with persistent endometritis has increased neutrophils that can release neutrophil extracellular traps (NETs). NETs proteases (elastase) may modify uterine environment and predispose to fibrogenesis. The aim was to evaluate PGF2α or PGE2 pathways in in vitro collagen type I (COL1) deposition by mare endometrial explants (type I/IIA or type IIB/III) challenged with elastase. PGE2 and PGF2α were assessed by EIA, EP2 by qPCR and COL1 by immunoblotting. Increased PGF2α and COL1 were only detected in FLP type I/IIA endometria ($P < 0.05$). Impaired PGE2 occurred in FLP and MLP type IIB/III endometrium and MLP type I/IIA ($P < 0.05$). Increased COL1 and low EP2 were only seen in FLP endometria ($P < 0.05$). FLP endometria may be more prone to fibrosis when triggered by pro-fibrotic stimuli, since it was when a putative pro-fibrotic association of high PGF2 and low EP2 under NETs proteases action was noted. NETs components induced changes on prostaglandins mediators may instigate PGF2α or PGE2 vias to become additional pathways in mare endometrial fibrogenesis.

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Endometritis Coexisting with Carcinoma Metastases to the Uterus in Mare.

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A mammary gland squamous-cell carcinoma (SCC) with metastases to uterus was found in an 18-year-old thoroughbred mare. Clinical examination demonstrated depression, anorexia, ventral edema and abdominal distension. Enlargement and purulent discharge from udder were observed. The diagnosis of the mammary gland SCC was based on a histological examination and presence of similar tissue in lymph nodes, vulva, vagina and uterus. After euthanasia, tissue samples were collected from SCC primary tumor and metastases, fixed for immunohistochemistry, cut into slices and labeled with primary, secondary antibodies and NuclearGreen or Hoechst. Slides were examined under confocal microscope LeicaTCSSP8 (qualitative) and scanning cytometer TissueFaxsPLUS (quantitative). Immune cells differentiating was based on expression of CD45/CD66 (granulocytes), CD45/CD14 (macrophages), CD45/CD3/CD4 (lymphocytes Th), CD45/CD3/CD8 (lymphocytes Tc). Both in the mammary gland (MG) and uterus (U), the tissue consisted of areas of normal glandular tissue and neoplastic glandular structures with irregularly shaped acini and tubules. Some cells were pyknotic and karyolytic, others have shown polymorphism, anisokariosis and atypia. Endometrial adenocarcinoma diagnosis was based on well circumscribed, non-encapsulated nodular tissue in uterine mucosa which infiltrated the myometrium. Massive inflammatory cells infiltration, haemorrhage and necrosis were present in MG and U where CD45 positive cells accounted 16.2% and 14.9% of all cells, respectively. Similar immune cells infiltration (MG:U) was observed: 62.2%:58.9% granulocytes, 20.1%:24.4% macrophages and 17.7%:16.7% lymphocytes (10.1%:8.9% Th and 7.6%:7.8% Tc). Mares with endometrial adenocarcinoma have been previously reported, but inflammatory infiltration was not described yet. Our findings confirm that immune cells reaction is similar in primary and metastatic tumors and contains a lot of non-activate immune cells such as tumor associated macrophages.

Plenary lecture II

Mechanisms linking metabolism with endometritis

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Robust endometrial defences are required to control bacteria in the uterus of cattle after parturition. The metabolic stress of lactation in dairy cattle increases the risk of postpartum uterine disease, including metritis and endometritis. The mechanistic links between metabolism and uterine disease are unclear, but metabolic stress may impair host tissue defences. Innate immunity is a key component of endometrial defence against bacteria. Cellular receptors recognize pathogen-associated molecular patterns, and activated cells release cytokine and chemokine inflammatory mediators. Innate immunity and cellular metabolism are highly integrated, and stressing one system might affect the other. Innate immune responses to the pathogen-associated molecular pattern, lipopolysaccharide, increase endometrial glucose consumption and induce the Warburg effect. Furthermore, limiting the supply of glucose or glutamine impairs cytokine and chemokine responses to pathogen-associated molecular patterns in the endometrium. Similarly, modulating AMP-activated protein kinase, which is the cellular energy sensor, blunts endometrial inflammatory responses to lipopolysaccharide. Metabolic stress also affects lipid metabolism, and manipulating the mevalonate pathway that leads to cholesterol synthesis, modulates inflammatory responses to pathogen-associated molecular patterns. However, other regulators of metabolism, including mammalian target of rapamycin, insulin-like growth factor-1, and ovarian steroid hormones have limited impact on endometrial inflammatory responses. In conclusion, glucose and glutamine fuel innate immunity in the endometrium, and metabolic stress perturbs the inflammatory responses to pathogen-associated molecular patterns.

Session III: Endometritis in cows

(Endo)metritis in modern dairy cows: a critical and innovative view

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Cow reproduction being the key driver in modern dairy industry, is currently under severe stress. Uterine health and receptivity are, among others, main determinants for the reproductive success of modern dairy cattle.

Multiple papers have been published to define uterine diseases like metritis and endometritis, but still there is a lot of controversy. Main challenge herein is to find an agreement between a scientifically sound definition and what practitioners encounter in the field. Furthermore, the old believe that chronic uterine disease should be seen as a problem of non-healing of clinical metritis, comes more and more under pressure. In the same context, some cows have been shown to suffer from both of these diseases, while others seemed to suffer from only one, which was confirmed by the fact that risk factors were slightly different. In this discussion comes also the discussion whether cervicitis should be seen as a distinct disease or rather is part of the larger metritis disease complex.

Relatively new in this context, is the occurrence of subclincal endometritis. Diagnosis of this disease is based on intra-uterine sampling and assessing of the relative amount of neutrophils in the swab by cytology. The disease is therefore sometimes referred to as cytological endometritis. Cows suffering from this disease experience difficulties to get in calf and need significantly more inseminations to become pregnant. Due to the fact that different authors sample cows at different time points after calving, there is a lot of controversy concerning both the threshold number of neutrophils to diagnose cows as diseased, as well as concerning the prevalence of the disease.

Recently, we did a large field study including >1.600 uterine swabs harvested by cytotape from 873 multiparous dairy cows at the moment of artificial insemination (AI). Using a ROC-curve, we were able to demonstrate that using this innovative but simple technique the threshold level was only 1%. Prevalence of cytologic endometritis (CYTO) at AI was 27,8%. Conception rate of the CYTO+ samples was 32,7% while it was 47% for CYTO– ones. A CYTO– sample had 1,8 more chances to become pregnant than a CYTO+ one. We also sampled 496 virgin heifers by cytotape at the moment of AI. Prevalence of cytologic endometritis in these animals was 7,86% with a difference in pregnancy rate of 24% (62 versus 38%). In virgin heifers a major risk factors to suffer from this disease, was the experience of a prior unsuccessful insemination.

The discussion that remains about the definition of uterine diseases is accompanied with a discussion about the treatment. Both for clinical, subclinical as well as cytologic endometritis there are multiple therapies applied in practice varying from purely expectative to an intra-uterine application of antibiotics.

A new discussion recently emerged about the role of bacteria in the uterine disease complex. While there is a general agreement about the role of pathogenic bacteria in clinical metritis, a lot of controversy remains about their role in for example cytologic endometritis. Multiple authors were unable to isolate the common and well known pathogenic bacteria in cows suffering from cytologic endometritis. The latter raised the question whether the diagnosed inflammation can be related to the metabolic stress the cows experience during the early postpartum period (metinflammation) and stimulated at least some scientists to test treatments only based on parenteral application of NSAIDs. Just as is known for coli mastitis, also for the metritis disease complex there seems to be an important role played by the inflammatory reaction of the host. Also the role of viruses like for example the BoHV, within the uterine disease complex is all too often neglected and needs further studies.

New in this whole discussion is also the potential presence of a specific microbiome in the uterus. While the uterus has so far been stated to be sterile, there are more and more studies using culture free identification techniques appearing to question this dogma. Whether the prevailing microbiome might be associated with the pregnancy success of the cow and whether disturbances in the prevailing microbiome may give rise to an inflammatory reaction of the endometrium warrants further research. Results may furthermore open perspectives for the use of innovative treatments like the intra-uterine application of probiotics.

Response of bovine endometrial epithelial cells depends on pathogenicity of bacterial strains

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Postpartum uterine bacterial infections are common in dairy cows leading to inflammatory processes such as clinical endometritis (CE) with a high prevalence. The endometrial epithelium plays a crucial role in the first immune response to invading bacteria by recognizing them and producing cytokines. Therefore, the objective of the present study was to evaluate the influence of distinct bacterial strains with different pathogenicity on the synthesis of pro-inflammatory factors in bovine endometrial epithelial cells in vitro. Various bacterial strains were isolated from healthy and inflamed uteri. Two strains of Trueperella pyogenes were included in this study: one strain (TP2) was isolated from the uterus of a postpartum dairy cow developing CE; and a second strain (TP5) was isolated from a uterus of a healthy cow. The two strains were compared in terms of their metabolic fingerprints, growth rate, and virulence gene transcription. Furthermore, Bacillus pumilus as a potentially pathogenic strain and several Lactobacillus species as commensal strains were isolated and characterized. Bovine endometrial epithelial cells were co-cultured with these bacteria in different multiplicities of infection (MOI = 1, 5 and 10). Total RNA was extracted from epithelial cells harvested at distinct time points (2, 4 and 6 or 8h) and subjected to RT-qPCR to analyze mRNA expression of selected pro-inflammatory factors. TP2 showed a higher growth rate, expressed more virulence factors (cbpA, nanH, fimE, and fimG), and elicited a higher mRNA expression of pro-inflammatory factors prostaglandin-endoperoxide synthase 2 (PTGS2), CXC ligand 3 (CXCL3), and interleukin 8 (IL8) in bovine endometrial epithelial cells compared with TP5. Presence of B. pumilus resulted in a significant higher mRNA expression of IL1A, IL6, IL8, CXCL1-3 and PTGS2 in co-cultured cells compared with untreated controls. Maximum increase was mainly detected after 2h. Presence of L. ruminis and L. amylovorus resulted in increased IL1A, IL6, IL8 and PTGS2 mRNA levels and the release of IL8 and prostaglandin F2α in endometrial epithelial cells compared with control cells. In contrast, L. buchneri did not significantly influence the expression and release of these factors. In conclusion, particular strain characteristics of T. pyogenes were found to be important for the development of CE. In addition, these results suggest that not only pathogens but also potential pathogens/opportunist contaminants may play a role in the inflammatory response of endometrial cells. Such approaches will help to better understand the interaction between epithelial cells and bacterial strains with different pathogenicity (pathogenic, potentially pathogenic, and commensal). This will enable to develop new strategies to treat such inflammatory diseases in the reproductive tract, e.g. endometritis. This study was supported by the DFG (GA 1077/5-1).

Genetically selected heifers differ in incidence of metritis

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Metritis in dairy cows has a large impact on economics, use of antibiotics and animal welfare. Genetic selection offers a promising tool for breeding cows with less susceptibility to illnesses. This study aimed at comparing periparturient performances of heifers selected via single nucleotide polymorphism typing for alternative parental chromosome 18 haplotypes associated with favourable (Q) or unfavourable (q) udder health. Holstein Friesian heifers (n=36, 18Q/18q) were supervised 21 days (d) antepartum, around calving until necropsy on d39 ± 4 postpartum. In case of disease, heifers were treated according to good veterinary practice. Groups were compared using Chi-squared test and unpaired t-test. No differences were detected concerning day of calving post insemination (Q: d278.6 ± 3.1 vs. q: d275.7 ± 7.8), day when macroscopic uterine involution was completed (Q: d19 ± 6.7 vs. q: d20 ± 3.8) or uterine weight at necropsy (Q: 0.669 ± 0.095kg vs. q: 0.622 ± 0.123 kg). Q heifers showed more functional corpora lutea during necropsy ($P<0.01$). The incidence of retained fetal membranes did not differ ($P>0.1$), but Q heifers showed less incidence of metritis and clinical mastitis compared to q-heifers ($P<0.01$). In conclusion, genetic selection should be considered for breeding cows with better periparturient clinical performance. The underlying mechanisms still need further investigation.

Reproductive performance, energy balance, ovarian function, and incidence of subclinical endometritis in cows diagnosed with metritis in early lactation

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Post partum uterine inflammatory diseases are prevalent in bovine dairy herds and responsible for major economic losses. In Israel, dairy cows are routinely examined by farm veterinarians at 5-12DIM (days-in-milk); metritis is diagnosed in case of fetid, watery to purulent vaginal discharge is present with an enlarged uterus. Our objective was to compare healthy cows to those suffering from metritis, in regards to energy balance, ovarian function, incidence of subclinical endometritis, and reproductive performance. Sixty-six Holstein-Friesian cows were enrolled in a case-control study (n=33 healthy; n=33 metritis). Clinical examinations (trans-rectal and vaginal examinations) and samplings (endometrial cytology & blood) were performed at 5-12, 30-37 and 60-70DIM. Ovarian function was assessed by neck tag activity records (SCR heat-detection), serum progesterone (pending) and anti-mullerian hormone (AMH). Body-Condition-Scores, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) were analyzed. Health and reproduction data were recorded. Statistical analysis was performed with Statistix-8 Software, paired t- test, Repeated Measure ANOVA, Kruskal Wallis-ANOVA, or Fisher's exact test, as applicable. As compared with healthy cows, cows in the metritic group tended to have higher incidence of subclinical endometritis at 60-70DIM (healthy:9% vs Metritic:27.3%, P=0.10); reduced reproductive performance (Pregnancy-rate at 150DIM: 60% vs. 33.3%; waste days: 47±9d vs. 64±10d; P<0.05); as well as lesser energy-compared milk yield by 90DIM (4062±134L vs. 3883±108L;P=0.08). Overall, serum NEFA, BHBA and AMH did not differ; however, AMH was significantly higher in multiparous cows as compared to primiparous cows (P<0.05). Among primiparous cows, the first detected estrus tended to be earlier in healthy as compared to metritic cows (45±6 vs. 73±12DIM), with more estruses detected by 70DIM (1.5±0.3 vs. 0.8±0.3). Cows that demonstrated estrus prior to 50DIM had better reproductive performance as compared to cows that demonstrated estrous later (Pregnancy-rate at 150DIM: 63.6% vs. 25%; P<0.05). In summary, early metritis (5-12DIM) may be associated with alteration of ovarian activity, higher incidence of subclinical endometritis and poorer reproductive performance later in lactation.

Inflammasome-dependent IL-1β production by bovine endometrial stromal cells and polarized epithelial cells.

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Following calving, all cows experience massive inflammatory activity in the uterus caused by the influx of bacteria and mechanisms of tissue repair. Major mediators of the inflammatory response are the endometrial epithelial and stromal cells which respond by releasing pro-inflammatory cytokines. This is a healthy inflammatory response allowing the uterus to return to a state of homeostasis. However, 30% of cows develop endometritis, pathological uterine inflammation that compromises fertility. Here we investigate roles for endometrial cells in determining the switch from healthy to pathological inflammation and hypothesize that regulation of inflammasome activity is key.

We have identified that stimulation of uterine epithelial and stromal cells with nigericin in combination with LPS resulted in IL-1β expression in a time-dependent manner, with protein levels peaking after 6 hours in stromal cells (>4500 pg/ml). IL-1β expression was higher in basolaterally stimulated polarized epithelial cells compared to apically stimulated cells (529 pg/ml vs 385 pg/ml). Bioinformatic and qPCR analysis identified that inflammasome components are conserved within the bovine genome and expressed within endometrial cells. Treatment of endometrial stromal cells with an inhibitor of the NLRP3 inflammasome (MCC950) and a pan-caspase inhibitor blocked IL-1β production by stromal cells, indicating that IL-1β production is inflammasome-dependent within these cells.

The data suggests a role for inflammasome activated IL-1β in endometrial cell mediated inflammation. Inhibition of IL-1β by the NLRP3 inhibitor MCC950 denotes a novel target for therapeutic intervention in the treatment of the pathological inflammation associated with postpartum endometritis and warrants further investigation.

Serum alpha 1-acid glycoprotein concentration on day 7 postpartum as a potential biomarker for the development of purulent vaginal discharge in dairy cattle.

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Early prognosis of uterine disease would facilitate earlier therapeutic intervention and improve the associated reproductive outcomes in dairy cows. Alpha-1-acid glycoprotein (AGP) has been proposed as an early biomarker for uterine disease. We hypothesise that high concentrations may identify cows at risk.

In Study 1, serum was obtained at 7 days post partum (DPP) from 60 Holstein-Friesian cows. Cows were diagnosed retrospectively on 21DPP based on vaginal mucus score (VMS) and polymorphonuclear cell (PMN) percentage as healthy (VMS 0, <18% PMN), purulent vaginal discharge (PVD: VMS ≥2 and <18% PMNs), clinical endometritis (CE: VMS 2 or 3, ≥ 50% PMNs) or cytological endometritis (CYE: VMS 0 or 1, ≥ 50% PMNs).

In Study 2, serum was collected from 84 Holstein-Friesian cows on 0DPP and 7DPP. Animals were diagnosed on the basis of VMS as healthy or PVD using the above criteria.

Using SAS 9.4, results were analysed using PROC MIXED with a Bonferroni adjustment. In study 1, similar AGP concentrations at 7DPP were detected in the healthy and CYE groups. However, cows subsequently diagnosed with PVD, CE or CYE had comparable AGP concentrations (P>0.05). In study 2, AGP concentrations did not differ between the groups on 0DPP (P>0.05). However, AGP concentrations were higher in PVD cows at 7DPP (1.35± 0.1mg/ml vs. 1.05± 0.1mg/ml, respectively; P = 0.03)

To conclude, 7DPP AGP concentrations may have prognostic value in identifying cows that subsequently develop early postpartum PVD.

Association between body condition score, uterine health status and ovarian cyclicity on early lactation dairy cows.

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It is well established that body condition score (BCS), uterine health status (UHS) and the postpartum anoestrous interval have important effects on cow fertility performance. The objective of this study was to evaluate the relationships between BCS, UHS and ovarian cyclicity (OC) during early lactation in seasonal-calving, pasture-based, dairy cows. A secondary objective was to compare the agreement between two techniques used to assess UHS. First and second lactation dairy cows (n=2858) from 35 dairy farms located in Munster, Ireland, were enrolled in the study. All cows were spring-calving (February to April) in either 2015 (n = 24 herds) or 2016 (n = 11 herds). All farms were visited every two weeks, and at each visit animals that were at week 3 (range 14 to 27 days in milk) and week 7 (range 42 to 55 days in milk) post-calving were examined. BCS was measured using a 1 to 5 scale in 0.25 increments. Transrectal ultrasound examinations (8.5 MHz transrectal transducer Ibex Pro, Ibex®, Colorado, USA) were conducted at each visit to determine OC by visualizing the presence of a corpus luteum on either ovary and to assess UHS. The UHS was assessed by both ultrasound exam (UHS_UE) and vaginal discharge score (UHS_VDS) (Metricheck® Simcro©, Auckland, New Zealand). The UHS_UE and UHS_VDS records were collapsed to give three UHS categories (score 1 = no infection; score 2 or 3 = mild infection; score >3 = severe infection). Fisher's Exact Test was used to test associations between these different categorical variables and was supplemented by logistic regression to calculate odds ratios and predicted probabilities. There were associations between BCS and OC and between BCS and UHS at week 3 (both P <0.01) and week 7 (both P <0.01). The likelihood of OC was less for cows that had BCS <2.75 (thin) and >3.25 (fat) compared with cows that had BCS between 2.75 and 3.25 at week 3 [41% (109/264), 36% (18/50), 46.0% (1171/2544)] and at week 7 [63.9% (268/419), 71.4% (20/28), 77.6% 1792/2309], respectively. The incidence of severe uterine infection at week 3 and the incidence of mild uterine infection at week 7 (both by UE) was greater for thin and fat cows compared with cows that had BCS between 2.75 and 3.25 [12.8% (34/264), 28.0% (14/50), 11.7% (298/2543)] and [76.7% (321/418), 71.4 (20/28), 70.9% (1638/2309)], respectively. The corresponding values for OC in cows having no infection, mild infection or severe uterine infection by UHS_UE were 80.0% (44/55), 48.3% (1187/2457) and 19.3% (67/346), respectively, for week 3 (P <0.0001) and 94.6% (710/750), 68.5% (1356/1979) and 53.8% (14/26), respectively, for week 7(P< 0.0001). The likelihood of OC was greater in cows having no uterine infection compared with cows that had mild and severe uterine infection (UHS_ VDS), at week 3 (P< 0.0001) and week 7 (P= 0.002) [55.5% (276/497), 48.7% (753/1544), 33.5% (216/644)] and [78.2% (848/1048), 74.0% (1120/1510), 64.9% (65/100)], respectively. The Kappa coefficient for

UHS was 0.21, indicating fair agreement between UE and VDS as diagnostic techniques for UHS. On average, low and high BCS caused poorer OC and UHS. Absence of uterine infection resulted in greater likelihood of OC. The results highlight the association between different phenotypes during the postpartum period.

The subsequent fertility performance for cows diagnosed with no infection, mild infection or severe infection is currently being investigated.

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Presence of bacteria in the endometrium and placentomes of pregnant cows

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Bacterial invasion of the bovine uterus during the postpartum period occurs in most cows, but the general consensus is that these bacteria are eliminated before the next pregnancy. The pregnant uterus has therefore hitherto been considered a sterile environment, but this assumption has now been challenged by recent studies in humans, which indicate that bacteria can be present in the placenta of term pregnancies without causing abortion. The aim of the present study was therefore to investigate whether bacteria are present in the uterus of pregnant cows. Specimens were taken from the inter-caruncular endometrium and from placentomes of slaughtered pregnant cows (n = 43; gestational age 28-263 days) and subjected to histology, fluorescence in situ hybridization and massive parallel sequencing. Bacteria were observed in the tissue from 90.7% (39/43) of the cows by fluorescence in situ hybridization. Fusobacterium necrophorum, Porphyromonas levii and Trueperella pyogenes were located within the endometrium, on the endometrial surface and in the caruncular stroma, but their presence was not associated with inflammation. Data from massive parallel sequencing of the 16S rRNA gene from a subset of 15 cows indicated that the most abundant bacteria were the families Porphyromonadaceae, followed by Ruminococcaceae and Lachnospiraceae. Our results indicate that the bovine uterus is not a sterile environment during pregnancy as previously assumed and that a cow can carry a pregnancy despite the presence of a few potentially pathogenic bacteria in the uterus.

Poster Presentations

Colonization of the bovine endometrium by Candida kefyr

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While fungal infection of the equine endometrium following antibiotic treatment has been reported, limited attention has been paid to such infections in non-pregnant cattle. Most studies on post partum uterine health in cattle have focused on bacteria, but the presence of fungi has been noticed in some studies. Here, we report the presence of Candida kefyr in the uterus of three Holstein cows from one herd. Uterine flushing samples and endometrial biopsies were taken at three time points after calving as part of a larger study. Microscopy of cytology slides from two of these cows and a third infertile cow in the herd revealed the presence of numerous yeast-like organisms and macrophages, which had phagocytized organisms. Histology showed fungi that colonized the endometrial surface, while tissue invasion was restricted to the most superficial part of the endometrium. Endometrial inflammation was unspecific. DNA was extracted from an endometrial biopsy and Sanger sequencing identified the organisms as C. kefyr. Probes targeting 18S rRNA of the K. marxianus group, including C. kefyr, were used for fluorescence in situ hybridization and revealed the presence of these fungi on the endometrial surface. Two cows were reexamined 21 days later, but fungi were no longer present. One of these cows became pregnant and delivered a normal calf at term, while the other was not bred. The infertile cow was examined at slaughter 201 days later but fungi were not recovered.

Optimization and characterization a novel model for culture of equine oviductal epithelial cells

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Establishment an optimal in vitro culture model for equine oviductal epithelial cells could be a step forward to gain a deep insight of oviductal function and the early embryonic development in equines. Therefore, we optimized a new isolation method of oviductal epithelial cells and cultured both (cells & explants) from follicular, early- and mid-luteal phases (5-6 independent replicates/group), using either fetal calf serum (10%FCS) or estrus mare serum (5%EMS). After culture, cells, explants and medium were collected at 0, 24, 48, 72 hrs, one week, and 10 days (cells), for total RNA isolation, and subsequent quantification of miRNAs & their target that are relevant to oviductal physiological function. Fresh collected tissue & cells served as control. Furthermore, oviductal explants were stained with haematoxylin-eosin (H&E) and measurement concentrations of prostaglandins (PGE2 & PGFα2) in medium, by ELISA. Data analysis was done using two-way ANOVA. We succeed to maintain epithelial morphology for 10 days, for both FCS & EMS, as shown by light and stereo microscope. Similarly, the presence of highly differentiated epithelium with basal nuclei and secretory granules were observed in H&E stained explants, until a week for both FCS & EMS groups. MiR-155, miR-223, miR-17, miR-24, miR-532-5p, miR-181b, miR-21, and let-7a as well as their targets; IGF1, OVGP1, PTGER2, CSF1, and VEGFA were differentially expressed according to the oestrous stage. Furthermore, the secreted PGE2 & PGFα2 revealed different dynamic patterns according to the oestrous stage. Our in vitro model maintained the morphological and oviductal physiological function. So, it could be used as an excellent model for multi-omics study and deciphering the first cross-talk between mother and embryo in mares.

Acetylcholine mediates contractility of inflamed porcine uterus through M2 and M3 receptors

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We studied the impact of inflammation on the acetylcholine (ACh)-induced muscarinic 2 and 3 receptors (M2R, M3R) conducted contractility of the porcine uterus. On day 3 of the estrous cycle, either 50 ml of saline or 50 ml of E.coli suspension (109 colony-forming units/ml) were injected into each gilt uterine horn. Eight days later, after euthanasia of gilts, uteri were collected. The Bonferroni test was used to compare mean statistical values of contractile parameters. Infected gilts developed severe form of acute endometritis. Compared to the period before treatment, ACh (10-5 M) increased contraction intensity in endometrium/myometrium ($P<0.001$) and myometrium ($P<0.05$) of saline-treated uteri, while decreased ($P<0.001$) in inflamed ones. After use of ACh (10-5 M) with M2R or M3R antagonists contraction intensity in both groups was reduced ($P<0.001$). This effect was stronger ($P<0.05$) in inflamed than saline-injected uteri. ACh (10-5 M) increased contraction frequency in endometrium/myometrium ($P<0.001$) and myometrium ($P<0.01$) from both groups, compared to the period before treatment, and it was reduced ($P<0.05$) in the presence of ACh (10-5 M) and M2R or M3R antagonists. Contraction frequency in endometrium/myometrium after E.coli injection was lower ($P<0.05$) in response to ACh (10-6 M) and M2R antagonist than in saline-treated uteri. In summary, in inflamed porcine uterus ACh mediates contraction intensity via M2R and M3R, while its contraction frequency is elevated mainly by M2R. Observed partly differential reaction of inflamed uterus to ACh may result from changes in expression or sensitivity of muscarinic receptors evoked by the local pathological state. Reduced contraction intensity of inflamed uterus in response to ACh can be essential for the course of inflammation of the uterus.

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The impact of inflammation on the protein expression of β-adrenoreceptors in pig uterus

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Uterine inflammation is a common reproductive disease occurring in domestic animals. This disorder may cause changes in density of noradrenergic nerve fibres in both endometrium and myometrium. The goal of this study was to reveal changes in the protein expression of β-adrenoreceptors in porcine inflamed uterus. On day 3 of the estrous cycle gilts were laparotomized and either 50 ml of E.coli (109 colony-forming units/ml; A group) or saline (B group) suspension were infused into uterine horns. In the pigs of the control group (C) only laparotomy was performed. The uterine samples (myometrium and endometrium), obtained 8 days later, were analyzed using Western Blot. The one-way of variance followed by the Bonferroni test was applied to compare the statistical values of the probes. Acute endometritis developed in all bacteria-inoculated gilts. In endometrium after E.coli injection, the protein expression of β2-adrenoreceptors decreased compared to the saline-treated ($P<0.001$) and control ($P<0.05$) tissues. There was an increase in the endometrial protein expression of β2-adrenoreceptors compared to β1 and β3-adrenoreceptors (group B; $P<0.001$), and of β2-adrenoreceptors in relation to β1-adrenoreceptors (group C; $P<0.01$). Moreover, in myometrium the protein expression of β1-adrenoreceptors was higher ($P<0.05$) after bacteria than saline intrauterine infusion. The protein expression of β2 and β3-adrenoreceptors also increased in myometrium in relation to saline-treated ($P<0.05$; $P<0,001$) and control ($P<0.001$) tissue. The myometrial protein expression of β2 and β3-andrenoreceptors after bacteria infusion and β2-adrenoreceptors after saline-treatment was higher ($P<0.001$) compared to β1-adrenoreceptors. This study showed that the inflammation changes the protein expression of β-adrenoreceptors in uterus, and suggests that the expression of these receptors is important for affecting of noradrenaline on inflamed uterus functions.

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Evaluation of usability of the Hemavet-Test as routine uterine cytology staining

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Staining and subsequent evaluation of cytological samples is a key element to achieve high sensitivity and specificity to diagnose endometritis in cow. Romanowsky type staining (oxidized methylene blue dyes and Eosin Y) is preferred for uterine cytology because it is a quick and easy method. Currently, different staining methods are available for cytology evaluation of uterine smears, one of them is for instance Diff-quick. Therefore, the usability of an another staining technic like the Hemavet-Test, which is commonly used in Poland for staining of blood smears, was evaluated as an uterine cytology staining after some modifications. Hemavet consists of 3 solutions: fixative, solution I and II and for endometrial cytology. Time of each washing in particular solutions was changed comparing to the manufacturer’s protocol and has been as followed: 10 second in fixative, 10 second in solution I, 5 seconds in solution II and rinsed 3 times in distilled water for 3 seconds each. Next excess of water was removed from a slide and let it to dry. Following, the slides have been dehumidified. The usability of the Hemavet-Test was evaluated with the help of 280 stained endometrial smears. In conclusion, the used staining method appears to be useful for histological evaluation, due to the fact, that only five slides could not be evaluated and further 12 ones had a moderate diagnostic value. An additional advantage is that Hemavet can be even used in samples containing a high number of red blood cells. Our results are promising to use this staining as a quick and cheap alternative for field veterinary practitioners.

The effect of transforming growth factor (TGF)-β1 on equine endometrial fibroblasts in vitro

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Endometrosis is a degenerative chronic process, characterized by fibrosis development in the endometrium in mare. This degenerative chronic condition is defined as an active or inactive fibrosis process that develops around the endometrial glands and/or in the stroma, often associated with pathological alterations in the endometrial glands within fibrotic foci. The concentration of endometrial TGFβ1 is known to be correlated to the severity of endometrosis. Fibrosis is characterized by the presence of fibroblasts, myofibroblasts, and deposition of extracellular matrix (ECM) components especially collagen type 1 (COLI), collagen type 3 (COLIII) and fibronectin (FN). The myofibroblasts are characterized by ability to excessive deposition of ECM, de novo α-smooth muscle actin (α-SMA) expression, contractility and resistant to apoptosis. The aim of the present study was to clarify the role of TGF-β1 in ECM component production and myofibroblast differentiation. The dose and time-dependent effects of TGF-β1 on α-SMA and ECM components expression in equine endometrial fibroblast were determined in vitro. Equine cultured fibroblasts (n=6) were stimulated with vehicle or TGF-β1 (1, 5, 10 ng/ml) for 24, 48 and 72 h. mRNA expressions of α-SMA, COLI, COLIII and FN in the fibroblasts were determined using real-time RT-PCR. The protein expression of α-SMA was determined using Western-blot. The secretion of COLI, COLIII and FN was determined using ELISA. Transforming growth factor-β1 affected in dose- and time-dependent manner on α-SMA and COLI, COLIII and FN expression. These findings suggest that in equine endometrium, the myofibroblasts may be induced from fibroblast in response to TGF-β1 and this cytokine seems to be a great stimulator of ECM component production.

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Incidence of endometritis in Holstein Friesian cows - a case study

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Uterine diseases occur in the entire postpartum period and are considered as the main cause of reproductive problems. Typically, 20%- 40% of dairy cows have clinical metritis or endometritis, which persists in up to 20%- 40% of animals as subclinical endometritis. Numerous factors are known to predispose cows to uterine infection, and due to this, the incidence of uterine diseases varies in particular herds but can be much higher in problem herds. The aim of our study was to determine the incidence of clinical and subclinical endometritis in a Holstein Friesian commercial dairy farm. Cows (n=97) were examined at 21 to 28 day after parturition from April to July, 2017 vaginoscopically. Animals were divided into four groups according to the severity of disease: E0- clear mucus, E1- mucus containing white flakes of pus, E2- less than 50% pus contained in mucus, E3- more than 50% pus contained in mucus. Cytologic samples were obtained by cytobrush from uterine area between left horn and body to determine the percentage of polymorphonuclear leukocytes (PMNs). The threshold of PMNs greater or equal 5% was used to diagnose subclinical endometritis and differentiate subclinical (E0SE) and healthy cows. The overall incidence of clinical and subclinical endometritis was E1- 23.7%, E2- 11.3%, E3- 9.3% and E0SE- 24.7%, whereas 31% of animals were diagnosed as healthy. Average number of PMNs in particular endometritis forms was E1- 39.1%, E2- 58.3%, E3- 72.7% and E0SE- 22.6%. In healthy group average PMNs was only 1.5%. In conclusion, 69% of cows had either clinical or subclinical endometritis which might cause subsequent fertility problems.

Cervical cytology is sufficient for diagnosis of subclinical endometritis in dairy cows

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Subclinical endometritis (SE) in dairy cows is characterized by elevated level of polymorphonuclear leukocytes (PMNs) in cytology samples obtained from uterus. Cytological samples can be collected from vagina, cervix or uterine horns and presence of PMNs in all parts of cattle reproductive tract was ascertained in the previous studies. The purpose of our preliminary study was to check possibility of diagnosis of SE only by using cytological examination of the cervix based on comparison of the number of PMNs in samples obtained from cervix and both uterine horns. A total of 117 bovine uteri without clinical signs of uterine disease from the abattoir were used for the study. Samples from cervix, left and right uterine horn were obtained by cytobrush after previous incision of cervix and both horns followed by Romanowsky staining. The number of PMNs greater or equal 5% of all cells in at least two among three sampling sites was used to diagnose SE. For statistical analysis Pearson correlation was performed using IBM SPSS Statistics 24. The disease was found in 17 cows while 100 were healthy. Average number of PMNs (mean±SD) in SE group was 21.4±19.7S in cervix, 16.2±13.7 in left horns and 15.7±14.8 in right horns, whereas in the healthy group it was 1.1±1.8, 0.8±1.2 and 0.8±1, respectively. There was a high correlation between percentage of PMNs in cervix, left and right uterine horn were significant in both groups ($p<0.001$). Due to this results only two cow would be misdiagnosed on the basis of cervical cytology. In conclusion, it seems that cytological examination of cervix might be used to diagnose subclinical endometritis in dairy cows.

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