ABSTRACs BOOK

Mechanisms of infection and immunity in the female genital tract

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Uterine disease is common after parturition in dairy cows and this often causes 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. The inflammatory response to microbes can be limited by treating endometrial cells with inhibitors that target cellular signalling pathways. Understanding the mechanisms of infection and immunity in the female genital tract is driving the discovery of novel approaches to treat uterine disease.

Comparative study of cellular and secretory function of bovine and equine endometrium

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As for other mammals, mare and cow fertility depends on adequate endometrium cellular and secretory dynamics during estrous cycle and post-partum. Vascular and non-vascular cell proliferation and apoptosis are hormone modulated physiologic processes essential for endometrium function. In the luteal phase, endometrium cell hypertrophy and hyperplasia might be necessary for histotroph production, essential for early embryo nourishment. During post-partum uterine involution, major tissue remodelling occurs, being complete on day 22 post-partum. Several hormones/cytokines influence endometrium prostaglandins (PG) production, throughout the estrous cycle in both species. In mares, oxytocin (OT) stimulates PGE2 and PGF2α by both stroma and epithelial cells. However, in the cow only epithelial cells are sensitive to OT action. Besides, nitric oxide (NO), via endothelial NO synthase, may be involved in the synthesis of PGE_2 during luteal growth, and $PGF_{2\alpha}$ at luteolysis, through PG synthases modulation. Interleukins also regulate PG secretion via *PG* synthases in equine endometrium. Cytokines are thought to regulate PGs secretion in the bovine endometrium. TNFα appears to stimulate both PGs production in the endometrium, as well as endothelial cell proliferation. However, there is no consensus about the role of interleukin (IL)s on PG secretion. Although IL-1α may stimulate *in vitro* luteolytic PGF_{2a} secretion during the estrous cycle, it acts *in vivo* only as a luteotropic factor in cows. IL-1a increased luteotropic PGE_2 and P4 output, inhibiting spontaneous luteolysis. Moreover, as well as PGs, leukotrienes (LT)s are synthesized from arachidonic acid (AA) in the female reproductive tract including endometrium in cows. We have recently showed that LTs caused changes in PG and P_4 secretion in reproductive tract, both *in vivo* and *in vitro*. 5-lipoxygenase and LT receptors are expressed in the bovine endometrium dependently on the day of cycle. Thus, not only PGs, but also LTs can be produced in the uterus and participate in the regulation of bovine uterine function. During the estrous cycle, the bovine endometrium exhibits characteristic morphological and functional changes. Production of PG by the endometrium and their influence on progesterone (P_4) and estrogens is the main mechanism responsible for the cyclic nature of the estrous cycle in cattle. Nevertheless, also exogenous estrogens including phytoestrogenes can act on endometrium. Phytoestrogens (isoflavonoids and coumestanes) and its metabolites act as endocrine disruptors, leading to disruption of the reproductive processes and to temporal infertility in both species. Coumestrol is a phytoestrogen that mimics E_2 action in equine endometrium. However, in contrast to E_2, only coumestrol was able to stimulate PGE_2 secretion by stromal cells. Phytoestrogenes are stronger stimulators of PG production than 17β-estradiol itself. Phytoestrogenes can disrupt the ratio of PGE_2 to $PGF_{2\alpha}$ which might lead to the non-physiological PG actions in bovine and equine reproductive tract during the estrous cycle. Further studies should be pursued for a better understanding of the physiologic regulation of cellular and secretory function in cow and mare's endometrium.

Bovine monocyte and macrophage heterogeneity in blood and uterine tissue

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Monocytes and monocyte-derived macrophages are important modulators of inflammatory processes. They take part in the initiation and the resolution of an inflammation as well as in tissue remodeling. Little is known about functional polarization of macrophages in the bovine system and especially in the uterus. We therefore addressed the phenotypical and functional heterogeneity of these cells in uterine tissue as well as the heterogeneity of their precursors in blood. Bovine macrophages can be classified according to their mutual expression of CD163 or S100A8/A9. In uterine tissues of cows with subclinical and clinical endometritis the abundance of S100A/A9 cells in subepithelial locations rises in case of inflammatory processes, suggesting that they resemble early inflammatory macrophages. In contrast, the relative numbers of CD163+ cells preferentially located in the connective tissue of the *Lamina propria* remain rather constant during metritis or endometritis. Some of the CD163+ cells show spindle-shaped morphology reminiscent of fibrocytes rather than of classical macrophages. Such monocyte-derived fibrocytes (MdF) differentiate *in vitro* under serum-free conditions, especially in the presence of Th2-type cytokines (interleukin- IL4, IL13). Their response towards lipopolysaccharide (LPS) is characterized by a more dominant expression of neutrophil-attracting chemokines (CXCL1, CXCL8) as compared to *in vitro* generated macrophages, which show a faster and stronger expression of IL1B, tumor necrosis factor α (TNF) and nitric oxide synthase 2 (NOS2). MdF and other macrophage types may develop in the context of a special uterine environment or derive from certain monocyte subsets. Evidence for the subset hypothesis comes from the observation that functionally heterogeneous bovine blood monocytes can be subdivided in classical, intermediate, and non-classical monocytes (based on differential CD14 and CD16 expression), which give rise to inflammatory as well as anti-inflammatory macrophages.

Impairment of the endometrial interleukin and prostaglandin systems in chronic degenerative endometritis in the mare

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Chronic degenerative endometritis, called as endometrosis, is associated with decreased fertility and early embryonic loss in the mare. Endometrosis is mostly observed in older mares and is often associated with increased susceptibility to infection. The aim of the study was to characterize expression of interleukins (IL) and prostaglandins (PG) systems in the course of endometrosis. Additionally, the influence of IL-1α, IL-1β and IL-6 on PG secretion and *PG synthases* mRNAs transcription in endometrial tissue during endometrosis was investigated. The endometrial samples were obtained at the early (n=12), mid (n=12) and late (n=12) luteal phases and at the follicular (n=12) phase of the estrous cycle. Within each of these stages of luteal phase, 4 samples represented each Kenney's categories, such as category I endometrium (no degenerative changes), II (moderate degenerative changes) and III (severe degenerative changes). The endometrial transcription of specific *PG synthases* (*PTGS-2*, *PGES* and *PGFS)* mRNAs and PGE_2 and $PGF_{2\alpha}$ concentration were determined. Additionally, the transcription of *IL-1α*, *IL-1β*, *IL-6* and their receptors mRNAs and their immunolocalization and protein expression were investigated. Furthermore, the endometrial samples (n=5 samples within categories I, II and III; mid luteal phase of the estrous cycle) were stimulated with IL-1α (10 ng/ml), IL-1β (10 ng/ml), IL-6 (10 ng/ml) for 24 h. After the stimulation, transcription of *PTGS-2*, *PGES* and *PGFS* mRNAs and PG concentration was determined. The estrous cycle-dependent IL and PG system expression patterns and endometrial secretory function are largely disturbed in category II and III endometrium compared to category I. This may result in serious endometrial microenvironment alterations. These disturbances might determine the impairment of endometrial physiology and promote mare sub-fertility or infertility, by affecting estrous cycle regulation and embryo survival or implantation.

Effect of preimplantation factor (PIF*) and lipopolysaccharide on the inflammatory response of equine endometrial explants *in vitro*

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AIMS: PIF is secreted only by viable mammalian embryos and is essential for achieving maternal immune-tolerance without immune-suppression. In human endometrium PIF coordinates and supports implantation and modulates immunity. Transposed to non-pregnant models, PIF displays immune-control and regenerative features in neuroinflammation and diabetes. The aim was to test the hypothesis that PIF exerts anti-inflammatory properties towards equine endometrium challenged with *Escherichia coli*-derived lipopolysaccharide (LPS) using an established endometrial explant culture model of uterine inflammation. METHODS: Luteal (n = 8), follicular (n=3), transitional (n=4) and anoestrus (n = 4) stage endometrium was collected from slaughtered mares. Explants were cultured in triplicate in serum-free medium alone (control) and with 0, 50 or 100nM synthetic PIF(sPIF; 25-100nM = human physiologic dose) and ± LPS (3µg/ml). Media samples were collected at 24 and 72 h and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 secreted into cultured medium were used as markers of inflammation determined by radioimmunoassay. RESULTS: Overall mean $PGF_{2\alpha}$ secretion by control explants was greater than PGE_2 at both sampling points: $P<0.001$, 24 hours 463.9 ±58.2 vs. 15.7 ±2.7 ng/ml; $P<0.001$, 72 hours, 1441.5 ±106.2 vs. 53.8 ±5.8 ng/ml. When compared to the control, LPS stimulated secretion of PGE_2 by 3.1 and 2.4 fold at 24 and 72 hours, respectively, in explants from mares at all stages ($P<0.001$). LPS stimulated secretion of $PGF_{2\alpha}$ only at 24 hours in follicular stage explants (1.8 fold change from control; $P<0.05$). sPIF alone did not alter prostaglandin secretion by explants compared to control. In follicular stage explants both 50 and 100 nM sPIF abrogated LPS induced $PGF_{2\alpha}$ secretion at 24 hours post treatment ($P<0.05$). CONCLUSION AND PRACTICAL SIGNIFICANCE: The effects of LPS and sPIF on prostaglandin secretion by endometrial explants are modulated by stage of oestrous cycle. PIF treatment exerts an anti-inflammatory effect in follicular tissue abrogating LPS-induced secretion of $PGF_{2\alpha}$. Data advocates sPIF anti-inflammatory role in non-pregnant endometrium, which complements PIF's essential role in supporting embryo implantation.

Crosstalk between leukotrienes, cytokines and *Escherichia coli* during induced inflammation of uterus in cow

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Leukotrienes (LT)s are produced/secreted in uterus, and proinflammatory cytokines such as tumor necrosis factor alpha (TNF) and interferon gamma (INFgamma) enhance mRNA expression of LT receptors and 5-lipoxygenase. Nevertheless the development of inflammation is not known. The aim of this study was to determine the dynamic profile of inflammation concerning the crosstalk between bacteria and the action of LTs and cytokines in uterus. Endometrial uterine explants (Day 8-10 of the estrous cycle; each 50 mg) were collected *post mortem* (N=6) and incubated for 2, 12 and 24 h with *E.coli* (isolated briefly from metritic cows; 10^6CFU/ml) and/or LTB4 and C4 (10^{-6}M) and/or cytokines (TNF+INFgamma; each 10 ng/ml). Bacteria proliferation rate, Toll Like Receptor 4 (TLR4) mRNA expression in the slices and PGE2 and F2alpha levels in the medium were determined.
The highest bacteria proliferation rate was observed after 12 h of experiment ($P<0.01$). TLR4 mRNA expression was increased in slices incubated with *E.coli*, cytokines and LTs after 2 and 12 h ($P<0.05$) but not after 24 h of stimulation ($P>0.05$). Incubation of slices with *E.coli* increased the output of both PGs (especially PGE2) after all time-selected periods ($P<0.05$). Induction of inflammation was enhanced by LTs and cytokines, and the highest level of PGs after 24 h of incubation with *E.coli*, LTs and cytokines was determined ($P<0.05$). The received results, where LTs action modify inflammation after short and long time of experiment indicate that LTs are involved in inflammatory process in uterus, both in the step of early immune response connected with activation of TLR4 and cytokine action and specific immune response connected with lymphocyte activation.

Effect of neutrophil extracellular traps and cytokines on the expression of genes involved in mare endometrium secretory function and fibrosis

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Neutrophils extracellular traps (NETs) are DNA molecule complexes carrying various nuclear and cytoplasmatic proteins, like elastase (ELA), cathepsin G (CAT), myeloperoxidase (MPO), among others. They are released as anti-microbial and pro-inflammatory response. Besides cytokines, fibrosis may me induced by NETs in mare endometrium chronically stimulated by bacteria infection. Fibrosis is related with Transforming Growth Factor beta (TGFβ1) fibrogenic action with abnormal deposition of collagen proteins, mainly type I and type III. NETs and cytokines effect on expression of genes involved in secretory function and fibrosis was assessed. Mares (n=5; mid-luteal) endometrium explants were cultured (24h, 48h, 72h) with NETs components: ELA, CAT, MPO; cytokines: Connective Tissue Growth Factor - CTGF, Tumor Necrosis Factor alpha - TNFα; oxytocin-OT. Prostaglandin- endoperoxide synthase 2 (PTGS2), $F_2\alpha$ and E_2 synthases (PGFS, PGES), Tissue inhibitor metalloproteinase 1-TIMP1, TGFβ1 and its receptors I (TGFRI) and II (TGFRII), types I and III, alpha 1 collagen (COL1, COL3) mRNA transcription were evaluated by real-time PCR. (i) PTGS2: increased with CTGF, TNF (72h; $p<0.05$), ELA, MPO (24-48h; $p<0.05$), CAT, CTGF, TNF (24-72h; $p<0.05$). (ii) PGFS: increased with CTGF (24h; $p<0.05$), MPO (24-72h; $p<0.01$), decreased with CTGF (24-48h; $p<0.01$). (iii) PGES: increased with CTGF and OT (48h; $p<0.05$). (iv) TIMP1: increased with CAT (48h; $p<0.05$), and OT (72h; $p<0.01$). (v) TGFβ1: increased with OT (48h), MPO (72h; $p<0.05$), decreased with TNF (24-72h $p<0.05$). (vi) TGFRI: increased with ELA, CTGF (48h; $p<0.01$), TNF (24-48h; $p<0.05$), and decreased with CAT (72h; $p<0.05$). (vii) TGFRII: increased with MPO (24-72h; $p<0.05$) and TNF (24-48h; $p<0.01$); (viii) COL1: increased with CAT, CTGF (48h; $p<0.01$); and all treatments (24-48h; $P<0.01$) except OT and TNF. (ix) COL3: increased with CTGF (24h, 72h; $p<0.05$), MPO (24-72h; $p<0.05$), decreased with CAT, CTGF, TNF (48h; $p<0.05$). All NETs components up regulated gene transcription of TIMP1, TGFβ1 and its receptors, which may suggest they are important players in fibrosis pathogenesis. A long-term incubation with NETs components and cytokine CTGF induced high transcription level of type I or type III collagen genes, characteristic of fibrosis. These results indicate that NETs and cytokines are involved in mare endometrial fibrosis establishment.

Role of BoHV-4 in bovine uterine infections

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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. In uterine isolates from animals with reproductive disorders, 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. Since bovine herpesvirus 4 possess a special tropism toward endometrial stromal cells, a simian virus 40 (SV40) immortalized endometrial stromal cell line (SV40BESC) was established and demonstrated 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 treatment. As a result, an increase of BoHV-4 replication and cytopathic effect was observed in BoHV-4 infected and TNF-α treated SV40BESCs. The 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. 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 binds TNF-αR1 on the surface of BoHV-4 infected endometrial stromal cells, inducing BoHV-4 *IE2* gene expression and enhancing 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).

The bacterial communities in the reproductive tract of dairy cows vary by farms

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The dairy industry is the largest agri-business in Ireland. This industry is based on small/medium farms that together hold a current livestock of over 1.1million dairy cows. A major challenge of this industry is the incidence of post-partum endometritis, a multifactorial condition that is likely to be associated, among others, to alterations of the bacterial communities, the immune status of the animals and differences between farms. However, little is known about the bacterial communities residing in the reproductive tract of dairy cows and if these communities vary among farms. In this study, we compared the community profiles in animal from three farms using culturable and non-culturable methodological approaches, the later relying on Terminal Restriction Fragment Length Polymorphism (TRFLP) of the bacterial 16S rRNA. Our data clearly showed the presence of significant differences in the communities of farms with different incidence of post-partum endometritis and warrant the study among a larger number of samples and farms.

Effect of preimplantation factor* on the secretion of prostaglandin $F_{2\alpha}$ from bovine endometrial tissue following a lipopolysaccharide challenge *in vitro*.

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AIM: Endometritis is a common inflammatory uterine disease in dairy cattle caused by entry of bacteria into the uterus. Secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from endometrial tissue is increased during the pro-inflammatory immune response caused by bacteria. Preimplantation factor (PIF) is a pregnancy specific peptide secreted by the embryo that has immune modulatory roles during pregnancy and has been shown to exert its effects within models of inflammatory based autoimmune diseases. The experimental aim was to assess if synthetic PIF (sPIF) has immune modulatory roles in an *Escherichia coli* model of $PGF_{2\alpha}$ secretion in endometritis. METHODS: Bovine endometrial tissue from cows (n=12) with stage I corpus luteum present was cultured in media alone or stimulated with lipopolysaccharide (LPS; 1µg/ml), sPIF (50, 100, 500nM) or LPS (1µg/ml) and sPIF (50, 100, 500nM). Media was sampled at 24, 48 and 72h and analysed by radioimmunoassay for $PGF_{2\alpha}$ concentrations. Serum was extracted for all cows from blood samples taken after slaughter and analysed by ELISA for progesterone concentration. RESULTS: Cows were split into two groups based on progesterone concentration at slaughter, with 8 cows having a lower concentration (1.93 ± 0.34 ng/ml) and 4 having a higher concentration (10.39 ± 2.43 ng/ml). There was a significant increase in $PGF_{2\alpha}$ secretion over time in both groups ($P<0.001$). There was no effect of progesterone concentration group on $PGF_{2\alpha}$ secretion ($P>0.05$). Within individual time points in both groups, there was a significant increase in $PGF_{2\alpha}$ secretion in response to LPS treatment ($P<0.05$). There was no effect of PIF on $PGF_{2\alpha}$ secretion at any time point in either group ($P>0.05$). Variance between cows in response to PIF was large. CONCLUSION: LPS significantly increased $PGF_{2\alpha}$ production, showing a strong pro-inflammatory immune response. However, PIF had a highly variable response and therefore no overall significant effect on the pro-inflammatory response to a LPS challenge in bovine endometrial tissue. The effect of PIF on other pathways integral to the innate immune response of the bovine endometrium to LPS remains to be investigated.

The putative role of interleukins and cyclooxygenase 2 in the development of subclinical/clinical endometritis in cows

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Cows with clinical or subclinical endometritis show typical signs of ~~an~~ inflammation with increased expression of pro-inflammatory factors in the endometrium. The interleukins (IL1A, IL1B, IL8) were higher expressed in the endometrial epithelial cells in cows with clinical/subclinical endometritis compared to healthy cows suggesting them as marker genes of this disease. However, IL6 and cyclooxygenase 2 (COX2) were not differently expressed depending on the uterine health status. In addition, in cows during the early puerperium all these IL and COX2 showed a peak expression in the endometrium on day 17-24 post partum. To test the influence of bacteria on the inflammatory process, several strains were co-cultured with bovine endometrial epithelial cells in vitro in different multiplicities of infection (MOI). Commensal strains, e.g. *Lactobacillus vaginalis*, did not affect the viability monitored by trypan blue staining of the cells for up to 96h. In contrast, potential pathogenic strains, e.g. *Bacillus pumilus*, caused death of >95% of the epithelial cells within 24h. After 2h, 4h and 6h co-incubation time, total RNA was extracted and subjected to real-time RT-PCR. Compared to an untreated control, the co-culture with *L. vaginalis* did not result in an increased expression of the interleukins or COX2. In contrast, epithelial cells co-cultured with *B. pumilus* showed a significantly higher expression of IL1A, IL6, IL8 and COX2 with MOI of 1, 5 or 10 at each point of time, respectively. The highest expression was observed after 2h co-culturing. These results show that potential pathogenic bacteria cause death of cells compared to commensal bacterial strains. However, endometrial epithelial cells are capable to respond in a very fast manner to activate the immune system. This may result in an increased influx of polymorphonuclear cells into the uterus.

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Effect of postpartum suppression of ovulation on uterine involution in dairy cows

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The objective of this study was to investigate the effect of time of first postpartum ovulation after calving on uterine involution in dairy cows with and without uterine puerperal disease. Transvaginal follicular puncture (FP) of follicles > 6 mm suppressed ovulation and development of a CL until Day 42 after calving. Fifty-three lactating Holstein Friesian cows (3.4 ± 1.2 yr old, parity 2.5 ± 1.0 [median ± mean absolute deviation]) were divided into groups based on the presence (UD+) or absence (UD–) of uterine disease and whether follicular puncture was carried out (FP+) or not (FP–). Uterine disease was defined as the occurrence of retained fetal membranes and/or metritis. This resulted in the following groups: UD–FP– (n = 15), UD–FP+ (n = 13), UD+FP– (n = 13), and UD+FP+ (n = 12). A general examination, vaginoscopy, transrectal palpation, and transrectal B-Mode sonography of the reproductive organs were conducted on Days 8, 11, 18, 25 and then every ten days until Day 65 after calving. After hormonal synchronization of ovulation (cloprostenol between Days 55 and 60 postpartum and GnRH 2 d later), cows were inseminated in the next spontaneous estrus. On average, the cows ovulated on Day 21.0 ± 6.0 (UD–FP–), 50.0 ± 4.0 (UD–FP+), 16.0 ± 3.0 (UD+FP–), and 48.0 ± 2.0 (UD+FP+) postpartum. Calving-to-conception interval and first-service conception rates were not affected by FP ($P > 0.05$). Healthy cows with FP had smaller ($P < 0.05$) uterine horn and cervical diameters assessed sonographically than cows without FP. Follicular puncture reduced the prevalence of purulent vaginal discharge and uterine size assessed transrectally in UD+ cows ($P < 0.05$). The results showed that suppression of an early ovulation by transvaginal follicular puncture improved uterine involution in cows with and without uterine disease.

Retained placenta as an important trailblazer for uterine infections: observations on the control of bovine parturition and placental release

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Dystocia and placental retention are important causes of severe postpartal uterine inflammation in cattle. However, there are still many open questions concerning the mechanisms controlling parturition including the timely release of the placenta. Important steps of the signal cascade leading to parturition previously revealed in the sheep have also been confirmed in cattle, such as a prepartal rise in fetal cortisol inducing an increase of placental CYP17, which results in a collapse of placental progesterone production. However, different from the sheep in prepartal cows the placenta contributes only marginally to maternal progesterone levels and the prepartal decline in maternal progesterone is clearly associated with luteolysis. The observation that around the time of luteolysis cyclooxygenase 2 is significantly up-regulated in the bovine trophoblast but not in the endometrium suggests that in prepartal cows luteolytic prostaglandins are primarily of placental origin. Blockage of progesterone receptors by an antiprogestin in late pregnant cows readily induced a complete opening of the cervix, but was invariably associated with tedious labour, long-standing placental retention and placental immaturity, which is inconsistent with the concept that a complete withdrawal of progesterone at the feto-maternal interface is a key event for placental detachment. Observations in peripartal cows suggest that around the time of fetal expulsion endometrial prostaglandin synthesis is substantially up-regulated, which returns to low levels soon after undisturbed parturition but may persist or even increase in case of significant trauma or infection. The high prostaglandin levels measured in postpartal cows challenge the concept that the application of uterotonic drugs may be of significant use to support uterine involution during the early puerperal phase.

Fungal endometritis in mares

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Fungal endometritis is an uncommon condition in mares, accounting for less than 5% of diagnosed endometritides. It is generally accepted that fungal infection is opportunistic and will only establish in a chronically disturbed uterine or vaginal environment; pneumovagina, persistent endometritis and repeated intrauterine antibiotic therapy are commonly cited as predisposing factors. Since the exact conditions that allow fungal colonization of the uterus are obscure, there are currently no treatments proven to offer a high likelihood of resolution, and recidivism is common. Uterine infection with fungal organisms is therefore a considerable therapeutic challenge with a poor prognosis both in terms of speed of recovery and in terms of future breeding potential, since the organisms invade deep into the endometrium where they instigate fibrotic degeneration. While both the identity of the causal organism and the duration of infection may affect the response to treatment, it is advisable to simultaneously correct any (suspected) predispositions (e.g. pneumovagina) and to treat against a potential reservoir of infection in the caudal reproductive tract (vagina and clitoral fossa). In the author's experience, intrauterine infusion with non-specific chemicals such as 2% acetic acid or hydrogen peroxide for 1-3 days or with anti-fungals such as clotrimazole (500mg) or nystatin (1.2 million units) daily for 5-7 days yields a resolution rate of 20-30% per treatment cycle. Recently, a combination of a single treatment with 2% acetic acid followed by six consecutive days of intrauterine and intravaginal clotrimazole has yielded better results. Resolution of fungal infection is often followed by a streptococcal endometritis that also requires treatment. If initial treatment is unsuccessful, a period of rest to allow spontaneous re-establishment of a normal uterine environment can be surprisingly effective.

Compartmentalization and dynamics of bacterial communities in the reproductive tract of thoroughbred mares

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Very little is known about the microbial communities residing in the reproductive tract of animals. Despite of the potential implications for the industry of horse breeding, the composition of the microbiota in the reproductive tract of mares is largely unknown. Most studies have focused on the analysis of clinical cases to isolate bacteria from vaginal discharges [1-2]. In a recent study, the presence of lactic acid bacteria in healthy mares highlighted their potential as probiotics in an analogous way as observed in the human vagina [3]. However, all these studies relied on culture-based analyses that are known to be biased in favor of a small percentage of the total microbiota and a throughput microbiological study of the horse reproductive tract is due. Here, we analysed the microbial community of the reproductive tract of thoroughbred mares using culture-dependent and independent approaches. A discriminant function analysis using data generated by Terminal Restriction Fragment Length Polymorphism (TRFLP) of the 16S rDNA of the non-culturable communities revealed the presence of different microbiota associated to each vaginal and endometrial mucosa. Given the physical compartmentalization of the reproductive tract, this result prompted to the determination of the phylogenetic affiliations in each compartment. One of the most striking differences was the presence of Actinomycetes such as *Corynebacterium spp.* and *Arcanobacterium spp.* which were found in 16S rDNA clonal libraries obtained from vaginal but not in endometrial samples. Interestingly, the vaginal and endometrial communities are mixed after breeding as observed by the appearance of Actinomycetes in endometrial samples. Our results show complex bacterial communities associated to the reproductive tract of thoroughbred mares. In addition, they highlight changes associated to breeding, when the risk of endometritis is the highest.

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The molecular and endocrine markers alternations in subclinical endometritis in mares

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Clinical and subclinical endometritis are leading causes of reduced reproductive efficiency in mares. Mares that do not became pregnant after repeated breeding without showing typical signs of clinical endometritis should be suspected of subclinical endometritis. Subclinical endometritis alters normal reproductive physiology. Components of bacteria activate Toll-Like Receptors on the endometrial cells, which affect the secretion of cytokines, chemokines and possibly metabolite of arachidonic acid. Therefore, to address the hypothesis that subclinical endometritis may affect immune-endocrine status of the equine endometrium, by TLR activation and prostaglandins (PGs) and leucotriens (LTs) pathways impairment, we checked: spontaneous secretion of prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2), leukotriene B_4 (LTB_4) and C_4 (LTC_4) as well as Tumor Necrosis Factor α (TNFα). In addition, mRNA transcription for Toll-Like Receptors type 2 and 4 in the endometrial fragments were determined. Eighty-four warm-blood mares, of known breeding history, were enrolled in this study. Based on histopathological assessment, mares were classified as suffering for slight subclinical endometritis, moderate/severe subclinical endometritis or being healthy. In addition, the Kenney category for each endometrial biopsy was determined. The ultrasound examination was done to check the stage of estrous cycle. One biopsy was obtained from each mare from the randomly selected uterine horn. The biopsy was divided in–three parts, as following: (i) first fragment was plunged in RNAlater and used for further gene analysis; (ii) second fragment was immersed in Dulbecco modified Eagle medium (DMEM) and used for spontaneous PGs, LTs and TNFα secretion analyses; and (iii) third fragment was preserved in buffered 4% formalin, for histopathological examination. The endometrial mRNA transcripts for TLR2 and 4 were analyzed by Real Time PCR method. The spontaneous secretion of $PGF_{2\alpha}$, PGE_2, PGI_2, LTB_4, LTC_4 and TNFα in 4h tissue culture at 37°C was assessed by immunoenzymatic method (EIA). The Kenney category and the infiltration of endometrial fragments with polymorphonuclear leukocytes were examined in routinely hematoxylin/eosin method. In the group of moderate/severe endometritis the PGE_2 and TNFα secretion was uniquely increased in comparison with control or slight subclinical endometritis ($P<0.05$). Prostacyclin secretion was increased in both groups of mares presenting subclinical endometritis when compare with control group ($P<0.05$). The secretion of both leukotriens did not change in examined groups. However, in the group of moderate/severe endometritis mares the LTC_4 secretion was slightly diminished in comparison to other groups. Interestingly, mRNA transcription analysis showed increase only for TLR2 in the moderate/severe endometritis mares ($P<0.01$). Obtaining results show that in subclinical endometritis, endometrial secretion of several arachidonic acid metabolites is disturbed. Increased endometrial secretion of PGE_2, PGI_2 and TNFα may alter the longevity of the estrous cycle and immune-endocrine environment in uterus.

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Evaluation of current diagnostic methods and treatment strategies in horses and cattle

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Endometrial sampling is not routine practice in cows, but endometritis diagnosis is based on clinical signs (discharge) and vaginal samples. In mares, bacteriological and cytological examination of the endometrium with swabs and cytobrushes has been used for a long time. However, recent studies suggest that a small volume lavage may be more sensitive, since the fluid is spread on a larger uterine surface as compared to the small contact area when a swab or a cytobrush is used. The cytobrush is preferred for cytology instead of the swab because it collects more material than a swab. In mares, endometritis caused by E.coli is often of subclinical nature and therefore more difficult to diagnose than, e.g. streptococcal endometritis. Endometrial biopsy is considered to be the most reliable diagnostic method, as it is based on histology and therefore allows also for the detection of chronic inflammation and degenerative changes. Special guarded biopsy forceps are required if also bacteria are cultured. In cows, chronic post partum endometritis is the indication for treatment, whereas in mares post breeding treatments are most common. Prostaglandin (PG) injection(s) is the most common treatment in cattle and can be used also in mares to cause luteolysis and to evacuate uterine contents. However, in horses oxytocin is often preferred over PG. In equine practice, the use of antibiotics should be based on bacteriological diagnosis, but routine post breeding treatments with various antibiotics are still commonly done without any bacteriological sampling or diagnosis. The use of antibiotics is not that common in cattle, because antibiotics have no advantage over PG but result in withdrawal times of milk.

Abnormal vaginal discharge – Is it always endometritis?

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Recently, it was shown that a fairly high number of cows with abnormal vaginal discharge (AVD) did not reveal a concomitant positive uterine cytology suggesting that other causes than endometritis might be responsible for AVD. Therefore, the objective of this study was to determine whether or not cervicitis was an independent disease or occurred mostly together with endometritis. In addition, possible effects on fertility were evaluated. Dairy cows (n=514) from 33 dairy farms without AVD were included into the study. Cervicitis was diagnosed when the 2nd cervical fold was swollen and prolapsed with (C2) or without redding (C1). Endometrial biopsies and cytobrush samples from the endometrium were collected from 416 cows at 42 to 50 days post partum. Cervicitis was diagnosed in 52.5 % of the cows (28.4 % C1 and 24.1 % C2). Based on uterine histology and cytology (> 5 % or > 10 % PMN) as indicators of endometritis, 30.5 %, 76.8 % and 85.0 % of cervicitis cases occurred independently of uterine pathology. Reproductive performance was determined for 385 cows divided into three groups (C0=without cervicitis, C1, C2). Days to first service, days open and pregnancy index did not differ between groups (P>0.05). Conception rate was higher in group C0 and C1 than in C2 (41.2 % and 42.7 % vs. 30.8 %, respectively; P<0.05). Overall pregnancy rate was lower in cows with C2 than in the other groups (68.1 % vs. 83.7 % and 81.5 %, respectively; P<0.05). The results suggest that cervicitis is an independent disease and that severely affected cows (C2) may reveal a depressed reproductive performance.

Clinical and subclinical endometritis: meaning of the diagnosis and therapeutic perspectives

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The diagnosis of endometritis can be realized by: (1) Rectal palpation - the diagnosis of endometritis through palpation is inaccurate, with low sensitivity and specificity. Furthermore, it is almost impossible to make the diagnosis of sub-clinical endometritis; (2) Cytology - the cytological diagnosis has been proposed as the gold standard for many years. There may be a high level of PMN without endometritis, as there can be animals without endometritis with a high level of PMN; and (3) Ultrasound diagnosis - the ultrasound diagnosis of endometritis is easy, fast and accurate, also resorting to ultra portable ultrasound unit. Regarding the presence of PMN and mucus, the uterine contents may be more or less echogenic. Purulent, muco-purulent endometritis and pyometra have a sonographic appearance like a snowstorm, while the mucometra is perfectly anechoic. Normally these inflammations are accompanied by the presence of one or more persistent corpora lutea, compact and/or with cavity. It is easy to make an accurate diagnosis of sub-clinical endometritis, as long as the assessment is not limited exclusively to the endometrial thickness (> 8mm) and the thickness of the uterine lumen (> 3 mm). The diagnosis based on these two parameters is inaccurate, due to the high number of false positives (very low sensitivity). All cows in proestrus, estrus and metaestrus are potentially diagnosable as sub clinical endometritis due to the fact they present more than 8 mm of endometrial thickness exceeding 3 mm in thickness and the uterine lumen. Evidently the ultrasound diagnosis must take into consideration the follicular map and the presence of a corpus luteum (cyclic or anoestrus). Besides assessing the amount of liquid present in the uterus, it should be also considered the type of material (echogenicity). The anechogenicity evidences absence of endometritis (if we exclude the mucometra), while the presence of a snowstorm more or less dense indicates uterine inflammation. The diagnostic process must be based on some objective observations, such as the presence of certain artifacts (shadow) compared to others (specular reflections). This allows differentiating the endometritis from a potentially normal uterus. Endometritis therapy is certainly one of the most debated issues in the context of reproductive management of dairy cattle. Three different therapeutic approaches can be considered: (1) Use of intra- uterine antibiotic medications – its utilization might be unclear since more than 90% of the endometritis present after the day 40 post-birth is due to inflammation and infection; (2) Use of prostaglandins (PG) - used to drain the uterus. Utilization of PGs is suggested with or without presence of corpus luteum. However, use of PG are not consensual regarding both time of administration (negatively affect the reproductive performance before the end of the voluntary waiting period - the day 12), and efficacy; and (3) Irritation solutions - normally Lugol's 3-5%. The risk with these solutions (if used repeatedly or at higher concentrations) is the uterine fibrosis, which *de facto* irreversibly affects the fertility. Recent studies have analyzed scientifically therapeutic procedures in use for endometritis, allowing to clarify the following assumption: neither prostaglandins nor intra-uterine antibiotics are an adequate method to fight endometritis. Another important aspect is the spontaneous healing of endometritis, where about 70% heals spontaneously in the first two months of lactation. Is the real goal to treat endometritis or to get pregnant the cows? If the goal is to get pregnant the cow, treatment for endometritis loses their meaning. The incidence of endometritis is important, since it is an indirect parameter to assess whether the management of transition period is adequate. It may be possible to use this parameter as an indicator of the ecological environment and animal husbandry in which the cow's life, it is also an indicator of human management of the cows, before, during and after calving.

Diagnosis of endometritis in the mare – Relation of sampling techniques to bacteriological results

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The aims of this study were to compare three systems for collecting uterine cytological samples (Knudsen catheter, commercial cotton endometrial swab, cytology brush) with respect to number of PMNs in the uterine smear and to examine the correlation between bacteriological findings from the uterus and the number of PMNs. Samples were taken from 340 warm-blood mares. In each mare two methods for collecting cytological samples were performed. In 279 mares samples for uterine cytology and bacteriological culture were taken. Differences among sample methods were analyzed using the Wilcoxon-Mann-Whitney-test. Correlations between bacteriological findings and number of PMNs were analyzed by the Spearman rank correlation coefficient. The pair wise comparison of the three sampling systems showed that:

- The Knudsen catheter delivered a significantly higher number of smears that failed to be utilizable because of insufficient cell material.
- The endometrial swab method caused a significantly higher number of cell deformations, whereas the cytology brush showed the highest number of erythrocytes.
- The Knudsen catheter method allowed the detection of PMNs more often ($p < 0.0001$) than the endometrial swab, but not more often than the cytology brush.
- The cytology brush allowed a more frequent detection ($p < 0.0001$) of PMNs than the endometrial swab.

Correlation between bacteriological and cytological findings:

- There was a significant correlation between a positive cytological result (smears with more than 0.5 % PMNs) and culture of β-hemolytic streptococci ($p = 0.002$) only using the cytology brush.
- There was a positive correlation between the number of PMNs and the number of colonies of β-hemolytic streptococci ($rs=0.2$, $p = 0.01$).

Local and Systemic Immune Profiles at Day 7 Post-Partum Identify Dairy Cows at Risk of Uterine Disease.

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Postpartum uterine infections are a leading cause of compromised fertility, which is the single biggest threat to the profitability of the Irish cattle sector. A reliable diagnostic test for uterine disease soon after calving would facilitate early and targeted therapeutic intervention. This study used a combination of high-throughput next-generation sequencing and traditional research techniques to identify potential prognostic markers of uterine disease in the post-partum dairy cow. A total of 182 genes were differentially expressed between uterine biopsies from inflamed cows (high degree of neutrophil influx into the epithelium) and healthy controls 7 days post-partum (DPP), and 1219 genes were differentially expressed between groups 21 DPP (n=15, FDR <0.1). Interleukin 17 has been associated with the pathogenesis of many inflammatory diseases was consistently significantly upregulated in the endometrium of inflamed cows at both 7 and 21 DPP (log_2 fold change of 2.8-3.6). Goseq analysis identified significant perturbation of multiple immune response and calcium signalling pathways between groups. The transcriptomic profile in healthy cows reflects a transient immune response 7 DPP, preceding a proliferative and repair response by 21 DPP which contrasts with a sustained inflammatory response in endometrial samples from inflamed cows. Additionally, miRNA profiling identified significant differential expression between time points and 11 miRNAs differentiated the inflamed and healthy cow samples 7 DPP. Microbial populations present on swabs taken from the uterus were identified using Terminal Restriction Fragment Length Polymorphism (T-RFLP) also identified different profiles between inflamed and healthy cows. At a systemic level, significant differences in haematological profiles were apparent with reduced numbers of circulating neutrophils and eosinophils in inflamed cows 7 DPP ($P<0.05$). Elevated levels of circulating serum proteins including HP (>1.7 mg/ml) and SAA (>100 μg/ml) indicated a significant acute phase response in inflamed cows 7 DPP. No significant differences in metabolite profile (BHB, urea, glucose and NEFA) were detected. Combined, we have identified a profile of sustained inflammatory responsiveness in the endometrium of a sub group of cows, which prevents the usual restoration of uterine homeostasis and is associated with significant systemic haematological, cytokine and acute phase protein changes. Specific cellular and molecular signatures identify animals at risk of developing uterine disease 7 DPP and validation of these biomarkers in a larger population of post-partum cows is now warranted.

Comparison of the biopsy and cytobrush technique for diagnosis of subclinical endometritis in the mare

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Endometritis is the most important cause of infertility in brood mares. During the breeding season, a timely diagnosis and an efficacious treatment is therefore essential for a successful breeding outcome. There are no precise criteria for interpretation of inflammation after bacteriological and cytological evaluation of uterine samples collected using the cytobrush. The objective of this study was to compare bacteriological and cytological results obtained from mares uterus by endometrial biopsy (EB) and cytobrush (CB) techniques and relation of those findings to histopathological examination of the endometrium for presence of endometritis. Samples were collected from 69 warmblood mares suspected for subclinical endometritis and from 15 young healthy mares. Samples for CB and EB were collected, smeared on a microscopic slide and cultured for bacterial growth. EB samples were additionally stored in formaldehyde for histological analysis. Bacteriological cultures and cytological samples obtained from CB and EB were classified as negative (no uterine pathogens in monoculture; < 2% PMNs) or positive (uterine pathogens in > 90% of grown colonies; > 2% PMNs) for endometritis. In histopathological examination infiltration of one or more PMNs per five fields (400x magnification) was considered as evidence of endometritis. The number of bacterial growth from the EB was 44 and from CB was 35 ($P>0.05$). Using the histopathological examination as the "best standard" for diagnosing of endometritis, the sensitivity of bacterial growth and cytology from an EB were 0.63 and 0.73 respectively. The sensitivity of bacterial growth and cytology from samples obtained by CB were 0.50 and 0.71 respectively. The specificity of bacterial growth and cytology from EB were 0.54 and 0.96 respectively. The specificity of bacterial growth and cytology from samples obtained by CB were 0.73 and 0.85 respectively. The positive predictive value of bacterial growth and cytology from EB were 0.74 and 0.98 respectively and from samples obtained by CB 0.80 and 0.91 respectively. The negative predictive value of bacterial growth and cytology from EB were 0.40 and 0.63 respectively and from samples obtained by CB 0.40 and 0.58 respectively. In conclusion endometrial culture may not identify all mares with endometritis, but its association with cytological evaluation of the endometrium enhances the diagnostic accuracy of endometritis in the mare. The bacteriological culture and cytology results obtained from CB are comparable to those obtained from EB. Based on our findings, the CB technique may be recommended for collection of materials from the mares's uterus during endometritis diagnosis.

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A retrospective analysis of cows referred to the Clinic for Ruminants, Berne, for fertility problems (2005-2012): Results of biopsies, microbiology and outcome

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In the years 2005-12, 140 Eringer cows were referred to our clinic due to fertility problems. These were 33% of all Eringer cows referred to our clinic as compared with 0.1% of all other breeds referred for fertility problems. Veterinarians supposed that Eringer cows were suffering from increasing fertility problems. The Eringer is known since 3000 B. C. and is an undemanding mountain breed used for milk and meat production. As these cows are also used for cow fights, selection is preferably done according to hot temper and muscle mass, thus, neglecting fertility. Therefore, a doctoral thesis was designed to retrospectively analyze the fertility variables of this outstanding Swiss breed. Mean interval from calving to first insemination was 86 days, days open were 146 days, conception rate was 39.1% and inter-calving interval was 431 days. The aim of the study was to: a) find the causes of fertility problems in the clinic patients; and b) compare fertility variables of the population as calculated by Pfister (2009) to those of our clinic patients. The median (25% / 75%) age of the clinic patients was 6.3 yrs (4.9 / 7.9), 16% were heifers, 62% were in 1^{st} - 3^{rd} lactation, 22% $\geq 4^{th}$ lactation. The last calving was 509 days (408 / 619) before presentation at the clinic. Routine examination of the cows (including heifers) consisted of cytological and bacteriological assessment of uterine swab, histological assessment of uterine biopsy, and patency testing of the oviducts. All samples were taken in diestrus (presence of corpus luteum and plasma progesterone value $\geq$ 1.5 ng/ml). Sixteen cows were slaughtered without consecutive insemination due to poor fertility prognosis based on these examinations. (a) From the remaining 124 cows, microbial culture of uterine swabs was positive in n=37 cows. *Trueperella pyogenes* was found in 10 cows (8 cows never got pregnant again, 2 cows aborted in 4^{th} month of pregnancy). *Pasteurellaceae* were found in 3 cows (never pregnant again). Furthermore, *E.coli*, *Streptococcus ssp*., *Enterococcus faecalis* and *Histophilus somni* were isolated. Inflammatory cells were seen on smears of n=44 cows. Uterine fibrosis was found in 44 biopsy samples, 16 of these samples exhibited massif perivascular and periglandular fibrosis. Deep inflammation of uterine tissue was found in n=8 biopsies. Moreover, in 9 cows the Fallopian tubes were blocked (7 cows never got pregnant again and 2 cows got pregnant after embryo transfer). Knockout criteria for future fertility of the cows referred to our clinic were positive culture of *T. pyogenes* from uterine swabs and missing patency of the oviducts (although the latter could be used as embryo recipients). From the 124 inseminated animals at the clinic, n=39 (32%) were confirmed as pregnant, including 6 cows which had an abortion 3 to 5 months later. From the 85 cows not pregnant at clinical discharge n=13 (15.3%), pregnancy was achieved later (between 3 and 130 days after clinic discharge). (b) In the clinic patients, mean intercalving interval was 835 days compared with 431 days in the entire Eringer population. Days open for all cows calving (n=46) was 525 days compared to 146 days in the entire Eringer population. Conclusions: In 57% of our patients, we could find the reason for the fertility problem with the examinations described. Data of the clinic patients clearly indicate that these cows are not representative for the entire breed as to fertility parameters.

Cytological and microbial metagenomic analysis of puerpural metritis in Israeli dairy cows

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Puerpural metritis is a common disease affecting milk production, fertility and welfare of dairy cows. The disease is highly prevalent and affects 30-40% of calving dairy heifers and cows in Israeli dairy farms. The etiology and the pathogenesis of the disease are currently not well understood although microbial involvement is usually suggested. The objectives of this study were to describe and analyze the cytology and microbiology of the post partum uterus in healthy and diseased cows. To this end, 27 cows were clinically examined 5-10 days after calving and uterine condition was determined by vaginal and rectal examination. Endometrial samples were taken from all cows using double guarded swabs, which were processed for both cytology and microbial metagenomic analyses. Cytospin slides were stained using DiffQuick and Gram stains and blindly analyzed using light microscopy for the presence and morphology of cells and bacteria. Significantly more normal and apoptotic neutrophils, and large aggregates of mainly gram negative bacteria were observed in samples from metritis cows. Albeit, phagocytosis of bacteria by neutrophils was significantly higher in healthy cows. These observations correlated well with the results of 16S rDNA deep sequencing analysis where microbial communities in metritis cows was dominated by members of the phyla bacteriodetes (77%) and fusobacteria (11.2%), while in healthy cows microbial communities were more diverse and dominated by firmicutes (30%), proteobacteria (34%), bacteriodetes (15%) and fusobacteria (8%). In this study, by specifically sampling the endometrium, we demonstrate major differences in microbial communities and inflammatory response of clinically diagnosed healthy and metritis cows.

Subclinical endometritis in dairy cattle – a short review

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Subclinical endometritis is defined as an elevated proportion of polymorphonuclear neutrophils (PMN) in the endometrium of clinically healthy cows, i.e. cows with no vaginal discharge. This definition has been suggested by Kasimanickam et al. (2004) and is generally accepted since 2006. Endometrial samples can be obtained with a small brush (cytobrush-technique) or by low-volume uterine flushing. A threshold value for the proportion of PMN is still under discussion. Thresholds described in the literature range from 5 to 18% PMN and more. It has been discussed if the stage of the estrus cycle influences the proportion of PMN in the cytobrush-sample. However, recent studies demonstrated that PMN did not vary with the stage of the estrous cycle. The reported prevalence of subclinical endometritis ranges ~~from~~ between 12 % and 50%, depending for example on the time postpartum. The impact of subclinical endometritis in fertility has been previously studied. Pro-inflammatory factors, e.g. interleukins, tumor necrosis factor, which have a negative effect on fertility and embryo quality, have been found elevated in endometrial tissue in cows with subclinical endometritis. Most of clinical trials found a negative effect on reproductive performance (e.g. days open, conception rate), whereas some studies did not confirm these results. In most studies cows were examined within the postpartum period, i.e. before the beginning of the breeding period. However, recent studies revealed that subclinical endometritis can also be present at the time of artificial insemination and decreases conception rates in these cows. Additionally, there is some evidence that the proportion of PMN in the endometrium affects the number and quality of embryos that can be obtained from superovulation and uterine flushing. Further research should confirm these results. Furthermore, little is known about the prevalence and impact of subclinical endometritis in repeat breeder cows. One study described that subclinical endometritis might contribute to this condition, whereas another study could not confirm these findings.

Epidemiology of endometritis and associations with reproductive performance and milk production in dairy cows in Israel

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Endometritis and metritis are terms used to describe abnormalities in the uterus of postpartum dairy cows. Case definition and time postpartum at which diagnosis is made have varied greatly between studies. Consequently, reports on the epidemiology and losses associated with these pathologies have been inconsistent. Within "Hachaklait" herd health program, all cows undergo a physical examination 5 to 12 d postpartum. Cows with endometritis (abnormal uterine discharge) are treated on a weekly basis until the condition has resolved. In 140 large farms, annual median incidence of endometritis was 46.4% and 41.3% in heifers and older cows, respectively. Nearly 30% of the variability in incidence of endometritis could be attributed to the veterinarian. The odds to heal from endometritis by 30 d postpartum were greater in cows without ketosis, twins or stillbirth (OR 1.4, 1.5 and 1.4, respectively) when compared with cows with these traits. Endometritis was associated with a 32% reduction in odds to conceive at first insemination in older cows, and 20% reduction in the hazard to conceive by 180 d in all cows. It was also associated with a 30% increase in hazard to leave the herd by 60 d postpartum. Estimated milk loss associated with endometritis was 105 kg and 201 kg in the first 180 d of lactation for heifers and older cows, respectively. Endometritis diagnosed 5 to 12 d postpartum is a common finding under Israeli circumstances and is associated with impaired reproductive performance and milk production and an increased hazard to be culled by 60 d in milk.

Disruption of ovarian function in mastitic cows and possible approaches to improving fertility in cows with uterine or mammary disease

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Inflammatory diseases such as endometritis and mastitis disrupt cow fertility. We examined mastitis effects on reproduction. (a) Naturally occurring subclinical mastitis induced delayed ovulation in 30% of cows exhibiting low and delayed preovulatory LH surge; the remaining 70% manifested normal interval to ovulation. A similar proportion (1/3) of subclinical mastitic cows exhibited low mRNA expression of steroidogenic genes in theca and granulosa cells reflected in low follicular steroids. (b) Induction of short-term mastitis by a single injection of Gram+ toxin or Gram- endotoxin caused immediate or carryover disruptive effects on follicular functions. In contrast, induction of long-term subclinical mastitis induced different responses: both toxins caused carryover decline of follicular steroid concentrations and immediate decrease in follicular growth. (c) Oocyte developmental competence of the ovarian pool of germinal-vesicle-stage oocytes was impaired in naturally occurring mastitic cows, as expressed in a decline in blastocyst-formation rate, but not cleavage rate. The magnitude of the decrease was associated with elevation of somatic cell count rather than bacterial type. Fertility studies aimed at improving conception in cows with inflammatory disease revealed that the 'Ovsynch' protocol improves conception rate of subclinical mastitic cows, but does not affect cows manifesting endometritis postpartum. In contrast, induction of two cycles by GnRH and $PGF_{2\alpha}$ before AI and adding progesterone (CIDR) after AI improved summer conception rate of endometritic cows but lowered that of mastitic cows. Improvement of fertility should be based on specific treatments for specific, designated subgroups of infected cows; this approach might lead to better breeding results.

Subclinical Endometritis in the Repeat-Breeding Cows

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The aims of this study were: a) to evaluate the incidence of subclinical endometritis (SE) means cytobrush cytology in repeat-breeding cows; b) to analyze mRNA expression of proinflamatory factors in repeat-breeding cows with and without SE, by RT-PCR. The analyzed proinflamatory factors were: tumor necrosis factor α (TNFα); inducible and epithelial nitric oxide synthases (iNOS and eNOS); cyclooxygenase-2 synthase (COX-2), prostaglandin $F_{2\alpha}$ and E_2 synthases (PGFS, PGES). 112 of 902 cows from 8 dairy herds were clinically selected as repeat-breeders. Using the cytobrush method, SE was diagnosed in 40,2% cows with the threshold of 10% PMNs and in 17,5% of cows when the threshold of 5% PMNs was applied. Biopsy samples of the endometrium were obtained from randomly chosen repeat-breeding cows with SE (n=10) and without SE (n=10). Statistically significantly higher ($p<0,05$) expression of TNFα, iNOS and COX-2 genes was detected in repeat-breeding cows with SE when compared to cows without SE. There was no significant difference in the level of eNOS, PGFS and PGES. Our study demonstrated a high prevalence of SE in repeat-breeding cows and suggests possible involvement of TNFα and iNOS and COX-2 pathways in this disorder. In contrast, the role of prostaglandins $F_{2\alpha}$ and E_2 has not been fully confirmed.

Impact of subclinical endometritis on fertility in cows, evaluated by three thresholds

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Clinical examination and cytobrush method were used in fourth and sixth week postpartum to select 222 cows with subclinical endometritis. To categorize the cows as positive (CE+) or negative (CE-) three cytological thresholds (per cent of polymorphonuclear leucocytes) were used: 1) >18% at Exam I and >10% at Exam II; 2) >8% at Exams I and II; 3) >5% at Exams I and II. Following, reproductive performance was calculated separately for all groups: first insemination pregnancy rate, number of services per conception, number of days with the cervix opened (days open), and total pregnancy rate on day 300 postpartum. Prevalence of subclinical endometritis ranged from 48.0% to 65.9% at Exam I and from 26.0% to 34.7% at Exam II, according to the threshold used. The first insemination pregnancy rate was statistically lower in all CE+ groups at Exam I (Number of inseminations per conception was higher ($P<0.05$) in each CE+ cows with exception of group CE+ created on the basis of third threshold at Exam II. The number of days open was significantly greater for cows with subclinical endometritis compared to control animals only for the first threshold at Exam I ($P < 0.01$), or when the first ($P < 0.05$) or the second threshold ($P < 0.05$) were used at Exam II, although the number of days open was numerically higher in all cows categorized as CE+ in Exam I and Exam II. Interestingly, overall pregnancy rates were similar in cows with subclinical endometritis and control cows in contrast to the differences in the above described fertility performance. It can be stated that the threshold > 18% polymorphonuclear leucocytes seems to be the most reliable, when applied in the fourth week postpartum.

Synthesis of prostaglandins and leukotrienes and their effect on the contractility of the inflamed porcine uterus

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Uterine inflammation is the most frequent reproductive disorder in livestock with consequences ranging from no effect on reproductive performance to permanent sterility. Endometritis occurs mainly after parturition as a consequence of impaired uterine involution and/or immunological response. Studies conducted on cows, pigs and laboratory animals have shown endometritis to lead to considerable disturbances in the hypothamic-pituitary-ovarian axis function. Changes in the secretory activity of an inflamed uterus and their importance to the function of the pathologically-changed organ are not fully recognized yet. Using pigs as an animal model, we investigated the synthesis of prostaglandins (PGs) and leukotriens (LTs) and their role in the contractility of the inflamed uterus. The levels of $PGF_2\alpha$ and PGI_2 metabolites, as well as LTB_4 and LTC_4 were increased in the peripheral blood of gilts after intrauterine inoculation of *Escherichia coli.* An enhancement in the contents of $PGF_{2\alpha}$, PGE_2 and PGI_2 in uterus with an acute inflammation was revealed and found to depend on the intensity of the inflammatory process, its duration and the type of uterine tissue affected. Elevated amounts of both PGE_2 and $PGF_2\alpha$ in the inflamed porcine uterus coincided with an increase in the expression of cyclooxegenase-2, microsomal PGE synthase-1 and 9-ketoreductase (enzyme which converts PGE_2 to $PGF_{2\alpha}$). Similarly, an increase in PGI_2 content was associated with an elevation in PGI synthase and PGI receptor expression. It was revealed that ovarian function was relative to the degree of endometritis/$PGF_{2\alpha}$ and PGE_2 production. The amounts of LTB_4 and LTC_4 as well as 5-lipooxygenase, LTA_4 hydrolase and LTC_4 synthase mRNA and/protein expression all increased in the inflamed uterine tissues. Our results show that in the endometrial samples from the inflamed uteri, lipopolysaccharide, and pro- and anti-inflammatory cytokines up-regulated the expression of enzymes involved in LTB_4 and LTC_4 synthesis, and the secretion of these LTs. On the other hand, LTB_4 and LTD_4 decreased the release of $PGF_2\alpha$ and PGE_2 from the endometrium with inflammation, while LTC_4, LTD_4 and LTB_4 enhanced nitric oxide production. Moreover, our research determined that the levels of cysteinyl LT type 1 (CysLT(1)R) and type 2 (CysLT(2)R) receptors, as well as LTB_4 type 1 (LTB4R1) receptor mRNA and/or protein expression were markedly changed in the inflamed uterus. It was also found that PGE_2, acting through EP_2 and EP_4 receptors, reduced the contractility of the analyzed inflamed organ. In turn, $PGF_{2\alpha}$ enhanced the contractile activity of the myometrium, not only through its own specific receptors but also by PGE_2 receptors. The intensity and/or frequency of contractions of the pathologically-changed uterus were also increased by PGI_2 as well as in response to LTC_4 and LTD_4. The obtained data indicate that PGs and LTs produced locally in an inflamed uterus can be important to the course of the inflammatory response and functions of the pathologically-changed organ. However, further studies aimed at determining the mechanisms underlying the changes in PG and LT synthesis in inflamed uteri and the influence of these factors on the inflammatory process and affected organ function are necessary.

Robustness and plasticity of arterial blood supply to the uterus and placentas in the multi-fetal mouse pregnancy

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The mouse is the most common animal model used to study development, genetics, as well as pregnancy-related diseases. Previous studies suggested that in mice, maternal blood flow to the uetrus and placentas occurs via a single arterial uterine loop generated by arterial-arterial anastomosis of the uterine artery to the uterine branch of the ovarian artery, resulting in counter bi-directional blood flow. However, using *in vivo* MRI and fluorescence imaging, we provide here experimental evidence that each placenta is actually supplied by two distinct arterial inputs stemming from the uterine artery and from the uterine branch of the ovarian artery, with position-dependent contribution of flow from each source. Moreover, we report significant positional- and inter-fetal dependent alterations of placental perfusion. Maternal blood flow to the placentas was dependent on litter size and was attenuated for placentas located centrally along the uterine horn. Distinctive apposing, inter-fetal hemodynamic effects of either reduced or elevated maternal blood flow, were measured for placenta of normal fetuses that are positioned adjacent to either pathological, or to hypovascular *Akt1*-deficient placentas, respectively. Our results underscore the critical importance of confounding *in utero* effects on phenotype presentation, in general and in the setting of genetically modified mice. The unique robustness and plasticity of the uterine vasculature architecture, as reported herin, can explain the ability to accommodate varying litter sizes, sustain large-litter pregnancies and overcome pathologic challenges. Remarkably, the dual arterial supply is evolutionary conserved in mammals bearing a single offspring (This research study was published in PLoS One 2012;7:e52273)

Imaging of the placentas in the multifetal mouse pregnancy

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The mouse is the most common animal model used to study development, genetics, as well as pregnancy-related diseases. Therefore, researchers are constantly seeking for enhanced imaging methods for the mouse placenta and fetus. Ultrasound scanning is a noninvasive method used clinically and in research for longitudinal evaluation of dynamic relationships among the mother, placenta, and fetuses, as it does not appear to alter the progression of pregnancy. However, only recently, high-quality microultrasound technology suitable for use in pregnant mice has become widely available. Fluorescence microscopy provides additional possibilities for imaging of the placenta structure in mouse models. Several ex-vivo methods have been described; however, recent intravital imaging techniques offer an opportunity to further understand the anatomy and function of maternal circulation in the mouse multi-fetal pregnancy. Magnetic resonance imaging (MRI) techniques are well suited to acquire imaging data related to placental anatomy, vascular physiology and function. Several MRI methods nowadays are available, such as T1- and T2-weighted imaging, contrast-enhanced MRI (e.g. GdDTPA or biotin-BSA-GdDTPA), Blood Oxygen Level Dependent (BOLD) methodology, and more recently, Bi-Directional Arterial Spin Labeling (BD-ASL) MRI techniques. Contrast-enhanced micro-CT is also a valuable research tool for in-vivo and ex-vivo imaging of embryo implantation and placenta development in the mouse. However, exposure to radiation during pregnancy might be a concern. Other techniques utilizing bioluminescence reporter genes for placenta imaging (e.g. Luciferin - Luciferase), as well as phothodynamic imaging have also been reported, but are not fully established for mouse placenta imaging. Over the last years, there has been significant progress in non-invasive imaging of mouse pregnancy, and studies utilizing experimental methods for imaging have revealed compelling facts about the biological structure and function of the placenta.

Pathogenesis of canine pyometra: *Escherichia coli* determinants of virulence and uterine innate immune response

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Response to uterine infection includes innate and acquired immune defense mechanisms. The innate mechanisms rely on germ-line-encoded pattern recognition receptors (Toll-like receptor; TLR) that recognize and interact with conserved pathogen-associated molecular patterns (PAMP) synthesized by microorganisms and, thereby, initiate a cascade of signaling events that include an early inflammatory response (Horne *et al*., 2008). Toll like receptors 1-7 and 9 genes are transcribed in the endometrium of bitches throughout the oestrous cycle, which indicates that TLR-mediated immune surveillance is an important component of the defense mechanisms within the uterus (Silva *et al*., 2012). Differential endometrial TLR transcription and expression occurred during the oestrous cycle, suggesting a regulatory role of ovarian steroids. In healthy canine endometrium, mRNA and protein expression of *TLR2* and *TLR4* were up-regulated at the late diestrus and anestrus and was the lowest in early diestrus. The decreased mRNA and protein expression observed at the early diestrus might be favourable to implantation but might also be linked to the high prevalence of pyometra at this stage of the oestrous cycle. *Escherichia coli (E. coli)* is the most common bacterium isolated in canine pyometra cases and its presence is normally associated with highly severe systemic signs and a potentially life-threatening situation. *E. coli* isolated from pyometra belongs to the highly virulent phylogenetic group B2 and exhibited a high number of virulence factor genes and pathogenicity island markers. These genes probably enhance the virulence and pathogenicity of the strain in the canine genital tract by facilitating colonization of the endometrium, enhancing tissue damage and increasing the amount of free iron available for bacterial growth (Mateus *et al*., 2013). *E. coli* pyometra is associated with an up-regulation of mRNA and protein expression of *TLR2* and *TLR4* genes in the endometrium. This up-regulation leads to the endometrial up-regulation of PG synthesis genes (*COX-2*, *PGES* and *PGFS*). This, in turn, results in a higher endometrial concentration of PGE_2 and $PGF_{2\alpha}$, which may further regulate the local inflammatory response (Silva *et al*., 2010). Uterine production of inflammatory mediators, in response to TLRs activation, is mediated by both MyD88 dependent and independent signaling pathways as several components of these pathways are transcribed in canine endometrium during healthy diestrus and pyometra. As a result, the transcription of several inflammation-related genes (*IL-1β, IL-6, IL-8, IL-10, CXCR1*) are up-regulated in the canine pyometra endometrium.

Horne *et al.* Reproduction (2008) 135, 739–749. Silva *et al.* J Reprod Immunol (2010) 84, 66–74. Silva *et al*. J Reprod Immunol 96 (2012) 45– 57. Mateus *et al.* Vet Mic (2013) doi:10.1016/j.vetmic.2013.07.018.

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