INTERNATIONAL CONFERENCE ON BIOLOGY AND PATHOLOGY OF REPRODUCTION IN DOMESTIC ANIMALS

September 28th-30th, 2015

Gdańsk, Poland

PROGRAM & ABSTRACT BOOK

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Committee for Biology of Reproduction, Division II: Biological and Agricultural Sciences, Polish Academy of Sciences

Japan Society for the Promotion of Science

Monday, September 28th

Satelite meeting I: CUTTING-EDGE REPRODUCTIVE PHYSIOLOGY - A PATH TO PREGNANCY

Plenary session I:

Chairs: Dariusz J. SKARŻYŃSKI and Kiyoshi OKUDA

9^{00}-9^{15} **Opening Remark & Review**: Kiyoshi OKUDA *(Lab. of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, Japan)*

9^{15}-10^{00} **Plenary lecture I: Kei-Ichiro MAEDA: Brain mechanism controlling mammalian reproduction: An overview** *(Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan)*

10^{00}-10^{45} **Plenary lecture II - Adam ZIĘCIK: Early pregnancy recognition - lessons learned from genes expression studies** *(Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland)*

10^{45}-11^{15} Coffee break

Chairs: Koji KIMURA and Izabela WOCŁAWEK-POTOCKA

- **Gamete and embryo development**

11^{15}-11^{35} Ken SAWAI, *Iwate, Japan*: Molecular mechanisms involved in segregation of inner cell mass and trophectoderm lineages in bovine and porcine embryos

11^{35}-11^{55} Zofia MADEJA, *Poznań, Poland*: The effect of Wnt/β-catenin signalling on bovine preimplantation development – prospects for bovine ESC derivation

11^{55}-12^{10} Paweł KORDOWITZKI, *Neustadt am Ruebenberge, Germany*: Effects of resveratrol supplementation during *in vitro* maturation and *in vitro* fertilization on developmental competence of bovine oocytes

12^{10}-12^{25} Nobuyuki SAKURAI, *Iwate, Japan*: Effects of downregulating OCT-4 and CDX2 transcripts on early development and gene expression in bovine embryos

- **Gamete transportation and oviduct functions**

12^{25}-12^{45} Yuki YAMAMOTO, *Okayama, Japan*: How is oviductal motility controlled? Production mechanisms of local factors which regulate smooth muscle contraction and relaxation in cattle

12^{45}-13^{05} Anna DUSZEWSKA, *Warszawa, Poland*: Functional morphology of cattle oviduct: comparison of morphology of bovine epithelial cells (BOECs) at an elevated temperature

13^{05}-13^{20} Yoshihiko KOBAYASHI, *Okayama, Japan*: Region-specific roles of endothelins in the bovine oviduct: Regulation of nitric oxide synthesis and spontaneous waves of contraction and relaxation

13^{30}-14^{30} Lunch

Satelite meeting I: CUTTING-EDGE REPRODUCTIVE PHYSIOLOGY – A PATH TO PREGNANCY

- **Maintenance of pregnancy: role of corpus luteum and uterus**

Chairs: Adam ZIĘCIK and Naoko INOUE

14^{30}-14^{50} Ryosuke SAKUMOTO, *Tsukuba, Japan*: Changes in the gene expression profiles of bovine corpus luteum during early pregnancy

14^{50}-15^{05} Kaya WATANABE, *Obihiro, Japan*: Role of *Bmal1* clock gene on corpus luteum formation of pregnancy in mice ovary

15^{05}-15^{20} Kazuhisa HASHIBA, *Okayama, Japan*: An increase in the level of α2,6-sialic acid inhibits galectin-1 binding to glycan during luteolysis

- **Mechanisms of maternal recognition of pregnancy and implantation failure**

Chairs: Marta SIEMIENIUCH and Ken SAWAI

15^{20}-15^{40} Koji KIMURA, *Okayama, Japan*: Evaluation of an alternative embryo transfer strategy to mitigate early embryonic loss and differential gene expression in endometria of fertility and sub-fertile cattle

15^{40}-16^{00} Izabela WOCŁAWEK-POTOCKA,*Olsztyn, Poland*: The effect of lisophosphatidic acid (LPA) on the embryo-maternal cross-talk in cows

16^{00}-16^{20} Katarzyna BUSKA-PISAREK, *Wrocław, Poland*: Early embryo-maternal communication in natural pregnancy and after embryo-transfer in mice

16^{20}-16^{35} Ken-Go HAYASHI *Tsukuba, Japan*: Temporal expression of vascular endotherial growth factor family members in the bovine endometrium during peri-implantation period

16^{35}-17^{00} Coffee break

Satelite meeting II: EQUINE REPRODUCTION IN A PILL – LECTURES FOR PRACTICIONERS

(*session with Polish translation*)

14^{30}-15^{30} Jutta KLEWITZ-SIELHORST (*Dipl. ECAR, Tierklinik Domäne Karthaus, DE*) Placentitis - recent diagnostic and treatment tools for the high-risk pregnancy in the mare - pregnancy maintenance

15^{30}-16^{30} Jesper NIELSEN (*Ansager Large Animal Hospital, DN*) Breeding soundness evaluation of the stallion - Methods for evaluation of semen and pathology. Diagnosis, treatment and prognosis of endometritis

16^{30}-17^{00} Coffee break

17^{00}-17^{30} Monika SIKORA (*Veterinary Faculty, University of Life Sciences, Wroclaw, PL*) Diagnostics of changes in the endometrium of infertile Icelandic horse mares

17^{30}-18^{00} Roland KOZDROWSKI (*Veterinary Faculty, University of Life Sciences, Wroclaw, PL*) Cytological evaluation of the mare endometrium

18^{00}-18^{30} Andrzej RAŚ (*University of Warmia and Mazury, Faculty of Veterinary Medicine, Olsztyn, PL*) Postpartum complications in a mare and their possible impact on further fertility

18^{30}-19^{00} General discussion

- **Biology and pathology of placenta**

Chairs: Takashi SHIMIZU and Tomasz JANOWSKI

17^{00}-17^{20} Marta SIEMIENIUCH, *Olsztyn, Poland*: Ovarian and placental molecular mechanisms responsible for pregnancy maintenance and prepartal luteolysis in cats

17^{20}-17^{40} Anna RAPACZ-LEONARD, *Olsztyn, Poland*: Expression of equine Major Histocompatibility Complex class I during pregnancy, parturition and fetal membrane retention

17^{40}-17^{55} Joanna JAWORSKA, *Olsztyn, Poland*: Does equine fetus express only maternally inherited Major Histocompatibility Complex I (MHC I) in order to protect long lasting pregnancy?

20^{00}-22^{00} Welcome reception

Tuesday, September 29th

9^{00}-9^{15} Opening Remark & Review Dariusz J. SKARŻYŃSKI *(Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland)* & **Tomasz JANOWSKI** *(University of Warmia and Mazury, Faculty of Veterinary Medicine, Olsztyn, Poland)*

9^{15}-10^{00} Plenary lecture III - Martin SHELDON: **Mechanisms linking bacterial infections of the endometrium to disease and infertility** *(Institute of Life Science, Swansea University School of Medicine, Swansea, United Kingdom)*

ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS

- **Workshop I: Endometritis in cows**

Moderators:

10^{00}-10^{30} Stephen Le BLANC: "Diagnosis and management of reproductive tract: inflammatory disease in dairy cows" *(Department of Population Medicine, Ontario, Canada)*

10^{30}-11^{00} Claire WATHES "Impact of negative energy balance and disease as risk factors for endometritis" *(Department of Production and Population Health, Royal Veterinary College, North Mymms, Hatfield, United Kingdom United Kingdom)*

Satelite meeting I: CUTTING-EDGE REPRODUCTIVE PHYSIOLOGY – A PATH TO PREGNANCY

- **Central regulations of reproductive functions**

Chairs: Kei-Ichiro MAEDA and Anna DUSZEWSKA

10^{10}-10^{30} Naoko INOUE, *Nagoya, Japan*: Brain mechanism underlying ovulation in mammals

10^{30}-10^{45} Yuta SUETOMI, *Nagoya, Japan*: Molecular Cloning and Identification of the Transcriptional Regulatory Domain of the Goat Neurokinin B Gene *TAC3*

10^{45}-11^{00} Satoshi OHKURA, *Nagoya, Japan*: Electrophysiological Technique for Monitoring the Hypothalamic Mechanism

11^{00}-11^{30} Coffee break/**Poster session**

Oral presentations for about 15 min including discussion

11^{30}-11^{45} Karen WAGENER, *Vienna, Austria*: Diversity and health status specific fluctuations of intrauterine microbial communities in postpartum dairy cows

11^{45}-12^{00} Raul Miranda CASO-LUENGO, *Dublin, Ireland*: Temporal and spatial analysis of the microbial communities in the reproductive tract of the endometritic cows

12^{00}-12^{15} Mohammed IBRAHIM, *Berlin, Germany*: Bovine endometrial pro-inflammatory response differs depending on the strain of *Trueperella pyogenes*

12^{15}-12^{30} Evgeniya SHILOVA, *Ekaterinburg, Russia*: Manifestation of infectious diseases of the reproductive organs in cows on the Urals region dairy farms

12^{30}-12^{45} Joseph LIM, *Meath, Ireland*: Analysis of systemic changes in cows with subclinical endometritis

12^{45}-13^{00} Agnieszka BARYCZKA, *Olsztyn, Poland*: A preliminary study on the prevalence of subclinical endometritis in cows with clinical endometritis after treatment with cephapirin, prostaglandin F2α or self-cured

Regulating Pulsatile GnRH Release in Goats

11^{00}-11^{30} Coffee break/**Poster session**

11^{30}-11^{45} Sho NAKAMURA, *Tokyo, Japan*: Neonatal kisspeptin is required for defeminization of the brain mechanism controlling sexual behaviors in male rats

11^{45}-12^{00} Youki WATANABE, *Nagoya, Japan*: Involvement of preoptic kisspeptin neurons in estrogen positive feedback to induce luteinizing hormone surge in both female and male Japanese monkey

12^{00}-12^{15} Kana IKEGAMI, *Nagoya, Japan*: Involvement of cell-to-cell communication via gap junctions in NKB-NK3R signaling-induced synchronous discharges of KNDy neurons

- **Ovarian functions (Follicular growth, Follicular functions, Ovulation mechanisms, Luteolysis)**

Chairs: Ryosuke SAKUMOTO and Dariusz SKARŻYŃSKI

12^{15}-12^{35} Takashi SHIMIZU, *Obihiro, Japan*: Effect of endotoxin on ovarian follicle function in domestic animals

12^{35}-12^{55} Anna ZIELAK-STECIWKO, *Wrocław, Poland*: Genomic portrait of ovarian follicle growth regulation in cattle

12^{55}-13^{15} Maciej MURAWSKI, *Kraków, Poland*: Ovarian and endocrine function after hormonal induction of ovulation in seasonally anovular goats

13^{15}-13^{30} Antonio GALVAO, *Olsztyn, Poland*: Nodal promotes vascular regression via Thrombospondin-1 pathway during luteolysis in the mare

13^{30}-13^{35} Closing remark & Review: Dariusz J. SKARŻYŃSKI *(Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland)*

13^{00}-14^{30} Lunch

- **Workshop I: Endometritis in cows**

14^{30}-14^{45} Osvaldo Bogado PASCOTTINI, *Ghent, Belgium*: Prevalence of subclinical endometritis at artificial insemination and its effect on subsequent conception rate in dairy cows: some preliminary results

14^{45}-15^{00} Lara GORRITZ-MARTIN, *Hannover, Germany*: Relationships between systemic levels of progesterone, 17ß-estradiol, and $PGF_{2\alpha}$ on the immunolocalization and expression of the oxytocin, progesterone, estrogen α, and prostaglandin F receptors in postpartum bovine uteri challenged with LPS

15^{00}-15^{15} Ruth CLAMP, *Aberystwyth, United Kingdom*: Effect of preimplantation factor in response to a LPS challenge on bovine endometrial IL-6 secretion and expression

15^{15}-15^{30} Erin WILLIAMS, *Edinburgh, United Kingdom*: Is *E. coli* off the hook? New perspectives on microbial causes of uterine infection in the postpartum cow

15^{30}-15^{45} General discussion

15^{45}-16^{15} Coffee break/**Poster session**

- **Workshop II: Endometritis in mares**

Moderators:

16^{15}-16^{45} Mats H.T. TROEDSSON: **Our current understanding of the pathophysiology of equine endometritis** *(Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, United States)*

16^{45}-17^{15} Terttu KATILA Evidence-based treatment of equine endometritis *(Department of Clinical Production Animal Medicine, University of Helsinki, Finland)*

Oral presentations for about 15 min including discussion

17^{15}-17^{30} Vladimir HURA, *Kosice, Slovakia*: Monitoring of the incidence of fluid in the uterus after insemination and its impact on fertility in mares

17^{30}-17^{45} Mushtaq AHMAD, *Lahore, Pakistan*: Incidence of endometritis and pregnancy rate is affected due to age and prolonged estrus in ovulation induced thorough bred brood mares: A clinical study

17^{45}-18^{00} John NEWCOMB, *Walsall, United Kingdom*: Treatment in post-insemination endometritis in practice

18^{00}-18^{15} Mette CHRISTOFFERSEN, *Copenhagen, Denmark*: Infectious endometritis is associated with endometrial expression of lactoferrin in broodmares

18^{15}-18^{30} Anna RAPACZ-LEONARD, *Olsztyn, Poland*: Prostaglandins production in heavy draft mares that retain fetal membranes and those that deliver fetal membranes physiologically

18^{30}-18^{45} General discussion

20^{30}-23^{00} Gala Dinner

Wednesday, September 30th

ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS

- **Workshop II: Endometritis/Endometrosis in mares**

9^{00}-9^{15} Graça Maria FEREIRRA-DIAS, *Lisbon, Portugal*: How challenging is chronic endometritis in the mare

9^{15}-9^{30} Maria Rosa REBORDÃO, *Lisbon, Portugal*: Mare endometrosis and pro-fibrotic cytokines: what's new?

9^{30}-9^{45} Anna SZÓSTEK , *Olsztyn, Poland/Okayama, Japan* : MMP-2 and MMP-9 in equine endometrosis

9^{45}-10^{00} General discussion

10^{00}-10^{30} Coffee break/**Poster session**

- **Workshop III: Endometritis in dogs and cats**

Moderators:

10^{30}-11^{00} **Francis FIENI: How to treat uterine infections in the bitch medical or surgical therapy** *(LUNAM University, Oniris, Nantes-Atlantic National College of Veterinary Medicine, Nantes, France)*

11^{00}-11^{30} **Alain FONTBONNE: Sub-clinical endometritis as a cause of infertility or embryonic resorption in the bitch** *(Ecole Nationale Veterinaire d`Alfort, Paris, France)*

Oral presentations for about 15-20 min including discussion

11^{30}-11^{50} Rita PAYAN-CARREIRA, *Vila Real, Portugal*: Endometrial factors favoring pyometra onset in diestrus

11^{50}-12^{10} Kamil KACPRZAK, *Warsaw, Poland*: Changes in ovaries and uterus after aglepristone administration in the third week of luteal phase of non-pregnant bitches

12^{10}-12^{25} Dorota BUKOWSKA, *Poznań, Poland*: Microarray assay as a fingerprint in endometritis – pyometra in bitches

12^{25}-12^{40} Roman DĄBROWSKI, *Lublin, Poland*: Tissue Kynurenic Acid in Bitches with Pyometra

12^{40}-12^{55} Marta SIEMIENIUCH, *Olsztyn, Poland*: How members of innate immunological response in the feline endometrium may differ dependently on the stage of the estrous cycle, pyometra or medroxyprogesterone acetate treatment

12^{55}-13^{10} General discussion

13^{10}-13^{15} Closing Ceremony

13^{00}-14^{30} Lunch

Brain mechanism controlling mammalian reproduction: An overview

K.I. Maeda and H. Tsukamura

Laboratory of Theriogenology, The University of Tokyo and Laboratory of Reproductive Science, Nagoya University

Date back a number of decades, Geoffrey Harris, a British anatomist, predicted the presence of hypothalamic hormones controlling anterior pituitary hormone release. Based on his prediction, two research groups led by Guillemin and Schally contended for the first place to identify hypothalamic hormones including gonadotropin-releasing hormone (GnRH). Their findings of hypothalamic peptides has promoted a better understanding of the endocrine mechanism controlling reproduction. On the other hand, the discovery of GnRH threw up a new mystery on us: what controls GnRH releae in the brain?

It is well accepted that the surge- and pulse-modes GnRH releases regulate the gonadal activity through controlling gonadotropin release. The surge is generated by a positive feedback action of estrogen and progesterone released from mature follicles. Pulses are fine-tuned by a negative feedback action of ovarian sex steroids. Brain mechanisms underlying the GnRH surge and pulse and the steroidal feedback action have been largely unknown for many years. In 2001, a neuropeptide called kisspeptin was found as a ligand for an orphan G-protein-coupled receptor, GPR54 from the human placenta. The discovery of the peptide now provides a clue to neuroendocrinologists to unlock the mystery of the mechanism regulating GnRH release.

Kisspeptin neurons have two distinct populations in the brain in most mammalian species examined so far. In rodents, one is located in the anterovetral periventricular nucleus (AVPV) or preoptic area (POA), which has been considered to be a center regulating GnRH surges. The other is located in the hypothalamic arcuate nucleous (ARC), which might be an essential part of the GnRH pulse generator. The ARC population of kisspeptin neurons is of special interest because they contain two other peptides, such as neurokinin B and dynorphin, and also referred to as 'KNDy neuron'.

We might be close to unveiling the brain mechanism to develop a new concept on the reproduction. The concept would be a key to develop a new approach to control reproduction or treat reproductive disorders.

Early pregnancy recognition- lessons learned from genes expression studies

A.J. Zięcik, M.M. Kaczmarek, E. Przygrodzka, A.A. Andronowska

Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland

Process of maternal recognition of pregnancy (MRP) begins from the appearance of embryo signals and ability of mother to receive and recognize these signals leading consequently to maintenance of corpus luteum (CL) function and embryo development.

In pigs, presence of semen and embryos in the reproductive tract can affect proluteal environment before MRP occurrence. According to a paradigm, estrogens produced by porcine conceptuses alter $PGF_{2\alpha}$ release from an endocrine to exocrine direction protecting CL against luteolysis. Lipids originating from conceptuses and endometrium are an integral part of MRP and affect $PGF_{2\alpha}/PGE_2$ ratio in favor of PGE_2. However, the most important events take place at post-$PGF_{2\alpha}$ and PGE_2 receptor signaling pathways and 'two-signal switch' hypothesis will be presented.

Analysis of mRNA expression of 70 genes involved in the regression or maintenance of porcine CL was performed. Among canonical pathways identified by Ingenuity Pathway Analysis, TNFR1 and apoptosis signaling pathways as well as the enhanced infiltration of immune cells into CL as early as on Day 12 of the estrous cycle were shown. Also altered expression of numerous chemokines produced by immune system components were found in the porcine CL on Days 12 and 14 of the estrous cycle.

Microarray studies concerning transcriptomic changes in the porcine endometrium during MRP revealed that among 589 accurately annotated genes the expression of 266 and 323 was up- or down-regulated, respectively. In contrast, the expression of only 110 and 179 differentially expressed genes (DEGs) was found on Days 12 or 16 of pregnancy. When RNA-Seq method was used 2500 and 1900 DEGs was noted on Days 12 and 14 of pregnancy. Significant changes in the profile of global genes expression in the endometrium at the time of MRP were found, while bioinformatic analysis identified several different functional networks.

Micro(mi)RNAs present in the uterus regulate the expression of putative target genes in the endometrial cells. For example, miR-125b affects diverse biological functions related to pregnancy outcome through the control of expression of *LIF*, *LIFR* and *IL-6R* genes involved in the acquisition of endometrial receptivity, immune system function or embryo development and implantation. Bioinformatic analysis suggested a number of miRNA-mediated specific processes and pathways important for endometrial remodeling, associated with embryo attachment, implantation and placentation.

Molecular mechanisms involved in segregation of inner cell mass and trophectoderm lineages in bovine and porcine embryos

K. Sawai

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Successful offspring production from *in vitro* produced embryos, such as *in vitro* fertilization (IVF) or somatic cell nuclear transfer, has accelerated progress in these areas. However, *in vitro* production (IVP) of bovine or porcine embryos is still inefficient compared with that of other mammals, such as mice. One of the reasons for the decreased development of the bovine or porcine IVP system is limited knowledge concerning the molecular mechanisms involved in early embryonic development. Therefore, to improve the IVP system for bovine and porcine embryos, it is important to focus on the molecular mechanisms underlying the regulation of early embryonic development.

In mouse embryos, differentiation of inner cell mass (ICM) and trophectoderm (TE) is regulated by various transcription factors, such as Oct-4 and Cdx2, but molecular mechanisms that regulate differentiation in bovine and porcine embryos remain unknown. To evaluate gene transcripts involved in segregation of ICM and TE lineages in bovine and porcine embryos, we examined the relative abundances of *OCT-4* and *CDX2* transcripts in these embryos. In bovine and porcine embryos, *OCT-4* transcript levels in ICM lineages were significantly higher than that in TE lineages. In contrast, the *CDX2* levels were lower in ICM lineages than that in TE lineages. These findings suggested that OCT-4 and CDX2 are involved in differentiation of ICM/TE linages in bovine and porcine embryos.

To clarify the necessity of OCT-4 for functional characterization in bovine and porcine embryos, we attempted OCT-4 downregulation of early embryos by RNA interference. Injection of specific siRNA resulted in a distinct decrease in *OCT-4* mRNA and protein expressions in bovine and porcine embryos. Although bovine and porcine embryos injected with OCT-4 siRNA were able to develop to the morula stage, these embryos failed to form blastocysts. When OCT-4 siRNA was injected to one blastomere of 2-cell stage embryos, blastomeres derived from OCT-4 siRNA injection were difficult to contribute TE linage. In bovine embryos injected with OCT-4 siRNA, *CDX2* and *FGF4* expression levels were significantly decreased.

In conclusion, our results indicated that OCT-4 is essential factor for differentiation of both ICM and TE linages in bovine and porcine embryos.

The effect of Wnt/β-catenin signalling on bovine preimplantation development – prospects for bovine ESC derivation

Z.E. Madeja

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With growing prospects of the use of stem cell research in medicine and animal biotechnology, cell lineage formation in species of economic significance, such as cattle is of increasing interest. The main aim was to investigate the effect of WNT signalling (by GSK3 inhibition) during the preimplantation period of bovine development, and to verify the subsequent potential of primary inner cell mass (ICM) outgrowths to support bovine ESC (bESC) maintenance in culture. The WNT pathway is associated with stem cell control in vertebrates and its role in pluripotency maintenance has been proven for several mammalian species. The precise effect of the WNT activity during the development of cow embryo has not been studied in detail. The overall conclusion is that the WNT pathway is present and active in bovine preimplantation embryos, and that it influences development, but the actual correlation between the WNT activity and pluripotency potential of the ICM and bESC has not been looked at. This is a novelty arising from our work. Therefore, before testing ESC culture conditions that have proven successful for the model species and switching to the combination of the inhibitors (such as the 2i/3i systems) we believe that it is important to understand the roles of the crucial signalling pathways independently of one another. This is important in the context of literature showing that depending on the species, the action of the GSK3i may be enhanced by different factors (LIF for mouse and rat and FGF for human).

Thus, we have investigated the effect of WNT activation on pluripotency marker gene expression in the ICM and the trophectodern (TE) and to study the derivation potential of primary bESC lines from blastocysts obtained in the presence of the GSK3i. WNT activity exerted a positive effect on pluripotency gene expression in developing bovine embryos, manifested by up-regulation of OCT4, NANOG, REX1, SOX2, c-MYC and KLF4 in the ICM and down-regulation of CDX2 in the TE. The results of bESC derivation experiments allowed us to speculate that the derived cell lines may share features of both naïve and primed ESCs. Similarly to mouse epiblast stem cells and human ESC the derived lines grew as flat, mono-layer colonies intolerant to passaging as single cells. JAK/STAT signalling was indispensable for proper colony formation and proliferation, yet LIF alone was inefficient to support self-renewal. Concomitant with the naïve state of mouse ESC, WNT activity supported by LIF had beneficial effects on cell culture propagation, survival after passage, morphology and pluripotency related marker gene expression.

Founding: NCN-4429/B/P01/2010/39 and FNP- HOM/2009/B

Effects of resveratrol supplementation during *in vitro* maturation and *in vitro* fertilization on developmental competence of bovine oocytes

P. Kordowitzki, S.M. Bernal, D. Herrmann, P. Aldag, A. Lucas-Hahn, H. Niemann

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Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin - isolated from various plant species, particularly grapevine peel. Recently, resveratrol gained scientific interest because of its strong antioxidant effects it may have health benefits, including protection against cardiovascular diseases. In addition, it has been shown to increase lifespan in several species and activates the SIRT1 gene. The aim of this study was to investigate its effects in bovine early embryo development. We employed three different resveratrol concentrations during *in vitro* maturation (IVM) and *in vitro* fertilization (IVF). Bovine oocytes (n=1648) were collected from slaughterhouse ovaries and subjected to IVM medium supplemented with 0.2µM, 1µM, and 20µM Resveratrol® (Sigma-Aldrich, Buchs, Switzerland) for 24 h followed by IVF with the same concentrations of resveratrol for 19 h. IVM and IVF medium without resveratrol (control) and DMSO supplementation as vehicle control were included in this experiment. Presumptive zygotes were cultured *in vitro* until day 8 to assess embryo development. Maturation rates, cleavage and blastocyst formation were determined. Maturation rates did not differ significantly (0.2µM: 64.2±7%; 1µM: 82.3±4%; 20µM: 68.8±2%; control: 74.6±5% and vehicle control: 70.2±6%, respectively, $p \leq 0.05$). Oocytes cultured in 1µM resveratrol supplemented maturation medium showed distinct detachment of cumulus cells. Cleavage was reduced in the 0.2µM and 20µM group (0.2µM: 44.21±2%; 1µM: 58.4±3%; 20µM: 40.9±5%; control: 56.6±2% and vehicle control: 55.2±6%, respectively, $p \leq 0.05$). Blastocyst development was impaired in the low and high resveratrol concentration group compared to the other groups (0.2µM:11.3±1%;1µM: 28.4±6%; 20µM: 8.2±4%; control: 22.7 ±4% and vehicle control: 20.8 ±2%, respectively, $p \leq 0.05$). These preliminary results indicate that very low and high concentrations of resveratrol impair the development to the blastocyst stage. In conclusion, a 1µM resveratrol supplementation during IVM and IVF seems to improve the developmental competence of oocytes, which is reflected not only in elevated blastocyst rates but also in the higher degree of expansion of cumulus cells after IVM and the maturation rates.

Effects of downregulating OCT-4 and CDX2 transcripts on early development and gene expression in bovine embryos

N. Sakurai[1], K. Takahashi[2], N. Emura[2], T. Hashizume[1, 2], K. Sawai[1, 2]

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[2] *Faculty of Agriculture, Iwate University, Japan*

In murine embryos, functions of Oct-4 and Cdx2 in differentiation of the inner cell mass (ICM) and the trophectoderm (TE) have been revealed in detail. Oct-4 and Cdx2 repress the expression of each other and are exclusively expressed in the ICM and the TE, respectively. Oct-4 regulates expression of genes involved in pluripotency or segrigation of the ICM such as Nanog and Fgf4. Cdx2 is required for correct differentiation of TE through repressing Oct-4 and Nanog and inducing TE-specific genes. However, little is known about roles of OCT-4 and CDX2 in bovine preimplantation embryos. To elucidate roles of these factors during the early development of bovine embryos, we attempted downregulation of OCT-4 and CDX2 using RNA interference. OCT-4 or CDX2 specific siRNA was injected into cytoplasm of zygotes obtained from *in vitro* fertilization. Developmental competencies until day 7 or 8 were evaluated. Gene expressions involved in the segregation and function of ICM or TE were evaluated at the morula stage on day 5. The blastocyst developmental rate of OCT-4 siRNA-injected embryos (16.3%) was lower ($P<0.01$) than that of siRNA-uninjected (40.6%) or control siRNA-injected embryos (36.1%). On the other hand, the blastocyst developmental rate of CDX2 siRNA-injected embryos (13.1%) was lower ($P<0.05$) than that of siRNA-uninjected (36.6%) or control siRNA-injected embryos (34.4%) on day 6. On day 8, CDX2 downregulated embryos developed to blastocysts, and there was no significant difference among the experimental groups (29.1 - 37.7%). However, the expanded blastocyst rate in CDX2 downregulated embryos (20.0%) was lower ($P<0.05$) than that in control siRNA-injected embryos (33.9%). OCT-4 downregulation resulted in decreases in *CDX2* and *FGF4*. Furthermore, an increase in *NANOG* and a decrease in *GATA3* were induced in CDX2 downregulated embryos. We conclude that OCT-4 and CDX2 are important factors for the early development and regulate the ICM/TE differentiation through the control of the gene expressions in bovine embryos.

How is oviductal motility controlled? Production mechanisms of local factors which regulate smooth muscle contraction and relaxation in cattle

Y. Yamamoto[1], Y. Kobayashi[1], Y. Yoshimoto[1], M. Kohka[1], I. Woclawek-Potocka[2], K. Kimura[1], K. Okuda[1]

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Oviductal motility is required for transport of oocyte and embryo resulting in successful fertilization and implantation in mammals. An ovulated oocyte is transported to the ampulla followed by fertilization within a day after ovulation. Then, the fertilized egg passes through the isthmus and reaches the uterus at 3-4 days after ovulation. The oviduct consists of epithelial, stromal and smooth muscle layers. The smooth muscle layer contains longitudinal and circular muscles, which work in a coordinated manner to transport the oocyte and the embryo. Oviductal motility is systemically and locally regulated by various factors. Previous studies have reported that oviduct cells produce local contractile factors including prostaglandin F2 alpha and endothelins, and relaxing factors including prostaglandin E2 and nitric oxide (NO). The objective of our research is to clarify the regulatory system of oviductal motility including the production mechanisms of these factors in cattle.

First, the expressions of regulating factors of oviductal motility were examined throughout the estrous cycle in the bovine oviduct. Some of them showed cyclical changes, which suggested that they were controlled by some other factors. We then developed isolation and culture systems of bovine oviduct epithelial and stromal cells to examine their functions in both ampullary and isthmic sections. The effects of ovarian steroids or oviductal local factors on the expressions of prostaglandins, endothelins and NO synthases were investigated using these systems. Several factors such as estradiol-17beta, progesterone and lysophosphatidic acid affected the expressions of regulating factors of smooth muscle motility. In addition, we found that these actions differed between the ampulla and isthmus in same types of cultured cell.

Our studies suggest that regulatory factors of smooth muscle contraction/ relaxation are produced during the optimal period and at proper location to transport the oocyte and early embryo in the bovine oviduct. Although the precise control of oviductal motility is essential for successful pregnancy, methods for diagnosing and treating of its functional abnormality have not been established yet not only in cows but also in other animals. Our studies should contribute to improving the fertility rates in mammals.

Functional morphology of cattle oviduct: comparison of morphology of bovine epithelial cells (BOECs) at an elevated temperature

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The oviduct is responsible for catching oocytes; migration of oocytes, sperm and embryos; fertilization and early embryo development. The system of *in vitro* culture of bovine oviduct epithelial cells (BOECs) with embryos was used to determine the mother-embryo interaction at an elevated temperature. The objective of this study was to evaluate the ultrastructure of BOECs cocultured with cattle embryos at the elevated temperature of 41°C.

Bovine oviducts and ovaries were collected *post mortem* from slaughtered cattle. Oviducts were obtained from cattle between 0 and 4th days of estrous cycle. Oviduct epithelial cells were cultured for 48 hours to form aggregates of BOECs consisting of secretory and ciliated cells. Then, BOECs were cocultured with cattle embryos at control (38.5°C) and experimental (41°C) temperatures for 168 hours. After coculture, samples of BOECs from both groups were taken for analyses: 1) viability (Trypan blue test), 2) cilia movement (subjective evaluation) and 3) ultrastructure (SEM, TEM methods) of: a) secretory cells (length of microvilli, number of granules) and b) ciliated cells (length of cilia, number of basal bodies). Additionally, the embryo development was analysed. Statistical analyses were performed by Statgraphics 5.0 Centurion using T-test for calculation the significance differences between the temperatures. Difference at $P<0.001$ was considered significant.

At control temperature (38.5°C), the cattle embryos cocultured with BOECs developed until the blastocyst stage in contrast with embryos at elevated temperature, in which development arrested until 8 cell stage and the difference was significant ($P<0.001$).

The percentage of viable cells was similar in the BOECs culture at control and elevated temperatures. Analysis of the cilia movement of ciliated cells indicated no difference between control and experimental temperatures. Also, there were no significant differences between the control and experimental group in the relative number of secretory granules and number of basal bodies in the secretory cells. There were no significant differences between groups in the length of cilia and number of microvilli.

The elevated temperature had no effect on the morphology of BOECs cocultured with cattle embryos.

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Region-specific roles of endothelins in the bovine oviduct: Regulation of nitric oxide synthesis and spontaneous waves of contraction and relaxation

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Endothelins (ETs) locally produced in the oviduct are not only molecules which contract oviductal smooth muscle, but also have been implied to be involved in development of embryos in mammalian oviducts. Previous *in vitro* studies showed that nitric oxide (NO) was required for survival of oocytes and embryos, suggesting that NO is a key molecule around fertilization in the oviduct. Since ET-1 stimulates NO synthesis in murine endothelium, this peptide is possible to regulate NO synthesis in other tissues including the oviduct. In the present study, we hypothesized that ETs play roles in two distinct aspects, regulation of NO synthesis and smooth muscle contraction in the ampulla and isthmus of the oviduct. To test above hypothesis, we investigated effects of ETs on NO synthesis in cultured oviductal epithelial cells isolated from bovine oviducts, and on spontaneous contraction of oviductal tissues by isometric contraction test. Immunohistochemical analysis was also performed to clarify distribution of ET receptors in oviductal tissues. Immunohistochemical investigation of ET receptors (ET-RA and ET-RB) revealed that the both proteins were expressed in epithelium of the oviduct. Only ET-RA was localized in oviductal smooth muscle. Expressions of iNOS mRNA and protein in cultured epithelial cells isolated from the ampulla was stimulated by ET-1 after 1 h incubation, while neither ET-2 nor ET-3 affected iNOS expression in the ampullary cells. In isthmic epithelial cells, none of ETs affected iNOS expression. Isometric contraction test showed that ET-1 and ET-2 but not ET-3 increased amplitude and tonus of the isthmic oviduct which have thick smooth muscle layer. Isometric contraction in the ampulla which is surrounded by thin smooth muscle was not observed with or without treatment of ETs. The overall findings suggest that ETs have region-specific roles in the oviduct. ET-1 stimulated synthesis of NO which is required for survival of oocytes and embryos in the ampulla where fertilization occurs, suggesting that ET-1 provides optimal micro-environment for the first days of pregnancy. By contrast, ET-1 and ET-2 suggest promoting spontaneous waves of contraction and relaxation of the oviductal smooth muscle in the isthmus, contributing successful transport of gametes and embryo.

Changes in the gene expression profiles of bovine corpus luteum during early pregnancy

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To evaluate functional changes of the corpus luteum (CL) during early pregnancy in cows, gene expression profiles of the CL at the time of maternal recognition were investigated. The CL tissues were collected from the cows on days 15 and 18 after artificial insemination, and a presence or absence of conceptus was checked macroscopically to determine whether the cows are pregnant or not. Microarray analysis, using a 15 K bovine oligo DNA microarray, demonstrated 30 and 266 differentially expressed genes in the CL on days 15 (P15) and 18 (P18) of pregnancy compared with the CL on day 15 (NP15) of non-pregnancy (n=4 for each group, >2-fold change relative to NP15; $P<0.05$). The gene expressions of *peroxisome proliferator-activated receptor delta (PPARD)* were the highest and *cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2)* were the lowest in P15 and P18 compared with NP15, and these microarray results were validated by quantitative real-time PCR analysis ($P<0.05$). In addition, transcripts of interferon-induced genes (*ISG15, OAS1, MX1 and MX2)* were more abundant in P15 and P18 than NP15, and both mRNAs and proteins for type I interferon receptors (IFNαR1 and IFNαR2) were expressed in the CL cells of P15, P18 and NP15.

Collectively, the different gene expression profiles may contribute to functional changes of the bovine CL during early pregnancy, and embryonic signals, *e.g.*, interferon-□ may act on the CL by a counter-current mechanism. The substances that exhibit changes in their expression levels in the pregnant CL may play a role(s) in regulating bovine CL function at the time of maternal recognition. Since PPARD induces progesterone (P4) production by stimulating cholesterol uptake in steroidogenic cells whereas CYP21A2 enzymatically catabolizes P4 to deoxycorticosterone, high levels of PPARD and low levels of CYP21A2 expressions in the early pregnant CL may contribute to support P4 production by bovine luteal cells.

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Role of *Bmal1*clock gene on corpus luteum formation of pregnancy in mice ovary

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Background: Circadian rhythms are associated with reproductive processes in mammals. It is known that *Bmal1* knock-out ($^{-/-}$) mice are infertility. Although their infertility is thought to be due to impaired corpus luteum steroidogenesis, the mechanisms are not clear. In the present study, we examined role of Bmal1 on oocyte quality and the corpus luteum (CL) formation of pregnancy.

Material and methods: Experiment 1: Oocytes collected from *Bmal1*$^{+/+}$ and *Bmal1*$^{-/-}$ female were used for IVF with sperm from *Bmal1*$^{+/+}$ males. Experiment 2: *Bmal1*$^{+/+-}$ and *Bmal1*$^{-/-}$ females were mated *Bmal1*$^{+/+}$ males, and female mice were killed on d 0.5 and d 3.5 of gestation. Collected blood was used for hormone measurements (progesterone and prolactin) and ovaries were used for quantification of mRNA expression and histological analysis.

Results: The percentage of oocyte fertilization and embryogenesis were not significantly difference between *Bmal1*$^{+/+}$ and *Bmal1*$^{-/-}$ oocytes. These results suggest ovulated oocytes in *Bmal*1$^{-/-}$ mice have normal capacity of fertility and embryogenesis. On d 3.5 of gestation (the day before implantation), serum progesterone levels and StAR and LHr mRNA expression in ovaries were lower in *Bmal1*$^{-/-}$ mice than in *Bmal1*$^{+/+}$ mice. In histological analysis, the CL were observed in Bmal1$^{+/+-}$ mice ovaries, but not Bmal1$^{-/-}$ mice. Generally, after cervical stimulation, prolactin (PRL) from pituitary plays an important role for the formation of pregnancy CL in mice. Therefore, we examined serum PRL concentration in *Bmal1*$^{-/-}$ mice on d 0.5 of gestation. Serum PRL concentration in *Bmal*1$^{-/-}$ mice on d 0.5 of gestation were lower than those in *Bmal1*$^{+/+}$ mice. In addition, expression of PRL-receptor mRNA in *Bmal1*$^{-/-}$ mice is shown the tendency to low as compared with *Bmal1*$^{+/+}$ mice.

Conclusion: Our results suggest that *Bmal1* may regulate secretion of PRL from pituitary and expression of PRL-receptor in the ovaries, and be involved in conversion of CL to pregnancy CL. Thus, Bmal1 is an important factor for the formation of pregnancy CL.

An increase in the level of α2,6-sialic acid inhibits galectin-1 binding to glycan during luteolysis

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Luteolysis is characterized by a reduction in progesterone (P4) production (functional luteolysis) followed by tissue degeneration (structural luteolysis) in the corpus luteum (CL). In various cell types, α2,6-sialic acid (α2,6-SA) is attached to N-glycan branch ends by α2,6-sialyltransferase (ST6Gal-I), where it inhibits binding of galectin-1 to glycan. Since galectin-1 increased the viability of bovine luteal steroidogenic cells (LSCs) in our previous study, we hypothesized that α2,6-SA is involved in survival of LSCs during luteolysis. In the present study, we examined 1) cyclic changes in the expression of ST6Gal-I and the level of α2,6-SA, 2) the factors that regulate the levels of ST6Gal-I and α2,6-SA, and 3) the interaction between α2,6-SA and galectin-1 in bovine LSCs.

CL tissues collected at the early (Days 2–3 after ovulation), developing (Days 5–6), mid (Days 8–12), late (Days 15–17) and regressed (Days 19–21) luteal stages. Cultured LSCs were treated with prostaglandin F2α (PGF2α: 1.0 μM), tumor necrosis factor α (TNFα: 2.3 nM) or interferon γ (IFNG: 2.5 nM) alone, or TNFα in combination with IFNG for 24, 48 and 72 h. LSCs were cultured with or without PGF2α for 72 h and washed. The cells were further incubated with or without galectin-1 (1.0 μg/ml) for 24 h, and their viability and P4 production were determined.

1) The levels of ST6Gal-I mRNA and α2,6-SA were higher at the regressed luteal stage than at the developing and mid luteal stages. α2,6-SA was detected mainly in the LSC plasma membranes. 2) The levels were increased by PGF2α in the LSCs cultured for 72 h compared with those without PGF2α treatment. 3) The viability of untreated LSCs was increased by further incubation for 24 h with galectin-1, while the viability of LSCs pretreated with PGF2α for 72 h was not changed by galectin-1. P4 production by LSCs that were pretreated or not pretreated with PGF2α for 72 h was not affected by further incubation for 24 h with galectin-1. These findings suggest that α2,6-SA expression stimulated by PGF2α contributes to structural luteolysis by inhibiting the galectin-1 binding in cattle.

Evaluation of an alternative embryo transfer strategy to mitigate early embryonic loss and differential gene expression in endometria of fertility and sub-fertile cattle

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Over the last few decades research has focused on the declining pregnancy rates that negatively affect calf and milk production, with significant economic losses for livestock producers. Consequently, numerous studies have sought to improve reproductive performance however few, if any, have identified truly effective solutions. Here this study aimed to determine the critical period of embryo loss after embryo transfer (ET) and investigate whether implementation of an alternative embryo transfer strategy could mitigate early embryonic loss. Moreover, we examined whether endometrial gene expression differs with respect to fertility status in the early post-estrus period.

After ET, half of the recipient cows did not become pregnant and approximately 60% of them returned to the estrus with normal length of cycle (< 25 days). In cows that failed to establish a pregnancy, the expression of interferon stimulated gene (*ISG*) *15* in peripheral blood mononuclear cells at day 18 post estrus, which reacted with IFN-τ effused from the uterine lumen, was significantly lower compared to those that maintained pregnancy. These results indicated that the majority of embryo loss occurred after ET occurs just before the period of maternal recognition of the pregnancy.

In a second experiment, the transfer of a later stage of conceptus (CT) was investigated as a strategy to bypass the critical period of embryo loss in order to improve pregnancy rates. Conceptuses were collected from superovulated cows on day 14 after insemination. An individual conceptus was non-surgically transferred to the uterine horn ipsilateral to the CL of a synchronized recipient known to be sub-fertile (defined by a failure to establish a pregnancy are > three standard ETs). Pregnancy rate after CT were not improved.

In third experiment, endometria were biopsied from the ipsilateral uterine horn of fertile and sub-fertile multiparous cows on day 7 post estrus. RNA was extracted from samples and gene expression analyzed via microarray and compared by hierarchical clustering. Hierarchy clustering analysis of the microarray data clearly differentiated fertile from sub-fertile females. Taken together, these data support that even early in the post-estrus period the endometrial gene expression differs in sub-fertile cows.

THE EFFECT OF LYSOPHOSPHATIDIC ACID (LPA) ON THE EMBRYO-MATERNAL CROSS-TALK IN COWS

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Lysophosphatidic acid (LPA) is a simple phospholipid with a vast variety of physiological and pathological actions on many cell types. In mammals, LPA exerts its action via several high affinity G-protein-coupled receptors (LPARs). So far many studies have been conducted on the influence of LPA on the regulation of reproductive processes in various animal species including cow. We found that LPA stimulated the insemination rate in cattle as well as progesterone (P4) and luteotropic prostaglandin (PG)E_2 secretion during estrous cycle and early pregnancy *in vivo* and *in vitro*. The presence of LPA as well as enzymes responsible for LPA synthesis and specific LPARs in the bovine endometrium, CL, follicle, oocyte and embryo was detected, indicating that these structures of the bovine reproductive tract are the sites of LPA synthesis and targets for its action during the estrous cycle and early pregnancy. However, during the estrous cycle and early pregnancy the most important place for LPA synthesis seems to be endometrium while the ovary and the embryo the most important targets for LPA action. We documented that LPA exerted auto/paracrine actions in the bovine reproductive tract. In the bovine CL, the luteotropic action of LPA resulted from its effect on the augmentation of P4 synthesis via the stimulation of 3βHSD expression. Moreover, we found LPA-dependent stimulation of IFNt action on 2,5'-oligoadenylate synthase (OAS1) and ubiquitin-like IFN-stimulated gene 15-kDa protein (ISG15) expression. On the other hand, at the time of luteolysis, LPA abrogated tumor necrosis factor (TNF)a and interferon (IFN)g as well as nitric oxide (NO) - induced inhibition of P4 synthesis in the bovine CL. LPA exerted this luteoprotective role during the CL luteolysis via the modulation of the cytokines and NO initiated apoptosis. In the bovine ovarian follicle, LPA stimulated estradiol (E_2) production and FSH action in granulosa cells *via* increased expression of the FSHR and 17β-HSD genes, which in turn accounted for the participation of LPA in ovarian follicle growth and differentiation. During oocyte maturation *in vitro*, the supplementation of the maturation medium with LPA improved oocyte maturation rates, decreased extent of apoptosis in COCs and sustained the expression of developmental competence related factors during oocyte maturation and subsequently affected gene expression profile at the blastocyst stage. We also demonstrated that LPA is an early bovine embryonic autocrine/paracrine signaling mediator, and its action during early embryo-maternal interactions led to embryonic survival.

In conclusion, the obtained data indicate that LPA can play the supportive role in embryo-maternal interactions in the cow.

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Early embryo-maternal communication in natural pregnancy and after embryo-transfer in mice

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Signals directed from embryo to mother are crucial for maintenance of pregnancy especially at early phase of gestation. During the first experiment we have compared two groups of mice: in pseudopregnancy and in pregnancy at 3,5 day after mating, before implantation of the embryo. Short signals are represented by the local actions in the uterus in response to embryo appearance, which were tracked by measuring the gene expression level of 9 chosen signaling pathways. Among 8 statistically relevant of all 84 examined genes , 6 were down-regulated and two up-regulated. Significantly altered genes belonged mainly to mitogenic (early-responsive genes) and NfκB pathway.

Embryo selection before and at the onset of implantation also depends on complex embryo-maternal interaction. Therefore in the second experiment we employed mouse non-surgical embryo transfer technique to investigate the differences in signaling between normal embryos and embryos with reduced biological potential achieved by TNFα treatment. Moreover, gene expression in murine preimplantation uterus differs depending on the quality of transplanted embryo. Transfer of biologically competent embryos alters 24 genes, among which 79% were up-regulated. Whilst transfer of TNFα treated embryos down-regulates expression of 41 genes, with only one gene with increased expression. Observed changes include crucial to implantation genes Ptgs2 and Hoxa10, NfκB, TGFβ, WNT and Hedgehog pathways.

The results of this study revealed that during natural pregnancy maternal response (on the level of signal transduction pathways) to embryo presence at preimplantation stage is discreet and subtle. Another conclusion that emerge from second experiment is differential gene expression depending on the quality of transplanted embryo. Those differences refer to the number of altered genes (24 and 42 in mice after transfer of normal and TNFα treated embryos respectively) and direction of regulation ranging from up-regulation in ET and down-regulation after ET with TNFα- treated embryos.

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Temporal expression of vascular endotherial growth factor family members in the bovine endometrium during peri-implantation period

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Vascular endotherial growth factor (VEGF) family is involved in regulation of angiogenesis and lymphangiogenesis in the female reproductive tract. This study aimed to determine mRNA expression patterns of VEGF ligands (VEGFA, -B, -C and -D) and their receptors (VEGFR1, -2 and -3) in bovine endometrium during the peri-implantation period. We also investigated the number of blood vessels and lymph vessels in the uterus. Endometrial tissues were collected from pregnant cows at Day 15, 18 and 27 after artificial insemination (Day 0 = day of AI) and non-pregnant cows at Day 15 and 18 of the estrous cycle (Day 0 = day of estrus). The mRNA expression levels of VEGF ligands and receptors were determined by quantitative real-time RT-PCR. In addition, we performed immunohistochemical staining for von Willebrand factor and lymphatic vessel endotherial hyaluronan receptor 1 to determine the number of blood vessels and lymph vessels in the tissue sections of ipsilateral and contralateral uterine horns to the ovary containing the corpus luteum of the pregnant and non-pregnant cows. In the pregnant endometrium, *VEGFA* and *VEGFR3* mRNA expression was higher but *VEGFB* mRNA expression was lower at Day 18 than the other days of gestation. *VEGFD* mRNA expression in the pregnant endometrium was decreased from Day 15 to 27. However, the mRNA expression of *VEGFA*, *VEGFC*, *VEGFR1* and *VEGFR3* in the pregnant endometrium was lower than the non-pregnant endometrium. In stroma of the ipsilateral endometrium of the pregnant cows, the number of blood vessels increased from Day 15 to 18 and was greater than that of the non-pregnant cows at Day 18. The number of lymph vessels in the ipsilateral myometrium of the pregnant cows tended to be increased from Day 15 to 18 ($P<0.1$). Our results demonstrated that the VEGF family is regulated in endometrium during the peri-implantation period as well as the estrous cycle, which may be associated with uterine function in maternal recognition of pregnancy and implantation.

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Ovarian and placental molecular mechanisms responsible for pregnancy maintenance and prepartal luteolysis in cats

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The length of the luteal phase in pregnant queens, depicted in elevated progesterone (P_4) levels, is assumed to be partially driven by luteotrophic and luteolytic factors of placental origin. The higher P_4 values reported for pregnant queens may result from P_4 supplementation by placental tissue.

Thus, the feline placenta was assumed to be an additional source of P_4. Both StAR and 3βHSD were immunolocalized in the placenta and CLs within each luteal phase. The successful extraction of P_4 from the placenta further supports its role as an additional source of P_4 in the cat. Placental P_4 concentrations seem to be dependent on the gestational age. In most species, the demise of CLs is connected to decreasing levels of serum P_4 and increasing levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); most likely as a prerequisite for prepartal luteolysis. However, the capacity of feline CLs to provide $PGF_{2\alpha}$ was limited during the pregnant and non-pregnant luteal phases. Therefore, the question, whether the feline placenta is capable of synthesizing prostaglandin $PGF_{2\alpha}$ during the course of pregnancy, was addressed. The placental PGFS, which is classified as an aldo-keto reductase 1C3 (AKR1C3), was analyzed at the mRNA and protein level throughout pregnancy and before parturition. Blood plasma was collected for immunoassaying P_4 and the $PGF_{2\alpha}$ inactive metabolite 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM). The PGFS protein was elevated, particularly at 2.5–3 weeks of pregnancy, compared to samples collected at 7-8 ($P<0.05$) or 8.5–9 weeks ($P<0.001$). The PGFS mRNA was significantly upregulated at the 3rd week of pregnancy and then gradually declined towards the end of gestation ($P<0.001$). Staining for PTGS2 showed distinct positive signals in placentas obtained during the last week before labor, particularly in the strongly invading trophoblast surrounding blood vessels, but also in decidual cells. Shortly after implantation, signals for PGFS were localized in the trophoblast cells. Near term, PGFS staining was seen mainly in decidual cells. Both placental $PGF_{2\alpha}$ and plasma PGFM were elevated towards the end of pregnancy compared to the values obtained at the 3rd week of pregnancy ($P<0.001$).

These findings led to the conclusion that even if PGFS (AKR1C3) was low just before term, with accompanying high placental $PGF_{2\alpha}$ concentrations and high levels of circulating PGFM, an enzyme responsible for prostaglandin metabolism, namely 15α-prostaglandin dehydrogenase (PGDH), might be involved in $PGF_{2\alpha}$ bioavailability. In addition, an involvement of aldo-keto reductase B5 (AKR1B5) in $PGF_{2\alpha}$ synthesis might also be considered, and especially the fact that AKR1B5 might be able to inactivate P_4 locally, despite elevated P_4 levels in the maternal blood.

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Expression of Equine Major Histocompatibility Complex Class I during Pregnancy, Parturition and Fetal Membrane Retention

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We hypothesized that the fetus will down-regulate MHC I expression to avoid maternal recognition during pregnancy, but will up-regulate MHC I expression during parturition so that the mother's immune system will reject fetal cells, facilitating release of fetal membranes. Moreover, we further hypothesized that lower expression of fetal MHC I during parturition will be associated with fetal membrane retention. To test these hypotheses, placenta samples were collected from 16 pregnant heavy draft mares in a slaughterhouse. We also took biopsies from mares during parturition (n=33) and anestrus (n=5). Mares in parturition were monitored for the time of fetal membrane expulsion. There were 13 FMR mares and 20 control mares. For qPCR primers were designed to detect all the best-known equine MHC I genes (Rapacz-Leonard et al. 2015). To detect MHC I protein, mouse anti-horse MHC I monomorphic antibodies were used (MCA1086GA Serotec and ab23491 Abcam) according to procedures described by Rapacz-Leonard et al. (2015). During pregnancy, MHC I mRNA was strongly up-regulated in the endometrium in the first month of pregnancy (at least 4.6 times higher than in any other month) (P=0.0008). After the first month of pregnancy, MHC I mRNA expression was down-regulated in the endometrium, but it was higher than during months 6–8 of pregnancy. In the allantochorion, MHC I mRNA expression was constant throughout pregnancy. With respect to MHC I protein, its levels were measured during months 3–8 of pregnancy and were barely detectable. To confirm the detection of MHC I, equine lymph node was used as a positive control. MHC I levels in the lymph node were at least 41 times higher than in placental samples (P=0.03). During parturition, MHC I mRNA expression in the allantochorion was higher than during months 6–8 of pregnancy, but its expression in the endometrium was the same as during those months of pregnancy. MHC I mRNA expression was at least 2.5 times higher in the allantochorion than in the endometrium. Moreover, MHC I mRNA expression in the allantochorion was 3 times higher in mares that retained fetal membranes than in mares that delivered fetal membranes physiologically (P=0.008). MHC I mRNA expression in the endometrium was very low when compared to the endometrium during anestrus (P=0.003). With respect to MHC I protein during parturition, its levels in the endometrium were 3.2 times higher than in the allantochorion (P=0.0005). Moreover, MHC I protein levels in the endometrium were 1.7 times higher in mares that delivered fetal membranes physiologically than in mares with fetal membrane retention (P=0.0079). MHC I protein levels in the allantochorion were 1.1 times higher in mares with fetal membrane retention than in mares with physiological delivery of fetal membranes (P=0.0159). In conclusion, maternal and fetal MHC I protein levels were very low during months 3–8 of pregnancy. Because MHC I mRNA was expressed during these months, this might indicate post-translational and/or transcriptional regulation. MHC I mRNA expression was up-regulated at parturition, which might indicate that fetus prepares the mother's immune system for fetal membranes recognition and rejection. However, because protein levels were determined with different systems, this needs further study. Interestingly, MHC I mRNA and protein expression were higher in the allantochorion of mares with fetal membrane retention than in mares that delivered the membranes physiologically, whereas MHC I protein levels were lower in the endometrium of mares that retained fetal membranes.

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Does equine fetus express only maternally inherited Major Histocompatibility Complex I (MHC I) in order to protect long lasting pregnancy?

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During pregnancy a mare has to maintain a semi allogenic graft for 11 months, while a fetus has to survive in immunologically hostile environment. In previous study, Rapacz-Leonard et al. (2015) indicated that the allantochorion (fetal part of the placenta) up-regulated the expression of MHC I throughout pregnancy. Moreover, this expression was higher during pregnancy than during parturition in both the allantochorion and in the endometrium (maternal part of the placenta). It remains unknown whether the expressed MHC I are of maternal or paternal origin, which would explain this up-regulated expression. To test this, we took samples of the allantochorion and the endometrium from 13 heavy draft mares in different months of pregnancy (3 – 8 months old). Samples were taken from a slaughterhouse, from the same mares as in Rapacz-Leonard et al. (2015) study. The studies involved amplification in PCR reaction of 10 MHC I genes in 2 experiments: first with DNA as a template and second with cDNA as a template. Presence of the products of the same base pair length in both parts of the placenta, suggested that both the mare and the fetus carried the same MHC I gene allele; whereas the absence of the product in one of the placenta parts suggested that the mare and the fetus carry different MHC I gene allele (the fetus inherited the allele from a father). In the first experiment (DNA PCR) in 64 combinations both the fetus and the mother had the same length of the amplified MHC I gene, which suggested that they share the same gene allele. In 8 cases the MHC I gene was only present in fetal part of the placenta, suggesting that it was inherited from the father. In the second experiment (cDNA PCR) expression of the maternal and fetal MHC I gene alleles was verified. The presence of the product indicated that there was expression of amplified MHC I gene. Out of 64 combinations from the first experiment where both the fetus and the mother had the same length of the amplified MHC I gene in 21 combinations the MHC I was expressed by the fetus and the mother, in 14 combinations was the MHC I gene was expressed only by the mother, in 5 combinations the MHC I gene was expressed only by the fetus and there was no expression in 24 combinations. In 8 combinations from the first experiment where the gene was present only in the fetal part of the placenta only in 1 combination the gene was expressed. Interestingly, 2 of the amplified MHC I genes were expressed in almost every mother-fetus combination (10 out of 13; 7 out of 13). Above results suggest that expressed MHC I in the allantochorion are mainly of maternal origin and that there is tight epigenetic control of MHC I gene expression of paternal origin.

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Brain mechanism underlying ovulation in mammals

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Mammals can be divided into two groups based on their ovulatory processes: one is the spontaneous ovulator and the other is the reflex ovulator. In the spontaneous ovulator, including mice, rats, pigs and cows, follicular development and ovulation occur in a cyclic manner. High level of estrogen produced by matured follicles induces gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) surge and then ovulation through estrogen-positive feedback action. On the other hand, in the reflex ovulator, including rabbits, ferrets and musk shrews, follicular development occurs without ovulation. They ovulate only when they receive the mating stimulus by males.

Kisspeptin is a newly found neuropeptide encoded by *Kiss1* gene in 2001. Kisspeptin receptor, *Gpr54* is expressed in GnRH cells. In the recent decade, it has been clarified that kisspeptin-GPR54 signaling plays a crucial role in initiating secretion of GnRH/LH in mammals, including both spontaneous and reflex ovulators. Preoptic area (POA)/anteroventral periventricular nucleus (AVPV) kisspeptin neurons are considered to be responsible for estrogen-positive feedback action. It has been suggested that POA/AVPV kisspeptin neurons are the GnRH/LH surge generator. In the spontaneous ovulation, high level of estrogen activates POA/AVPV kisspeptin neurons to induce GnRH/LH surge and then ovulation. On the other hand, in the musk shrew (*Suncus murinus*), a reflex ovulator, mating stimulus activates POA kisspeptin neurons to induce ovulation *via* GnRH release (*Inoue et al,* 2011). In this presentation, I would like to review the brain mechanism underlying spontaneous ovulation and reflex ovulation focusing on kisspeptin-GPR54 signaling.

Molecular Cloning and Identification of the Transcriptional Regulatory Domain of the Goat Neurokinin B Gene *TAC3*

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The pulsatile secretion of gonadotropin-releasing hormone (GnRH) from hypothalamus and the subsequent pulsatile gonadotropin release stimulate ovarian follicular development in mammals. KNDy neurons located in the hypothalamic arcuate nucleus (ARC) have been suggested to generate GnRH pulses. Neurokinin B (NKB), encoded by *TAC3*, in KNDy neurons is thought to be an important accelerator of pulsatile GnRH release. Despite the importance of NKB in mammalian reproduction, few studies have focused on how NKB and *TAC3* expression are regulated. The present study aims to clarify the transcriptional regulatory mechanism of *TAC3* in goats.

First, we performed 5'- and 3'-RACE and genome walking using the sample from Shiba goat to identify the full-length mRNA and the 5'-upstream sequence of goat *TAC3*, respectively. Next, we conducted the luciferase assay using the obtained 5'-upstream region to examine the transcriptional regulatory mechanism of goat *TAC3*. Five DNA constructs from -2706, -1837, -834, -335, and -197 to +166 (transcription start site was designated as +1) of goat *TAC3* were inserted into luciferase reporter vector pGL4-basic. The vectors were transfected into mouse hypothalamus-derived N7 cells and human neuroblastoma-derived SK-N-AS cells. Twenty-four hours after the transfection, the cells were treated with 0, 1 or 10 nM estradiol (E2) for additional 24 hours. The cells were lysed to measure luciferase activity.

By RACE method, we determined the full-length mRNA sequence of goat *TAC3* to be 820 bases including a 381-base coding region, with the putative transcription start site located 143-base upstream of the start codon. The deduced amino acid sequence of NKB was completely conserved among goat, cattle and human. We cloned 5'-upstream region of goat *TAC3* up to 3400 bases from the translation initiation site, and this region was highly homologous with cattle *TAC3* (89%). The luciferase activity gradually increased with the deletion of the 5'-upstream region, suggesting that the transcriptional suppressive region is located between -2706 and -336 bp and that the core promoter exists downstream of -197 bp. Estradiol treatment did not lead to significant suppression of luciferase activity of any constructs, suggesting the existence of other factor(s) that regulate goat *TAC3* transcription.

Electrophysiological Technique for Monitoring the Hypothalamic Mechanism Regulating Pulsatile GnRH Release in Goats

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Reproductive processes in female mammals, such as follicular development in the ovary, ovulation of dominant follicles, lactation and sexual/maternal behaviors, are under control of the brain. The release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is the top of neuroendocrine control hierarchy of reproductive functions and the pattern of pulsatile GnRH release is a key determinant regulating the gonadal activity. The basal release of GnRH is pulsatile and GnRH pulses are associated with synchronized electrical activity in the mediobasal hypothalamus (multiple unit activity; MUA), which is thought to reflect the rhythmic oscillations in the activity of the neuronal network, the GnRH pulse generator, which drives pulsatile GnRH secretion. However, the cellular source of this ultradian rhythm in GnRH release has not been fully elucidated.

Recent studies in the filed of reproductive neuroendocrinology reveal that a subset of neurons identified in the hypothalamic arcuate nucleus (ARC) coexpress three neuropeptides, kisspeptin, neurokinin B (NKB), and dynorphin; each of three peptides has been shown to play a critical role in the central control of reproduction. Growing evidence suggests that these neurons, KNDy neurons named after initial letters of three peptides, are conserved across a range of species from rodents to humans and play a key role in the regulation of pulsatile GnRH release. To determine whether the KNDy neuron signalling could be responsible for producing pulsatile GnRH secretion, we recorded MUA in the close vicinity of KNDy neurons in the posterior ARC, where the majority of KNDy cells are located in goats. Rhythmic volleys of MUA were found to be accompanied by luteinizing hormone (LH) pulses with regular intervals. Exogenous administration of NKB and dynorphin stimulated and suppressed, respectively, regular occurrence of rhythmic volleys of MUA. In contrast, administration of kisspeptin stimulated a sustained increase in LH secretion, without influencing MUA, suggesting that the GnRH pulse generator, as reflected by MUA, originated from outside of the network of GnRH neurons. Rhythmic electrophysiological activity could plausibly reflect the pacemaker activity of KNDy neurons, whose projections reach the median eminence where GnRH fibers project. These observations suggest that the KNDy neurons in the ARC may be the intrinsic source of the GnRH pulse generator.

Neonatal kisspeptin is required for defeminization of the brain mechanism controlling sexual behaviors in male rats

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Reproductive behaviors are sexually differentiated by perinatal testosterone and its metabolite estradiol in rodents. It has been well established that female rats show lordosis behaviors as a female-type sexual behaviors to accept male sexual behaviours. On the other hand, lordosis behaviors are suppressed in male rodents. Thus, the brain mechanism controlling sexual behavior is defeminized in males. Besides the critical role of kisspeptin in controlling gonadotropin-releasing hormone release in adults, little is known about the functional significance of kisspeptin in the differentiation of sexual behaviors. The present study aims to investigate the role of kisspeptin in defeminizing the brain mechanism controlling sexual behaviors by using *Kiss1* KO rats. Adult *Kiss1* KO male rats treated with preovulatory level of estrogen showed robust lordosis behaviors as are found in females. This suggests that kisspeptin plays a crucial role in the process of defeminization of the brain mechanism at the early development. Plasma testosterone levels at embryonic day 18 and postnatal day (PND) 0 in *Kiss1* KO males were similar to those in wild-type males. Estradiol benzoate treatment at PND0 reduced the lordosis quotients in *Kiss1* KO males to a level found in wild-type males. These indicate that testosterone is secreted normally and the downstream of estradiol works properly in *Kiss1* KO males. Kisspeptin replacement at PND0 reduced lordosis quotients in *Kiss1* KO males not in females, suggesting that kisspeptin is able to defeminize the brain mechanism in perinatal period in the presence of neonatal testosterone. Taken together, kisspeptin is required during perinatal period for defeminizing the brain mechanism controlling sexual behaviors in male rats through a HPG-independent mechanism.

Involvement of Preoptic Kisspeptin Neurons in Estrogen Positive Feedback to induce Luteinizing Hormone surge in both female and male Japanese monkey

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The brain mechanism responsible for behavioral and hormonal sexual dimorphism has been mainly proposed based on the knowledge accumulated in rodent models. Male rats never show estrogen positive feedback-induced luteinizing hormone (LH) surge, which triggers ovulation in female rats. To the contrary, the estrogen-induced LH surge is evident in male primates including human. These findings suggest that the central mechanism governing reproductive hormone in the primate is different from that in rodents. The purpose of this study is to investigate whether male Japanese monkeys conserve a brain mechanism mediating the estrogen-induced LH surge via activation of kisspeptin neurons. Adult male and female Japanese monkeys were gonadectomized and then were treated with estradiol-17b for 2 weeks followed by a bolus injection of estradiol benzoate. Both male and female monkeys showed an estrogen-induced LH surge. In gonadectomized monkeys sacrificed just before the anticipated time of the LH surge, the exogenous estrogen treatment significantly increased the expression of *KISS1*, a gene encoding kisspeptin, in the preoptic area (POA) of both males and females. Further, the estrogen treatment induced the expression of c-Fos, a marker of neural activation, in the POA *KISS1*-positive cells in males as well as female monkeys. This treatment failed to induce c-Fos expression in the arcuate nucleus (ARC) kisspeptin neurones in both sexes just prior to LH surge onset. Thus, kisspeptin neurones in the POA but not in the ARC might be involved in the positive-feedback action of estrogen that induces LH surge in male Japanese monkeys, as well as female monkeys. In conclusion, the present results indicate that estrogen-responsive kisspeptin neurons in the POA are responsible for LH surge generation in male Japanese monkey as well as females. The conservation of the LH surge generating system exist in adult male primates, unlike rodents, could be a consequence of the capability of estrogen to induce POA kisspeptin expression and activation.

Involvement of cell-to-cell communication via gap junctions in NKB-NK3R signaling-induced synchronous discharges of KNDy neurons

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The pulsatile secretion of gonadotropin-releasing hormone (GnRH)/gonadotropins luteinizing hormone (LH) is essential for follicle development and spermatogenesis in mammalian species. A cohort of neurons expressing kisspeptin, neurokinin B (NKB) and dynorphin A (KNDy neurons), located in the hypothalamic arcuate nucleus, are a putative intrinsic source of GnRH pulse generator and synchronous discharges of KNDy neurons might be obligatory for pulsatile GnRH secretion. Here we showed that cell-to-cell communication via gap junctions among KNDy neurons is required for NKB-NKB receptor (NK3R) signaling-induced synchronous discharges of KNDy neurons in vitro. GFP-visualized KNDy (KNDy-GFP) cells were collected from the fetal mediobasal hypothalamus of the transgenic mice expressing GFP under the control of the *Kiss1* promoter and cultured on a glass-base dish for 2 to 6 weeks. Intracellular Ca^{2+} concentrations were measured in individual KNDy-GFP cells using the fluorescent Ca^{2+} indicator Fura-PE3. Senktide, a selective agonist for NK3R, increased the frequency of the Ca^{2+} oscillations in cultured KNDy-GFP cells. The senktide-induced Ca^{2+} oscillations were synchronized in the neighboring KNDy-GFP cells. Further, gap junction inhibitors, 18β-glycyrrhetinic acid and mefloquine, attenuated senktide-induced Ca^{2+} oscillations in KNDy-GFP cells. These results obtained from the present study indicate that NKB-NK3R signaling is required for synchronous activities in neighboring KNDy neurons and that cell-to-cell communications via gap junctions may play a role in controlling synchronous activities of KNDy neurons, which in turn induces pulsatile GnRH release. This work was supported in part by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and Food Industry (to H.T.) and a Grant-in-Aid for the Japan Society for the Promotion of Sciences Fellows Grant 26-4247 (to K.I.).

Effect of endotoxin on ovarian follicle function in domestic animals

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Uterine bacterial infection commonly occurs in postpartum dairy cows and perturbs ovarian function. *Escherichia coli* is among the main types of bacteria causing endometritis, and much of the tissue pathology is associated with the bacterial endotoxin lipopolysaccharide (LPS). LPS has been detected in the follicular fluid of cows with endometritis, suggesting a relationship between uterine inflammation, LPS production and follicular function.

In follicles with a high level of LPS, the concentration of estradiol (E2) was lower and that of progesterone (P4) was higher when compared to those in follicles with a low level of LPS. In addition, transcripts of P450arom were lower in follicles with a high level of LPS. In vitro culture, LPS strongly inhibited E2 production in FSH-treated granulosa cells isolated from bovine small follicles rather than those of large follicles. Moreover, LPS stimulated the transcription of TLR4, CD14 and MD2 genes in FSH-treated granulosa cells of small follicles but not large follicles. These results suggest that granulosa cells of small follicle have a higher sensitivity to LPS than those of large follicles in bovine ovaries. In theca cells isolated from pre-selection follicles (PRF) and post-selection follicles (POF), LPS suppressed production of P4 and androstenedione in LH-treated theca cells of PRF and POF. In addition, LPS inhibited the expression of StAR and CYP17 genes in LH-treated theca cells.

In conclusion, LPS inhibits steroid production in granulosa cells and theca cells of follicles at different developmental stages in bovine ovary. The sensitivity to LPS on follicular cells depends on the follicular developmental stage. These findings highlight a possible mechanism of ovarian dysfunction in cows with endometritis.

Genomic portrait of ovarian follicle growth regulation in cattle

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Female reproductive cycles are characterised by cyclic patterns of ovarian follicle growth with few being selected to ovulate and the remainder undergoing degeneration. In mono-ovulatory species, such as cattle, ovarian follicular development is based on distinct waves of follicle growth. A wave is defined as the synchronous growth of a cohort of small follicles. From this cohort, one follicle is selected for continued growth and assumes dominance, while other subordinate follicles regress and undergo atresia by programmed cell death and apoptosis at various stages of development. Unquestionably, the fate of each follicle is controlled by endocrine factors (e.g. gonadotrophins, their receptors and steroids), as well as autocrine/paracrine factors (e.g. insulin-like growth factors, their binding proteins and transforming growth factor-β family members), but cellular and molecular mechanisms controlling follicle selection and apoptosis are not fully understood. The bovine genome codes for approximately 22 thousand genes, and the pattern of gene expression in ovarian cells controls follicle development at different stages (recruitment, selection, dominance, atresia) by coordinating these endocrine and intraovarian factors. It is well established that mRNA expression for *LHR*, *FSHR*, *CYP17*, *CYP19*, *INHA* and *INHBA* are critical for final ovarian maturation and their level of expression increases as follicular development progress (greater in dominant compared to subordinate follicle). Furthermore, several pro- and anti-apoptotic genes involved in the survival of follicles have been demonstrated (e.g. *Bax*, *Bcl-2*, *Fas*, *FasL*). Recent evidence indicates that gene expression is regulated by small molecules called microRNAs (miRNAs; gene silencing through translation repression or direct mRNA degradation). They have been identified in whole bovine ovaries, as well as in follicles of various size. Recently, a set of differentially expressed miRNAs between dominant compared to subordinate follicles have been shown to be involved in known molecular pathways (e.g. Wnt, TGF-beta, ErbB, GnRH, MAPK and oocyte meiosis signaling pathways) that determine the fate of ovarian follicle development. Identification of mRNAs and miRNAs involved in regulation of survival and apoptosis of bovine follicular cells enables better understanding of the mechanisms in charge of follicle development.

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Ovarian and endocrine function after hormonal induction of ovulation in seasonally anovular goats

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The primary goal of this series of experiments was to employ daily transrectal ultrasonography and hormone measurements to describe and compare ovarian activity and accompanying endocrine changes in seasonally anovular goats (July-August) receiving exogenous gonadotropins or buserelin acetate (GnRH analogue), with or without the 14-day pre-treatment with intravaginal sponges containing 45 mg of fluorogestone acetate (FGA). The effects of a single i.m. dose of equine chorionic gonadotropin (eCG, 500 IU), human chorionic gonadotropin (hCG, 200 IU) or buserelin (0.01125 mg) on ovarian function, circulating concentrations of ovarian steroids and periovulatory secretion of luteinizing hormone were evaluated. Each experiment utilized 12 goats of the Polish White breed that were randomly allocated to two equal groups (FGA-primed vs. untreated controls) and examined for a total of 42 days, with induced ovulations occurring 21 days into the observation period. Treatment of anestrous goats with FGA did not affect the number of emerging follicular waves (defined as cohorts of follicles growing synchronously to ovulatory sizes before regression or ovulation). In all experiments, control animals tended to have more ovarian antral follicles ≥5 mm attaining larger maximum diameters of the than FGA-treated goats, but had fewer ($P<0.01$) ovulations compared with their FGA-treated counterparts. The FGA-treated goats had significantly lower circulating concentrations of estradiol compared with controls. Within the FGA-primed anestrous goats, the greatest ($P<0.05$) number of growing antral follicles in the ovulatory wave and the highest ($P<0.01$) ovulation rate were seen in eCG-stimulated animals. All goats that had received FGA sponges before the treatment with eCG, hCG or GnRH analogue developed fully functional corpora lutea (CL) post-ovulation whereas all control animals only had short-lived CL secreting small amounts of progesterone. Lastly, persistent ovarian follicles were detected in goats after eCG and hCG treatments but not in the buserelin-treated animals. The present results clearly indicate the modulatory effects of a synthetic progestin on ovarian antral follicle development, estrogenicity and gonadropin responsiveness as well as on ensuing luteal function in anestrous goats induced to ovulate with exogenous gonadotropins or a GnRH analogue. The use of exogenous gonadtropins appeared to be associazted with the prolonged antral follicular growth post-treatment.

Nodal promotes vascular regression via Thrombospondin-1 pathway during luteolysis in the mare

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The local auto-/paracrine mechanisms regulating corpus luteum (CL) growth, maintenance and regression have been thoroughly studied in primates, rodents and ruminants. However, equine CL functions and regulations at molecular level is understudied. Recently, we have shown that Nodal, a Transforming Growth Factor Superfamily member, is expressed in the equine CL. Moreover, Nodal signaling pathway was associated with functional luteolysis, decreasing progesterone and promoting prostaglandin (PG) F2α secretion. However, the role of this cytokine on vascular regulation is yet to be characterized. Thus, we hypothesized that Nodal contributes for vascular regression during luteolysis establishment in the mare. To verify the hypothesis we investigated the *in vitro* role of Nodal in mid-CL isolated cells pro-angiogenic vascular endothelial growth factor (VEGF) A and its receptor 2 (VEGFR2), as well as the anti-angiogenic thrombospondin (TSP) 1 and its receptor CD36. Blood and CL samples (n=6) were collected *post mortem* at the abattoir from randomly designated cyclic mares (mid-luteal phase of the cycle). Enzymatically dispersed luteal cells were cultured in T25 culture flasks ($5.0x10^6$ cells/mL; for further mRNA and protein analysis). Cells were treated for 24h with: (i) no exogenous treatment; (ii) Nodal (0.1; 1 and 10ng/ml); (iii) PGF2α (10-7M); and (iV) luteinizing hormone (LH, 10ng/ml). The mRNA level was assessed by relative quantification real-time PCR and protein expression was quantified by western blotting. The lowest dose of Nodal (0.1ng/ml) increased VEGF mRNA transcription ($p<0.05$), whereas LH promoted VEGFR2 mRNA ($p<0.05$), comparing to control. Conversely, Nodal at 10ng/ml triggered TSP1 and CD36 mRNA and protein expression ($p<0.05$). The luteolytic agent PGF2α steadily increased TSP1 mRNA and protein ($p<0.05$) and CD36 protein ($p<0.05$), while LH decreased both mRNA and protein of aforementioned factors ($p<0.05$). Our previous results evidenced a significant raise on Nodal protein expression during the time of luteolysis. Presently we demonstrate that Nodal might effectively trigger vasculature demise in equine CL during luteolysis, acting on TSP1 signaling pathway. Furthermore, Nodal effects might be dependent on level of transcription/translation, since different doses promoted opposite results. Generally, increased expression of Nodal in late-CL seems to be determinant for angioregression during luteolysis in the mare.

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Placentitis - recent diagnostic and treatment tools for the high-risk pregnancy in the mare – Pregnancy maintenance

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Following the routine pregnancy examinations to detect the early conceptus and prevent implantation of multiple embryonic vesicles (~Day (D) 14-15), investigate embryonic development (~ D 25-30) and determine fetal gender (~ D 65) the equine pregnancy ceases to be the focus of veterinary attention until parturition approaches unless obvious complications arise. Mares with a history of abortion or placentitis or first signs of a high-risk pregnancy should be clinically, ultrasonographycally and endocrinologically monitored in regular intervals throughout pregnancy.

Placentitis is among the leading causes of abortion in the mare, affecting approximately 2-5% of pregnancies worldwide. Especially older, pluriparous mares with pneumovagina or cervical defects are predisposed to ascending bacterial infection –most commonly caused by Streptococcus equi subsp. zooepidemicus (S. zooepidemicus) or Escherichia coli. Local placental inflammation at the level of the cervical star area leads to placental separation without external evidence of disease. Bacteria readily cross the fetal membranes and colonize the fetal fluids, amnion and umbilicus, gaining access to the fetus via the umbilicus or fetal respiratory movements. Fetal cortisol levels increase and enhance placental and uterine prostaglandin production, resulting in abortion or premature fetal delivery. In most cases, precocious mammary development or premature lactation is the only clinical sign of disease prior to abortion.

In high-risk pregnancies transrectal and transabdominal ultrasonography of the fetus, the fetal heart rate, allantoic and amniotic fluid volume and echogenicity, cervical dimensions, the combined thickness of the placenta and the uterus (CTUP) and placental integrity support clinical signs (e.g. premature udder development, lactation and vulvar discharge) and biochemical indices (e.g. progestagens, estrogens, Serum Amyloid A). Colour Doppler sonography has become routine for the evaluation of high-risk pregnancies in human medicine and different authors described changes in uterine blood flow throughout pregnancy in the horse. Following the early diagnosis of placentitis, the mare should be immediately treated combining antibiotics, anti-inflammatory or immune-modulatory medication, and progestins to maintain pregnancy. To date, best results are achieved by the combination of trimethoprim sulfamethoxazole or penicillin-gentamycin, flunixin-meglumine and altrenogest. Research activities focus on the development of affordable, sensitive screening tests and identification of more effective antibiotic and anti-inflammatory therapy to prevent abortion and fetal compromise.

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Breeding soundness evaluation of the stallion - Methods for evaluation of semen and pathology

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Several situations occur when it is required to evaluate the potential fertility of a stallion including selection of new young stallions, before sale, before the beginning of a breeding season, to estimate the number of possible covers or inseminations, in connection with abnormal sexual behavior or in suspicion of a pathogen. It is however important to keep in mind, that no matter how many test or parameters that can be established in the laboratory about a given stallion or a semen sample, there is only one true parameter for fertility: A foal by the side of the mare next year.

Minimum examination procedure

A minimum examination procedure includes evaluation and palpation of the external genitalia, scrotum and testicles. Measurement of scrotal width and the size of the testicles are important as these measures can be used to establish the predicted daily sperm output (Squires *et al.* 2011). A rectal examination of the internal genitalia and sexual glands including ultrasound evaluation is also recommended by several authors (Pozor *et al.* 2006). Collection of two ejaculates with at least an hour in between the collections is normally recommended for further evaluation of semen in the laboratory. Blood samples and cultures for potential veneric (EIA, EVA, CEM etc.) diseases are recommended included in the evaluation.

Evaluation of semen

The semen can be evaluated by the use of simple light microscopy in most laboratories. A phase contrast microscope and a hemocytometer are used to calculate concentration and progressive motility. Stained spermatozoa can be evaluated in Eosin/Nigrosine slides to calculate viability and the proportion spermatozoa with normal conformation (Johansson *et al.* 2008). The use of automated fluoroscopy (Johansson *et al.* 2008) has been demonstrated useful and reliable to calculate concentration and viability in a semen sample as well as other automated instruments (CASA, Flowcytometer) can be useful in the further evaluation.

Fertility data – variation of data

Previous obtained fertility data of the stallion including pregnancy rate per cycle and number of live born foals are often more important in the evaluation of potential breeding soundness, than what can be found in a semen sample in the laboratory. These data however are often not recorded with any degree of precision and has to be evaluated with great care. The often low number of mares covered by a stallion often leaves a wide statistical variation to be included in the evaluation (Graham *et al.* 2005).

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Diagnosis, treatment and prognosis of endometritis

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Diagnosis of infertility problems in the mare based on samples recovered from the uterus has been used for almost a century (Schiebel, 1920). This concept has over the years remained almost the same, however the specific diagnostic techniques applied to analyze the recovered samples have been further developed. The methods used for diagnosing acute endometritis today includes endometrial swab, endometrial biopsy and endometrial flush, as well as the harvested material can be used for bacteriological, cytological and histological procedures (Nielsen 2005).
During the last decade it has become increasingly clear, that acute endometritis is not only initiated by pathogens. It is widely accepted, that uterine inflammation is a normal response to intrauterine deposition of semen. The normal mare will clear this inflammation within 48 hours post mating. The mare with compromised uterine clearance however will establish a more pathological condition including accumulation of inflammatory debris and fluid, which will persist for more than 48 hours (Troedsson 1999).

Diagnostic methods and procedures

Material from the endometrium and uterine lumen can be collected in at least three different ways: A guarded sterile cotton swab (Brook 1984), endometrial biopsy (Nielsen 2005) and uterine flush (LeBlanc et al. 2007). Each of these three methods has advantages and disadvantages. The guarded swab and the uterine flush can be used for diagnosing endometritis by cytology and bacteriology whereas an endometrial biopsy can be used for diagnosis by cytology, bacteriology and histology.

Bacteriology

Presumptive identification of bacteria and yeast can be made in laboratories in most practices. In a majority of cases pathogen identification can be performed based on colony morphology following incubation on blood-agar in atmospheric air for 24 and 48 hours. The sensitivity and negative predictive value of using a biopsy or an endometrial flush for bacteriology has been shown superior to the swab in several studies (Nielsen 2005, LeBlanc et al. 2007, Nielsen et al. 2010).

Cytology

Cells from the endometrial swab, biopsy or flush can be smeared on a microscope glass slide and stained. Examined by light microscopy the presence of polymorph nuclear cells (PMN´s) will indicate whether an inflammatory response is present in the endometrial tissue (Nielsen 2005).

Histology

Following preparation and staining PMN´s can, if present, be demonstrated in a histological slide. If PMN´s are present, an ongoing inflammatory response is present in the endometrial

tissue. Other histological abnormalities such as glandular structure, fibrosis and lymphatic lacunae, which are more related to chronic endometritis, will also become evident in the histological examination. The sensitivity and positive predictive value of the histological examination regarding inflammation is very high. The processing of the histological slides is however time consuming.
The histological abnormalities found in a slide from a biopsy can be used to classify the mare into different groups (I, IIa, IIb, III), that can be directly related to the chance for the mare to carry a foal to term (Kenney and Doig 1986). In a recent evaluation of the grading system proposed by Kenney and Doig at the authors own laboratory foaling rates of a total of 261 mares were compared to evaluation of endometrial histology. Foaling rates of the four groups (I, IIa, IIIb, and III) were found 88, 59, 52 and 42 percent respectively. This means that a mare in category I with no significant pathology found in the endometrium is respectively 5.5, 7 and 11 times more likely to carry a foal to term compared to a category IIa, IIb or III mare.

Fluorescent in Situ Hybridization (FISH)

The presence of Streptococcus Equi subspecies Zooepidemicus deep in the endometrium of the chronically infected mare has lately been described (Petersen et al. 2009). Using a specific oligonucleotide attached to a flourophore, streptococci could be visualized deep in the sub epithelial tissue and in the endometrial crypts. Disadvantages of this method are the use of an expensive fluorescence microscope and the reported low sensitivity (Petersen, unpublished data).

Treatment

The single factor, that has contributed most to an increase in pregnancy rates, has probably been the Caslick procedure. Mares that had vulvoplastic surgery performed, had a higher pregnancy rate than mares without this procedure (Nielsen et al 2008).
The use of uterine flush with 0.9 % saline or lactated ringers solution to help expel debris and inflammatory products from the uterine lumen has also been widely described. These uterine flushes are often used together with deposition of antibiotics and/or antifungal drugs in the uterine lumen. Blanchard et al (2003) provides an extensive list of dosages of antibiotic and antifungal treatment as well as possible disinfectants for uterine use.
Treatment with ecbolics such as oxytocin is often used in conjunction with uterine flush, antibiotic treatment and after breeding, and it has become a routine tool in the treatment of acute endometritis (Madil et al. 2002).
Down regulation of the inflammatory response by a single injection of dexamethasone at the time of breeding to susceptible mares has also been reported successful (Bucca et al. 2008).
The most inflammatory agent exposed to the uterus is often the semen itself. Management of the time of ovulation by ovulation-inducing agents is therefore often vital in the effort of reducing the inflammatory response to that of a single insemination or breeding.

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Diagnostics of changes in the endometrium of infertile Icelandic horse mares

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Endometritis is a common cause of infertility in barren mares. The diagnosis is based on transrectal manual and ultrasonographic examinations of the reproductive tract and on other samples or biopsies taken for cytological, bacteriological and histopathological examinations. The histological examination is considered to be the reference standard to diagnose endometritis in mares.

The aim of this study was to diagnose the changes in the endometrium of infertile mares. The research was conducted on 53 subfertile mares, aged 3 - 25, Icelandic horses, which had shown no discharge and no intrauterine fluid, but had been unsuccessfully inseminated during three oestrous cycles. Two endometrium biopsies were taken from all the mares from the base of either uterine horn with Kevorkian biopsy forceps. The endometrial biopsies were fixed in 4% formalin and examined for the presence of PMNs within the luminal epithelium, stratum compactum and stratum spongiosum, as well as for the Kenney-Doing score. The biopsies were considered as evidence of acute endometritis if there were more than three PMNs per five fields of high magnification (x400).

We found that in most biopsies there were differences in the number of PMNs and the Kenney-Doing score. The results of the histopatological examination were supplemented with cytological and bacteriological examinations collected with cotton swabs and with the fluid recovered from the uterine levage. Moreover, immunohistochemical examinations of all the endometrial biopsies were performed.

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Cytological evaluation of the mare *endometrium*

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Cytological examination of the endometrium is frequently used in diagnosis of uterine health in horses. The evaluation of inflammation in cytological smears is mainly based on determination of the numbers or percentages of polymorphonuclear cells present; nevertheless, it should be pointed out that there is a lack of agreement on guidelines for interpretation of cytology results. Additionally, nowadays samples for cytological evaluation of the endometrium can be collected in a variety of ways depending on physician preference, including the following methods: cotton swab, uterine cytological brush, low-volume uterine flush, and by preparing smears directly from the endometrial biopsy. The quantity and quality of the collected material is different among particular methods, and consequently results can also differ. It has been recently published that cytological evaluation of the mare endometrium should be based on counting numbers of polymorphonuclear cells in relation to epithelial cells rather than counting the number of polymorphonuclear cells per high power field, and the threshold level indicating endometritis for samples collected in estrus should be set at 2% of polymorphonuclear cells if specimens for cytological evaluation are collected using the cyto-brush technique and 1% of polymorphonuclear cells if specimens for cytological evaluation are collected using endometrial biopsy technique.

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Postpartum complications in a mare and their possible impact on further fertility

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Normal parturition and expulsion of fetal membranes in a proper time (0 min- 2 h p.p.) are good predictors of physiological puerperium. An outflow of lochial liquids usually finishes about 7 th day p.p. and in that time ovarian follicles rise and lead to so called foal- heat, that occurs 7-20 days p.p.

Postpartum period is often complicated. The most common abnormalities are uterine and vaginal trauma, uterine or rectal prolapse, retained fetal membranes, endometritis puerperalis, puerperal laminitis and mastitis.

Dystocia and fetotomy carry the risk of complications in the form of: uterine rupture during manipulation and extraction of sharp- edged fetal parts, haematomes of vulva and perineum, bleedings from endometrium and paralysis of the bladder. Uterus prolapse occurs rarely, usually several hours after dystocia and is usually accompanied by a rupture of mesometrium that leads to severe internal bleeding and death. Seldom there are cases of uterine rupture during labour and expulsion of bowels through the birth canal. In cases like that mares usually require euthanasia.

Injuries within the soft genital tract usually give chance to heal properly and allow the mare to return to reproduction when stitched within couple of hours. If the procedure is delayed long-term surgical treatment of fistulas is expected.

The most common postpartum complication is retention of fetal membranes. Some heavy breeds (like draft horses, Friesians) are genetically predisposed to this. Fetal membranes retention occurs more often after dystocia, twin pregnancy, placentitis and abortion. Because placenta loses its function directly after birth, it becomes subject of degenerative processes leading to slow decay and bacterial growth causing purulent endometritis. Toxic substances enter the bloodstream causing toxaemia and may lead to puerperal laminitis and sepsis.

Mastitis is far less frequent condition in mares than it is in cows because sucking foal empties the udder regularly. Symptoms are not characteristic and usually the first sing an owner sees is lameness and unwillingness to fed the foal. The udder is swollen, painful and the acute mastitis quickly turns into the chronic condition. It often leads to fibrosis and ulceration.

In summary, understanding causes of postpartum complications in mares, may improve their treatment and increase further fertility.

Mechanisms linking bacterial infections of the bovine endometrium to disease and infertility

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Postpartum microbial infections of the uterus often cause metritis and endometritis in dairy cattle. These uterine diseases are characterized by endometrial inflammation and tissue damage, the accumulation of pus in the uterus, and infertility. Bacteria cultured from the uterus of diseased animals include *Escherichia coli*, *Trueperella pyogenes*, and anaerobic species of bacteria. Although, more sensitive microbiological techniques yield additional bacterial species that may contribute to uterine disease. Irrespective of the species of invading bacteria, host cellular responses to infection depend on the balance between innate immunity and microbial virulence factors. The innate immune response by endometrial cells relies on Toll-like receptors binding pathogen-associated molecular patterns, including lipopolysaccharide and bacterial lipopeptides, which leads to the release of chemokines such as interleukin 8 to attract hematopoietic immune cells, and secretion of cytokines such as interleukin 6. The principal virulence factor of *T. pyogenes* is pyolysin, which forms pores in plasma membranes leading to cytolysis, particularly of endometrial stromal cells. Despite the tissue damage during microbial infection and associated with parturition, endometrial cells do not use their innate immune system to sense damage-associated molecular patterns. However, the combination of infection followed by tissue damage leads to release of the intracellular cytokine interleukin 1alpha from endometrial cells, which acts to scale the inflammatory response. Endometrial inflammation and tissue damage likely compromise reproductive physiology, but further work is needed to understand the precise mechanistic links between host-pathogen interactions and infertility.

Diagnosis and management of reproductive tract inflammatory disease in dairy cows

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Approximately 50% of cows are affected by at least one reproductive tract inflammatory disease (RTID: metritis, purulent vaginal discharge, endometritis, or cervicitis) in the postpartum period. Most cows experience a period of insulin resistance, fat mobilization, inflammation, and reduced effectiveness of immune function in early lactation. The mechanisms which influence the severity of these challenges and consequently the risk of RTID are increasingly understood, but it is not clear how to prevent these diseases through management. There are numerous links between fat metabolism, inflammation, immune function, and probably feed intake regulation. An excessive pro-inflammatory state early in the postpartum period appears to be a key feature of cows with endometritis about one month later. Aspects of innate immune function are commonly impaired in the transition period, particularly in association with elevated non-esterified fatty acid concentrations and to a lesser degree by ketosis. Changes in metabolism and immune function precede reproductive tract disease by several weeks. Implementation of nutritional and management best practices are likely to favor metabolic and reproductive health. Purulent vaginal discharge (PVD) is associated with an increase of approximately 30 days in the time from calving to pregnancy. PVD affects 15 to 20 % of cows at 4 to 5 weeks postpartum and can be accurately diagnosed by vaginoscopy, Metricheck device, or gloved hand inspection but ultrasound has low specificity and moderate specificity. The effect of PVD on pregnancy rate is mitigated by intrauterine treatment with cephapirin, whereas the effect of treatment with 1 or 2 injections of prostaglandin is equivocal. Endometritis is diagnosed by endometrial cytology using a low-volume flush or cytobrush. There is only fair agreement between the results of uterine cytology and histology, but the results of many large studies consistently show that 15 to 30 % of cows have > 5% neutrophils on cytology at 5 to 8 weeks postpartum, which is associated with an increase of 25 to 40 days to pregnancy. More than half of cases of endometritis do not have concurrent bacterial infection based on conventional microbiology. Endometritis and PVD are distinct conditions whose effects are additive. Practical cow-side diagnostic tools for endometritis are needed and effective treatment of endometritis remains unclear. Research is needed to increase understanding of RTID to develop more effective prevention, treatment selection criteria, and therapies.

Impact of negative energy balance and disease as risk factors for endometritis

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Dairy cows enter a period of negative energy balance (NEB) after calving during which they mobilize body tissues to support milk production. Changes in the synthesis, secretion and signalling pathways of somatotrophic hormones (insulin, GH, IGF1) and adipokines (e.g. leptin) are central to the regulation of these processes. As glucose supplies are limiting, lipids are preferentially used for energy production. This in turn causes oxidative damage to mitochondria in metabolically active tissues including the liver and reproductive tract. Following calving the endometrium also has to undergo significant tissue remodelling in order to reduce in size and repair surface erosion. Both epithelial and stromal cells possess pattern recognition receptors including toll-like receptors (TLRs) which detect pathogen-associated molecules such as bacterial lipopolysaccharide (LPS). LPS binding triggers two major downstream signaling pathways: (1) the MyD88-dependent pathway which activates NF-kB and MAPK signaling leading to the induction of inflammatory cytokines, and (2) the TRIF-dependent pathway (MyD88-independent) leading to the induction of type I IFNs via IRF3 activation and inflammatory cytokines also via NF-kB activation. A wide variety of both chemokines and antimicrobial proteins are also up-regulated to help clear the infection. The ability to mount an efficient immune response is, however, compromised after calving, leading to the development of uterine inflammatory disease. There are many significant associations between measures of NEB, including circulating non esterified fatty acids (NEFAs) and IGF1, analysis of immune cell populations within the endometrium and expression of genes associated with immune function. Additionally many dairy cows are exposed to a variety of viral infections. Cattle infected with non cytopathic bovine viral diarrhoea virus (ncpBVDV) appear increasingly susceptible to infection with bacteria so we investigated the response of endometrium to ncpBVDV using whole-transcriptomic gene expression arrays. ncpBVDV treatment alone showed interplay between induction and inhibition of immune responses. Down-regulation of inflammatory cytokines, chemokines, and serine proteinase inhibitors suggested mechanisms by which ncpBVDV may counter the host response. Comparison of combined treatment of BVDV+LPS vs. CONT+LPS revealed that many innate immune genes which typically respond to LPS were inhibited by ncpBVDV including those involved in pathogen recognition, inflammation, interferon response, chemokines, tissue remodeling, cell migration and cell death/survival. Many of these genes are also regulated by IFNT during maternal recognition of pregnancy. Infection with ncpBVDV may thus predispose cows to postpartum bacterial endometritis and reduced fertility.

Diversity and health status specific fluctuations of intrauterine microbial communities in postpartum dairy cows

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Reproductive disorders caused by bacterial uterine infections represent the most important reasons for substantial economic losses in dairy farming. A better understanding of fluctuations in uterine bacterial communities is required to interpret the interactions between pathogens, commensals and the host.

Therefore, Fourier-transform infrared (FTIR) spectroscopy was used to monitor the bovine microflora of postpartum (pp) dairy cows. A total of 40 (Farm A) and 170 cows (Farm B) were sampled at the day of calving and at day 3, 9, 15, and 21 pp using the cytobrush method. At day 21 pp the vaginal discharge was assessed by vaginoscopy and categorized in vaginal discharge scores (VDS) 0 to 3.

In total, the aerobic uterine microflora comprised a diversity of bacteria belonging to 202 different species. On both farms, *Trueperella pyogenes* and *Escherichia coli* were the most frequently detected bacteria followed by *Bacillus* spp., *Streptococcus* spp. and *Staphylococcus* spp. The infection pattern of *T. pyogenes* and *E. coli* were nearly identical on both farms. *T. pyogenes* was most prevalent at day 15 with 85.0% (Farm A) and 56.6% (Farm B) positive animals. The percentage of positive animals for *E. coli* was greatest at day 3 (60.0%, Farm A) and day 9 (45.1%, Farm B). *Streptococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. were most frequently detected at the day of calving with 23.8% to 59.0% positive animals. Regression analysis revealed that *T. pyogenes* infections on day 21 (Farm A) or on day 15 and 21 (Farm B) significantly increased the risk for VDS 2 and 3 at day 21. Depending on the day of sampling, various microbial interactions were observed. For instance, the presence of *S. uberis* at day 3 significantly increased the risk of *T. pyogenes* infections at day 9, possibly indicating synergistic interactions within the bovine uterus.

In conclusion, the uterine microflora comprised a variety of bacteria belonging to different taxonomic groups with so far unknown pathogenicity traits. The bacteria isolated in this study and the recorded FTIR spectral data provide a promising starting point for detailed investigations of intraspecies variability of uterine bacteria and their effects on host-pathogen interactions.

Temporal and spatial analysis of the microbial communities in the reproductive tract of the endometritic cows

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Bacterial colonization of the uterus affects a high percentage of animals during the postpartum period. Endometritic animals fail to clear this bacterial infection within three weeks postpartum. In order to understand the dynamics of microbiome and its relation with the health status of the reproductive tract of dairy cows at the peripartum period, this study relied on the analysis of 16S rDNA amplicons using terminal restriction fragment length polymorphism (TRFLP), a high-throughput and low-cost DNA profiling method, followed by deep sequencing (454 pyrosequencing) of selected samples. TRFLP showed that vaginal and uterine communities in the postpartum period appeared not to be significantly different. However, comparison of the communities at different times postpartum showed significant differences in animals that developed endometritis. The vaginal and uterine microbiomes at 7 days postpartum showed the greatest differences between cows that cleared the postpartum bacterial infection and those that developed postpartum endometritis. In contrast to healthy cows, a remarkable high similarity was observed between vaginal and uterine microbiomes in animals that developed endometritis at 7 days postpartum. To gain a deeper insight into the differences associated to the health status of the cows at 7 days postpartum, the microbiome was subsequently analysed by pyrosequencing of the variable regions v1 to v3 of the 16S rRNA. A massive reduction in bacterial diversity was associated to the dysbiosis occurring in the reproductive tract of animals that developed endometritis.

Bovine endometrial pro-inflammatory response differs depending on the strain of *Trueperella pyogenes*

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Uterine infection is a characteristic event of the postpartum period in cows. Among different bacterial populations colonizing the uterus, *Trueperella pyogenes* was found to be associated with clinical endometritis. The ability of a cow to defend against *T. pyogenes* depends on host innate immunity and virulence of invading bacteria. Aim of this study was to explore mRNA expression of pro-inflammatory factors in bovine endometrial epithelial cells in presence of two biochemically distinct strains of *T. pyogenes*. The bacterial strains (TP1 and TP2) were isolated from the uterus of a postpartum dairy cow with purulent vaginal discharge (TP1) and one without signs of clinical endometritis (TP2). To evaluate the potential effect of viable bacteria, bacterial endo- and exotoxins, endometrial epithelial cells isolated from five animals were co-cultured with each bacterial strain in form of alive, heat-inactivated (HI) or bacteria-free filtrate (BFF) at a multiplicity of infection of 1. Viability of endometrial cells was monitored using Trypan blue exclusion. After 2, 6 and 8h, RNA was extracted and subjected to RT-qPCR. Alive *T. pyogenes* of both strains caused death in more than 95% of the cells within 16h. In contrast, HI and BFF of both strains did not affect the viability of cells up to 72h. Compared with untreated controls, alive TP1 induced higher expression of *IL6* and *PTGS2* after 8h in co-cultured cells. Alive TP2 induced higher expression of *IL6* after 8h. However, alive TP2 induced lower expression of *CXCL3* and *IL6* after 2h. Comparing the effect of both strains on endometrial cells showed that alive TP1 induced higher expression of *CXCL3*, *IL8* after 2h and *PTGS2* after 8h compared with alive TP2. Lower expression of *IL6* was noticed in presence of BFF of TP1 strain compared with TP2 after 8h. In conclusion, the *T. pyogenes* strain recovered from a cow with clinical endometritis elicits higher pro-inflammatory response in endometrial cells than the strain isolated from a cow with a normal puerperium. These results may suggest that the strain of *T. pyogenes* could be a determinant factor in the development of clinical endometritis.

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Manifestation of infectious diseases of the reproductive organs in cows on the Urals region dairy farms

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Chlamydiosis, infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) has the greatest value among infectious pathology of the reproductive organs in cows on farms of the Urals region.

Chlamydiosis is the stationary and difficult to control infection causing significant decrease of reproduction indices. Reproductive disorders in cows associated with Chlamydophila abortus and include abortions, infertility, endometritis, obtaining non-viable calves. Examination at 21 farms with livestock more than 2,000 cows in the Urals region of the Russian Federation was carried. Antibodies to Chl. abortus was detected, range of seroprevalence was at the level from 56.4 to 83.3%. Seropositive animals marked with various pathologies of the reproductive tract such as habitual abortion (33%), abortion with fetal mummification (19%), abortion in the later stages of pregnancy (20%), 28% of cows clinical signs of disease not have been identified.

Infectious bovine rhinotracheitis was detected in 60.4% of cows and heifers. Clinical signs of pustular vulvovaginitis identified in 77.0% of cows and 57.0% of heifers. Seroprevalence of BHV-1 in unvaccinated animals was 54.5%, the abortion rate has been from 2.4 to 4.7%. Cows, which are used to live marked vaccine seroprevalence to field BHV-1 was 10%, the clinical signs of the disease not have been identified.

Bovine Viral Diarrhoea was detected in 11.8% of the examined farms. Persistently infection of BVDV in calves ranged from 8.8 to 9.0%. Clinical signs of disease were observed in 60.20% of nonviable calves as prenatal development defects (cleft lip, microphthalmia). The incidence of hidden abortions in cows 30-45 days of pregnancy was 17.11 - 20.23%. In BVDV-free farms this figure does not exceed 4,17-8.52%.

As a result, the Urals region infectious diseases of the reproductive organs in cattle are widespread and cause abortions at different stages of pregnancy, fetal mortality and other pathology, leading to decreased of reproduction indices.

Analysis of Systemic Changes in Cows with Subclinical Endometritis

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All cows experience an ascending influx of bacteria through the cervix during and soon after calving. While the majority clear the microbes; a significant proportion fails to do so, leading to prolonged endometrial inflammation, uterine disease and subfertility. One of the major causes of decreased fertility postpartum is endometritis and the diagnosis of sub-clinical disease is limited by the lack of sensitive and specific biomarkers. We hypothesise that early systemic immune changes associated with sub-clinical endometritis may provide prognostic indicators of cows which are likely to develop uterine disease. Uterine cytology, mucus scores and peripheral blood evaluations from postpartum cows (n=139) were performed at 7 and 21 days postpartum (DPP). Vaginal mucus was assessed on a scale of 0-4 (0 being no purulent material and 4 being >50% purulent or mucopurulent material by volume). Vaginal mucus assessment identified 61 cows (44%) with clinical endometritis at 21DPP. Currently cytological analysis is being performed (18% polymorphonuclear cells present to indicate subclinical infection). Once completed haematology, systemic metabolites, gene expression will be performed to compare healthy, subclinical and clinical cows (n=15/group); matched based on age, parity and calving date. The identification of systemic biomarkers associated with subclinical endometritis will improve reproductive health in cattle by aiding early detection and prevention of uterine disease.

A preliminary study on the prevalence of subclinical endometritis in cows with clinical endometritis after treatment with cephapirin, prostaglandin F2α or self-cured

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The aim of this study was to compare the prevalence of subclinical endometritis(SE) after different treatment methods of clinical endometritis vs self-cured cows (control group). Fourty nine cows between twenty one to twenty eight days after parturition with vaginal discharge were included into the study. Clinical endometritis was diagnosed on the basis of vaginoscopy, rectal palpation and ultrasound examination. Cows were randomly assigned into 3 groups according to the treatment method: group I – cows treated with PGF2α i.m. (n = 15), group II – cows treated with cephapirin i.u. (n = 18), group III cows left untreated (n = 16). Endometritis cases within each experimental group were divided into 2 subgroups on the basis of the character of vaginal discharge: mild – over 50% mucos (n = 29) and severe – over 50% pus (n = 21). Two weeks after treatment cows were clinically controlled and in animals still affected by endometritis, the treatment was repeated with the same method. Cytological samples were taken only from clinically healthy cows and subclinical endometritis was diagnosed by cytobrush using the threshold of 5% polymorphonuclear leucocytes. Totally, subclinical endometritis was diagnosed in 13,33% (n = 2) cows treated with PGF2α and in 16,67% (n = 3) treated with cephapirin , whereas in 31,25% (n = 5) of control cows. Taking into account a severity of endometritis we diagnosed similar proportion of cows affected by SE (mild - 20,69%, n = 6 vs severe – 20% , n = 4) independently from character of vaginal discharge before treatment.

From this study we conclude, that after clinical recovery, independently from the treatment method used, a number of cows with SE was similar and ranged between 13,33 – 16,67%, being slightly enhanced in untreated cows (31,25%). In this respect, we did not observe a difference between PGF2α vs cephapirin treatment. Similarly there was no relationship between severity of clinical endometritis and prevalence of SE.

Prevalence of subclinical endometritis at artificial insemination and its effect on subsequent conception rate in dairy cows: some preliminary results

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The primary objective of the present study was to determine the prevalence of subclinical endometritis during AI in both heifers and dairy cows and to assess its effect on the conception rate. Six experienced inseminators took endometrial cytology samples during AI using the newly developed CytoTape (CT) technique. Briefly, CT consisted of a 1,5 cm paper tape rolled on the top of a conventional AI catheter and covered with a double guard sheet. In total 2.935 Holstein Friesian cows (n=2.423) and heifers (n=512) were inseminated and sampled at the same time. After sampling, the top of the AI catheter was gently rolled onto a glass slide, air dried and stained using Diff-Quick®. For each slide, a total of 300 nucleated cells were counted, and the polymorphonuclear cell ratio (PMN%) was assessed at 400X. In total 98.29% of the samples were considered as qualitatively acceptable for further analysis. In the heifers, the mean PMN% was 0.31±1.49% while in cows it was 1.56±4.95%. Using 3% of PMNs as a threshold, SCE prevalence in heifers was 4.7% (n=24) while in cows it was 13.85% (n=329). Preliminary pregnancy results of 1.947 inseminations carried out by one single inseminator were assessed. An insemination was considered successful when pregnancy was confirmed by rectal palpation at least 45 d post-AI. Cows were defined to be not pregnant when they received a subsequent insemination or were diagnosed empty by rectal palpation. The overall conception rate in heifers (n=456) was 60.52%. In SCE positive heifers (n=24), this was 25% versus 62.5% (P=0.0003) in their negative counterparts (n=432). In cows, the overall conception rate was 43.58%. In SCE positive cows (n=217), the conception rate was 27.18% versus 46.36% (P<.0001) in SCE negative cows (n=1279). In conclusion, satisfactory endometrial cytology samples were harvested during AI using the CT technique. Subclinical endometritis diagnosed at AI significantly affects the fertility results. Based on the complete dataset, a PMN% threshold at insemination will be calculated, taking also other variables that are known to affect pregnancy results into account.

Relationships between systemic levels of progesterone, 17ß-estradiol, and $PGF_{2\alpha}$ on the immunolocalization and expression of the oxytocin, progesterone, estrogen α, and prostaglandin F receptors in postpartum bovine uteri challenged with LPS

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Uterine contractility plays a decisive role in uterine involution during the first few days after parturition. However, up to know it is not clear how uterine contractility is regulated during this time period. Therefore, the aim of this study was to investigate if there are relationships between progesterone (P_4), 17ß-estradiol ($E_{2ß}$), and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) and the receptors for oxytocin (OTR), progesterone **(**PR), estrogen α (ERα), and prostaglandin F (FPR), in inflamed postpartum bovine uteri. Inflammation was induced by intrauterine infusion of 5µg/mL LPS ad 1L 0.9%NaCl (37°C) in pluriparous 12 Holstein-Friesian cows after physiological placenta expulsion (6.0±1.8h after calving). Blood samples were collected from the jugular vein prior to the tissue collection (=3h after challenge). P_4, $E_{2ß}$, and PGFM averaged 0.36±0.14ng/mL, 87.6±39.5pg/mL, and 2.15±0.89 ng/mL, respectively. PR and ERα were localized in the nuclei of uterine cells, OTR in cytoplasm and FPR in both locations. PR was detected in compact and reticulated stroma and in myometrium. Cells from the surface epithelium, compact and reticular stroma, glands, vessel wall and myometrium were ERα, OTR and FPR-positive in different proportions (ERα, FPR) or intensities (FPR, OTR). The myometrial expression of ERα, PR, OTR and FPR mRNA transcripts was 22.9±0.7, 31.6±0.6, 24.6±0.9, and 27.3±1.9ΔCq, respectively. P_4 was positively associated with nuclear FPR in compact stroma ($p<0.05$; $r=0.69$); $E_{2ß}$ was negatively related with OTR mRNA transcripts ($p<0.05$; $r=-0.71$) as well as with the immunolocalization of ERα in compact stroma ($p<0.05$; $r=-0.67$) and positively related to the nuclear immunolocalization of FPR in vessel walls ($p<0.05$; $r=0.61$); PGFM showed positive relationships with PR mRNA transcripts ($p<0.05$; $r=0.70$) and the immunelocalization of OTR in glands ($p<0.05$; $r=0.81$) and of FPR in compact stroma and surface epithelium cytoplasm ($p<0.05$; $r=0.75$ and $r=0.61$, respectively). These results show that hormone receptors involved in uterine contractility are related to peripheral P_4, $E_{2ß}$, and PGFM concentrations in inflamed uteri after parturition. Further studies are needed to corroborate if there are causal relationships between peripheral concentrations of sexual hormones and their receptors to regulate uterine contractility after parturition.

Effect of Preimplantation factor in response to a LPS challenge on bovine endometrial IL-6 secretion and expression

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Preimplantation factor (PIF) is a 15 amino acid peptide that is secreted by viable foetuses as early as the two cell stage. PIF is able to alter the maternal immune response for the benefit of the embryo and translated to models of inflammation-based auto-immune disease has immune modulatory properties. The aim of this study was to investigate the use of PIF as an immune modulator within a model of bovine *E. coli* endometritis. The study used three different concentrations of lipopolysaccharide (LPS) to mimic different levels of *E. coli* infection. Bovine endometrial tissue (n=4) was cultured from uteri with a stage IV corpus luteum. Tissue was initially pre-treated for 24 hours with PIF (100 nM) or media alone before subsequent PIF and LPS treatment. Thereafter tissue was treated for 6 and 24 hours with: Control (medium alone), LPS (5, 50 and 500 ng/mL), PIF (100 nM) or LPS (5, 50 and 500 ng/mL) with PIF (100 nM). Tissue was collected post-treatment at 6 hours for RT-PCR analysis for IL6 expression. Culture media samples were collected from separate wells at 24 hours for ELISA analysis of IL-6 secretion. There was a significant increase in IL6 mRNA expression when tissue was stimulated with 50 and 500 ng/mL LPS ($P<0.05$). When PIF was added with 500 ng/mL LPS, IL6 gene expression was increased compared to the expression induced by 500 ng/mL LPS alone ($P<0.05$). There was no effect of PIF on IL6 expression with any other concentration of LPS ($P>0.05$). All concentrations of LPS significantly increased IL-6 secretion from explants ($P<0.001$). However, there was no effect of PIF on IL-6 secretion from explants ($P>0.05$). In conclusion, at gene expression level, PIF increased IL6 expression relative to the highest concentration of LPS alone, displaying a pro-inflammatory effect of PIF. However at a protein level, it was shown that PIF had no effect on IL-6 secretion from explants. It is possible that changes in protein secretion may be seen at earlier time points.

Is *E. coli* off the hook? New perspectives on microbial causes of uterine infection in the postpartum cow

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The microbial population in the uterus has been extensively studied in the dairy cow in order to elucidate the causes of postpartum uterine infections. Traditional culture techniques enabled the identification and semi-quantitation of the uterine microflora. Early studies using such techniques indicated that upwards of 90% of cows had microbial 'contamination' of the uterus after calving, and that this contamination persisted in around two thirds of animals. A number of different bacterial species were found to be present; the most commonly isolated bacterium being *Escherichia coli*, which dominates the uterine flora in the first few days postpartum and increases susceptibility of the uterus to subsequent infection with *Trueperella pyogenes*. Cows infected with *E. coli* in the early postpartum period have a higher risk of being diagnosed with abnormal vaginal discharge, and a higher risk of metritis and subclinical endometritis in subsequent weeks. Specific strains of *E.coli* have been shown to be particularly pathogenic to bovine endometrial cells and stimulate a host immune response. Furthermore, *E. coli* virulence genes associated with adhesion and invasion of the endometrium, and subsequent uterine disease, have been identified. It is not surprising therefore, that *E. coli* has long been considered a key modulator of postpartum uterine disease in dairy cattle.

Recent advances in culture independent techniques, based on analysis of genome sequences, has resulted in a growing literature of analysis of the postpartum uterine microbiome. These studies have shown that the bacterial diversity in the uterus between healthy and infected cows is much greater and more complex than described following traditional culturing techniques. Interestingly, although *E.coli* has previously been highlighted as being a key modulator of uterine disease, gene sequences related to this organism have not been found to be significant using culture independent techniques. This raises the question of whether the advent of new technologies has exposed an over-estimation of the importance of *E.coli* in the development of disease, as it suggests that other microorganisms, as yet unidentified, may have a more critical role in the development of postpartum uterine infections in dairy cattle.

So, is *E.coli* off the hook?

Our current understanding of the pathophysiology of equine endometritis

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Equine endometritis remains one of the most common causes of infertility in horse. While most mares are highly resistant to persistent endometritis in response to uterine contamination with bacteria and other antigenic substances, a subpopulation of mares fail to clear the induced inflammation in a timely fashion. These mares are considered to be susceptible to persistent inflammation, which may interfere with fertility. Based on the causing agent, equine endometritis can be divided into infectious or breeding-induced endometritis. Both conditions involve a rapid inflammatory response, characterized by the expression of pro- and anti- inflammatory cytokines and an influx of polymorphonuclear neutrophils (PMNs) into the uterus.

Mares resistant to persistent endometritis clear the inflammation within 24-36 hours after exposure to micro-organisms or semen. These mares have a rapid increase of pro-inflammatory cytokines in response to an inflammatory challenge, and an upregulation of inflammatory modulating cytokines within 6 hours after exposure. In conjunction with a sustained elevation of myoelectrical activity, these events are believed to be responsible for the transient nature of the inflammation. In contrast, susceptible mares fail to clear the inflammation in a timely fashion. They have been shown to have an imbalanced gene expression of pro- and anti-inflammatory cytokines during early stages of the inflammation, and have also been shown to suffer from an abnormal accumulation of intraluminal nitric oxide, which may be related to the impaired myoelectrical activity that has been proposed to cause delayed uterine clearance. As a consequence, these mares establish a chronic inflammation, which interferes with the establishment of pregnancy if the mare has conceived.

Recent studies on the expression of inflammatory mediators in resistant and susceptible mares at different time points after an induced inflammation have revealed interesting information, which suggest an underlying immunologic basis for susceptibility to persistent inflammation. However, the inflammatory pathways have yet not been fully studied, and a relationship between cytokine expression, nitric oxide, and myometrial contractions has not been established. In addition, seminal plasma has been shown to modulate breeding induced inflammation, but the proteins involved in this process or the molecular basis of the modulation are not fully understood. A genomic approach appears to be needed in order to better understand the characteristics of inflammatory pathway and ultimately the pathophysiology of the disease.

Evidence-based treatment of equine endometritis

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Many commonly used treatments of equine endometritis derive from clinical experiences. In a practice situation, it is impossible to have non-treated control animals. Even if there are control groups, treated with either placebo or with the standard drug, the numbers of animals are often too low to allow proper statistical comparison.

Routine treatments of equine endometritis are uterine lavage, ecbolic hormones, mucolytics and antibiotics. New additions are cell-wall extracts, platelet rich plasma (PRP) and dexamethasone.

Uterine lavage fluid (1 l of saline or Ringer's at a time) is distributed throughout the entire endometrial surface and effectively removes debris, pus, inflammatory by-products and fluid. In addition, it stimulates uterine contractions. Lavage is indicated before antibiotic administration because in the presence of pus many antibiotics are inactivated and lose their efficacy. Uterine lavage is commonly used after breeding to remove excess sperm and inflammatory by-products in order to prevent the post-mating endometritis to become persistent.

Ecbolic hormones, oxytocin and prostaglandins, induce uterine contractions and thereby facilitate uterine drainage. Their half-lives, duration of contractility and doses have been studied. They are routinely used after breeding and in connection with intrauterine treatments to improve uterine clearance.

Mucolytics or solvents include kerosene, dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC). Kerosene and DMSO are older treatments and have been replaced by NAC. The NAC-treatment has to be local, since parenteral administration is not effective. There are clinical studies showing improved pregnancy rates of problem mares after NAC-treatment. Normally the NAC-solution is flushed from uterus after 6 to 12 h, but it can also be left in the uterus.

Characteristics of antibiotics – efficacy against different microorganisms, dose, duration of minimal inhibitory concentrations, penetrance, route of administration, etc. – have been studied in many species and in many diseases; our practices in the treatment of equine endometritis are based on these studies, not so much in experiments carried out on mares with endometritis. Generally, antibiotics produce higher concentrations for a longer time in the endometrium and in the lumen after intrauterine administration as compared to parenteral treatment. However, some antibiotics are too irritant for intrauterine administration and have to be administered systemically.

Some clinical experiments have been done in small numbers of problem mares concerning local treatments with cell-wall extracts and PRP claiming positive effects. Pre-breeding treatment with intravenous dexamethasone improves pregnancy rates only in mares with recurrent or persistent accumulation of intrauterine fluid.

Monitoring of the incidence of fluid in the uterus after insemination and its impact on fertility in mares

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PPBEM – Post-breeding endometritis is the main cause of infertility in mares. Endometritis presents a normal condition in the period immediately post partum, however, the presence of fluid in the uterus later than 12 hours post partum is pathological. A total of 167 cycling mares of Slovak warmblood breed and Quarter Horse were used for the study**.** These were categorized into groups by age. In 25% young mare was found fluids in the uterus after insemination. In 20% mare after one or more foaling was found fluids in the uterus. In 34,4% mare with problematic reproduction history was detected fluids in the uterus. In 18,19% mare 10 years old, in 28,57 % between 11-17 years old and in 72,72% more than 17 years old were determined fluids in the uterus. The lowest percent of pregnancy was found in mare with pathological reproduction history. In young mare were found 37,5% of pregnancy, in mare 52,63% of pregnancy and older mare (more than 17 years old) 35,7% pregnancy all with fluids in the uterus. The highest percent of pregnancy (75,4%) were determined after inseminations with cool semen from 24 to 12 hours before ovulation opposite 12 hours before ovulation (60,9%) and opposite 12 hours after ovulation (53,2). Percentage of pregnancy was lower (37,03%) in mare after treatment with oxytocin i.m. 15 i.u. 6,12,24 hours after artificial insemination (AI) opposite mare after tratment with flushing of uterus by Ringer lactate (44,4%) or after combination therapy (oxytocin and flushing) (45,45%). This work was supported by Ministry of Education of Slovak Republic, VEGA 1/0366/15.

Incidence of endometritis and pregnancy rate is affected due to age and prolonged estrus in ovulation induced thorough bred brood mares: A clinical study

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The covering success is lower during transition periods of ovarian activity in mares. The success depends upon proper time of covering, age, quality of follicle, oocyte and season. The current study investigated the effect of age and duration of prolonged estrus upon the pregnancy rate and incidence of endometritis in ovulation induced thorough bred brood mares during long transition period. The mares exhibiting long estrus (>8 days, n=21) were scanned for size of dominant follicle and uterine edema; and received hCG (5000 iu) for ovulation induction (follicular size >35mm) and covered twice. The mares were categorized by age (A1: <8 and A2: >8yr), duration of estrus (E1: 8-15 and E2: >15 days, abnormally long). The mares were again scanned for ovulation success and changes in uterus at 5-8th day and later for pregnancy at 20-30th day post covering. The data for pregnancy and incidence of endometriosis with chi-square test. The strength of association was calculated with Phi coefficient of association. The pregnancy rate was recorded higher (P = 0.027) in A1 than A2 (58.3 vs 11.1% respectively) exhibiting strong negative association with age (Phi = -0.48). Similarly, the pregnancy rate was higher (P=0.02) in E1 than E2 (66.7 and 16.7% respectively) showing strong negative relation (Phi= -0.51) with duration of prolonged heat. The incidence of endometritis was lower (P= 0.001) in A1 group than A2 (8.3 and 77.8% respectively) and strong association with age (Phi= 0.708). However, the ratio of endometritis did not differ between E1 and E2. Interestingly, when A1 group was further split based upon duration of heat, the pregnancy rate was higher (P=0.028) in mares with heat <15 days containing strong negative association (Phi= -0.714), explaining that prolonged duration of heat decreases pregnancy rate even in young mares. Moreover, the endometritis occurred more in old mares (E2 group) when heat was more prolonged (>15 days) showing strong negative association (Phi= 0.598). It is concluded that young mares with less than 14 days of heat can be made pregnant with less incidence of endometritis in controlled breeding program during transition period. The higher age with prolonged duration of estrus decreases pregnancy rate with higher ratio of endometritis.

Treatment of post-insemination inflammation and endometritis in mares in clinical practice

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Mares are essentially resistant to infection during the follicular phase and become increasingly susceptible during the luteal phase as progesterone concentrations rise and particularly as the cervical canal begins to close. Therefore treatment to assist the natural defence mechanisms must be carried out during oestrus and during a narrow window of time after ovulation. Insemination close to or after ovulation, substantially reduces the available time for treatment compared with a mating 3-4 days before ovulation.

Mares visited once every two days are given intrauterine antibiotics at the first opportunity following mating, followed by a single intravenous 25 units of oxytocin the next day. Those which show significant depths of fluid are re-examined two days later and given further oxytocin injections for up to 3 days after ovulation. Mares known to be susceptible to infection and/or fluid retention are purposely mated before the arrival of the veterinarian so that antibiotics can be infused at as short an interval as possible after mating. The most serious 'problem' mares are mated just before arrival and first given oxytocin followed by antibiotics 30-60 minutes later and followed by further oxytocin that night and the next day. Matings are made at least an estimated 2-3 days before ovulation and are not repeated even if this interval exceeds 3 days. The longevity of sperm in the mare is generally underestimated

At the Clinic, all mares are routinely examined every 8+/-1 hours during oestrus and until after ovulation. Chilled semen is usually inseminated in the 8 hours before ovulation and frozen semen in the 8 hours after ovulation. Following mating or insemination, at the next examination, no more than 8-9 hours later, all mares are given a 1 litre saline lavage followed by oxytocin. Then 8 hours later, routine antibiotics (penicillin and framycetin), and further oxytocin 8 hours after that and repeated when fluid is still present.

When the use of chilled or frozen semen in known problem mares is unavoidable, then the lavage is performed within 4 hours of insemination followed immediately by oxytocin and then antibiotics about 10 minutes later. Digital dilation of a tight cervix is considered important especially when following oxytocin, fluid is seen accumulating anterior to the internal os. Either 75 mcg d-cloprostenol or 100 units oxytocin given subcutaneously are sometimes found to be more effective than intravenous oxytocin. When fluid returns by the time of the next examination, the whole procedure is repeated and 10 mg dexamethasone given daily. Acceptable pregnancy rates have been achieved following lavage as early as 1 hour post insemination. Antibiotic treatment is only repeated in mares where infection has been a previous problem.

Infectious endometritis is associated with endometrial expression of lactoferrin in broodmares

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Infectious endometritis has for decades in numerous papers been described as the major cause of infertility in mares. The inflammatory response secondary to uterine infection appears to be a major contributor to a suboptimal uterine environment. The aim of the present study was to characterize the endometrial gene expression of the glycoprotein lactoferrin in brood mares and to evaluate if the expression was associated with infectious endometritis. Endometrial biopsies were obtained from broodmares 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 as well as cytology 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 as the most frequently isolated agens (45%). Data was analysed using the non-parametric Kruskal-Wallis test. Expression of lactoferrin was significantly increased in mares with infectious endometritis compared to mares with no growth of uterine pathogens. Lactoferrin expression was also associated with positive cytology (>0.5% PMNs as described byNielsen, 2005). The results indicate that lactoferrin plays a role in the innate immune response and emphasizes its ability to function as a bacteriostatic and bactericidal agent.

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Prostaglandins production in heavy draft mares that retain fetal membranes and those that deliver fetal membranes physiologically

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In horses, the mechanism of release of fetal membranes is unknown. Understanding this mechanism is important for applying the correct treatment for fetal membranes retention (FMR). FMR is often treated with either oxytocin or prostaglandin injections to stimulate muscular contractions. Thus, we asked whether, at foal delivery, heavy mares that retain fetal membranes and those that release these membranes physiologically differ in terms of mRNA expression of prostaglandin synthases (COX-2, PGFS and PGES), protein content of prostaglandins (PGF2α and PGE2), and the tissue location of COX-2, PGF2α, PGE2, and receptors for PGF2α and PGE2 in the allantochorion and endometrium.

To answer these questions, we took samples from the allantochorion and endometrium of 33 Polish heavy draft mares immediately after foal delivery. Fetal membranes retention (FMR) was defined as failure to deliver these membranes within 3 h of foal delivery. 20 mares delivered fetal membranes physiologically and 13 mares retained them. To confirm that the tissues were correctly sampled, and to examine their characteristics, they were histologically examined and their characteristics were graded on a 6-point scale by a pathologist who was blinded to the study. For measurement of mRNA expression of COX-2, PGFS and PGES synthases, qPCR was used. To determine the tissue concentrations of PGF2α and PGE2, EIA was done. The location of COX-2, PGF2α, and PGE2, and receptors for PGF2α and PGE2 was shown by immunostaining.

Histology confirmed that sampling was done correctly. The allantochorion of FMR mares was more hyperemic and infiltrated by immune cells to a greater extent than in control mares ($P<0.05$). FMR mares expressed significantly less COX-2 mRNA in the endometrium and COX-2 and PGES mRNA in the allantochorion than control mares (endometrium—COX-2, 26 times less, $P=0.046$; allantochorion—COX-2, 6.3 times less, $P<0.001$; PGES, 2.2 times less, $P<0.001$). All other differences in mRNA expression were not significant. FMR mares had a significantly higher concentration of PGF2α in both placental compartments than control mares (endometrium—FMR 44 pg/mg, control 19 pg/mg, $P=0.017$; allantochorion—FMR 247 pg/mg, control 180 pg/mg; $P=0.046$). Although the differences were not significant, FMR mares had more than twice as much PGE2 in the endometrium than control mares (FMR 61 pg/mg, control 26 pg/mg, $P=0.057$), but somewhat less PGE2 in the allantochorion (FMR 470 pg/mg, control 520 pg/mg, $P=0.53$). Immunostaining showed the presence of COX-2, PGF2α, and PGE2, and receptors for PGF2α and PGE2 in the epithelial and endothelial cells in both the endometrium and the allantochorion.

These results indicate that the process of production of PGF2α and PGE2 differs in FMR mares and mares that deliver fetal membranes physiologically. The differences suggest that, in the context of FMR in heavy mares, it would be worth investigating tissue remodeling and immune processes that are modulated by these hormones.

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How challenging is chronic endometritis in the mare?

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As in other organs with chronic inflammatory diseases, fibrosis is a hallmark of this pathological condition in the mare endometrium. Chronic degenerative endometritis (endometrosis) has long been linked to infertility and large economic losses in equine industry. This apparently irreversible process has been a challenge for decades: while its complex physiopathological mechanisms are not completely understood, new therapeutic approaches to resolve this endometrium dysfunction are also under study. In fibrosis, excessive deposition of extracellular matrix (ECM) severely impairs tissue architecture and function, eventually resulting in organ failure. The exact cause for endometrium fibrosis has been a subject of our research for some time. While most mares are capable of fighting the physiologic endometritis that occurs after mating, others are not. Those mares - susceptible to chronic endometritis - have a recurrent influx of polymorphonuclear neutrophils (PMN) in the uterus and subsequent endometrium fibrosis development. Equine PMN, as the first line of innate immune defense, in response to bacteria causing endometritis in mares (*Streptococcus zooepidemicus, Escherichia coli* or *Staphylococcus capitis*) release their DNA and form neutrophil extracellular traps (NETs). Thus, formation of NETs that entangle bacteria might be a complementary mechanism to fight some bacteria causing endometritis. But, an intriguing dilemma is the dual role of NETs: while they kill microorganisms in mare uterus fighting undesirable infection, they also have pro-inflammatory properties and contribute for endometrium fibrosis. This fibrosis process might be primarily mediated by the induction of myofibroblasts, which produce large amounts of collagen I, the main component of ECM. Accordingly, the origin, developmental pathways, and mechanisms of myofibroblast regulation are attracting increasing attention as potential therapeutic targets. The fibrotic cascade, from initial epithelial damage to eventual myofibroblast induction, is mediated by complex biological processes such as inflammatory cells infiltration, and by inflammatory mediators such as NETs components (elastase, cathepsin-G, myeloperoxidase), and cytokines/growth factors like transforming growth factor-β (TGFβ), connective tissue growth factor (CTGF), platelet derived growth factor (PDGF), interleukins (IL), and others. Besides, the role of prostaglandins (PG) on endometrium fibrogenesis modulation should be considered, since PGF2α may cause not only luteolysis and/or early pregnancy loss, but also collagen deposition in mare endometrium. A number of stimuli and pathways modulating mare endometrosis establishment are still to be unraveled.

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Mare endometrosis and pro-fibrotic cytokines: what's new?

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Transforming growth factor beta 1 (TGF-β1) and its receptors I and II (TGFRI, TGFRII), Platelet derived growth factor (PDGF), Connective tissue growth factor (CTGF), Matrix metalloproteinases (MMPs) and Tissue inhibitor of metalloproteinase 1 (TIMP-1) are important in fibrogenesis. Endometrosis is a mare endometrial disorder where tissue structure disruption and dysfunction result from excessive deposition of extracellular-matrix (ECM), like type I and III collagens (COL1, COL3). Our studies on follicular phase (FP) endometrium explants treated with TGF-β1, PDGF or CTGF upregulated COL1 and COL3 gene transcription at different incubation times. Thus, the aim was to evaluate TGF-β1, PDGF and CTGF *in vitro* effects on expression of genes COL1, COL3, TGF-β1, TGFRI, TGFRII, MMP-2, MMP-9, TIMP-1 and TGF-β1, TIMP-1, TNFα, PGE_2 and PGF2α secretion in mare luteal phase (LP) endometrium. Endometrium explants (n=5) were cultured (24h, 48h, 72h) with TGF-β1 (1, 10ng/ml), PDGF (0.1, 5ng/ml), CTGF (100, 200ng/ml), TNFα (10ng/ml) or oxytocin (10^{-7} M). Real-time PCR was used for mRNA transcription evaluation. Protein expression was quantified in culture medium by Elisa. TGF-β1 (10ng/ml) raised mRNA of COL1 (24h, 72h), COL3 (72h), TGFRI (24h, 72h), TGFRII (72h) ($P<0.05$) and MMP-9 (24h, $P<0.001$; 72h, $P<0.05$). TGF-β1 protein expression was up-regulated at all times ($p< 0.0001$). CTGF effect on COL1 and COL3 mRNA was dose and time dependent. At 48h, CTGF (100ng/ml) increased COL1 and TGFRI and decreased COL3 mRNA ($P<0.05$). At 72h, high dose CTGF raised COL1, COL3 and TGF-β1 mRNA transcription ($P<0.05$). CTGF (100ng/ml at 48h; 200ng/ml at 72h) decreased protein expression of PGE_2 ($P<0.05$), raised PGF2α/PGE_2 ratio ($P<0.001$) and TIMP-1 protein ($P<0.05$). TNFα up-regulated COL1, TGF-β1, TGFRI, TGFRII, MMP-9 mRNA transcription ($P<0.05$), at 48h. TNFα protein expression was up-regulated at all times ($p< 0.0001$). In LP, the positive cross-talk between PDGF and TGF-β1 noticed in FP, was not detected. Besides TGF-β1 direct effect on its receptors, it directly induces MMP-9 expression. Since TGF-β1 is secreted in inactive form, MMP-9 may cleave latent TGF-β and activate it. CTGF appears to mediate endometrium fibrosis through stimulation of TGF-β1 signaling and TIMP-1 production. TIMP-1 might prevent MMPs ECM degradation and thus participate in ECM accumulation. Besides, a high PGF2α/PGE_2 ratio seems to generate an enabling cytokine environment to CTGF induced fibrosis. TNF may mediate endometrium fibrosis through TGF-β1 and MMP-9 actions. In conclusion, this study shows a possible involvement of TGF-β1, CTGF and TNF in the development of mare endometrial fibrosis during luteal phase.

MMP-2 and MMP-9 in equine *endometrosis*

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Endometrosis 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 in the mare. Extracellular matrix (ECM) accumulation in fibrosis seems to be the consequence of two processes: an increase in the expression and deposition of connective tissue proteins and/or a decrease in the degradation of ECM proteins. Collagen turnover and ECM remodeling are regulated by various matrix metalloproteinases (MMPs). Most MMPs are not constitutively expressed by cells *in vivo*, but their expression is induced by exogenous signals, e.g. cytokines (IL-1β, IL-6), growth factors, or altered cell-matrix and cell-cell contacts. The aim of the present study was to determine whether MMP-2 and MMP-9 are concerned in the pathogenesis of *endometrosis.* In Exp 1, the endometrial potential activity and mRNA expression of *MMP2* and *MMP-9* were determined. A total of 24 uteri from *diestrus* (n=6 for each category I, II A, II B, III) and 20 uteri from *estrus* (n=5 for each category I, II A, II B, III) were used. The expressions of *MMP-2* and *MMP-9* mRNA and potential activity of both MMPs were performed using quantitative RT-PCR and zymography, respectively. In Exp 2, the effect IL-1β and IL-6 on the expressions of *MMP2* and *MMP-9* mRNA and secretion in the course of *endometrosis* were determined. The endometrial explants from *diestrus* (n=20; category I, II A, II B, III) were incubated for 24 h with IL-1β (10 ng/ml) and IL-6 (10 ng/ml). *MMP-2* expression did not change in the course of *endometrosis* ($P>0.05$), but its potential activity was up-regulated in III category endometria ($P<0.05$). *MMP-9* mRNA expression was highest in category III endometria ($P<0.05$), but its potential activity differed depending on fibrosis degree ($P<0.05$). Interleukin-1β increased MMP-2 secretion only in category I endometrium ($P<0.05$), but did not affect MMP-9 secretion in any stage of *endometrosis*. The effect of IL-6 on MMP-2 and MMP-9 secretion differed according to *endometrosis* degree. Our findings suggest that the alteration in *MMPs* expression, their potential activity and secretion in *endometrosis* may lead to excessive ECM deposition.

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HOW TO TREAT UTERINE INFECTIONS IN THE BITCH: MEDICAL OR SURGICAL THERAPY

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In the bitch, purulent endometritis or pyometra is a frequent life-threatening disease, affecting mainly aging intact females. Uterine infection may be linked to hormonal imbalance or abnormal response to gonadal steroidal hormones estrogens and progesterone. During diestrus, the high level of progesterone secretion decreases myometrial contraction, induces the cervix to clossing and tends to decrease the efficiency of non-specific immunity, leading to the promotion of bacterial adherence, colonisation and growth. Bacterial contamination of the uterus appears to be a normal phenomenon in the proestral or estral birch. The uterine response to the presence of bacteria is largely based on innate immunity.
In most cases, pyometra is diagnosed at a late stage of the disease, because clinical manifestations are not characteristic, especially in case of closed-cervix pyometra. Endotoxemia, bacterial spreading and chronic inflammation frequently lead to electrolytes/acid-base imbalances, dehydration, hypoproteinemiaand anemia. So purulent endometritis or pyometra is a medical emergency that requires rapid intervention to prevent over whelming sepsis. Without treatment, the infection is fatal.

Ovariohysterectomy, combined with antibiotherapy and resuscitation procedures, is the radical treatment of choice. Ovariohysterectomy suppresses both the source of hormonal production (ovaries) and the primary infection site (uterus). This surgical intervention is particularly recommandedinag in bitches with pyometra at an early stage in a healthy bitch. When CEH is revealed at ultrasonography, surgical treatment should also be chosen. CEH is often associated with infertility.

When ovariohysterectomy cannot be performed (breeding female dogs, high-risk bitches), medical treatment may be considered. Medical treatment should be restricted to stable bitches in good condition. Aglepristone, alone (10 mg/kg injected SC 24 hours apart, followed by weekly injections at the same dose until complete recovery of the animal) or combined with cloprostenol (1 microgramme/kg, once a day for 2 to 5 days), or cabergoline (5 micrograms/kg orally once a day for 7 to 10 days) combined with cloprostenol (1 or 5 microgram/kg SC every third day for 7 to 10 days), have been found to be safe and efficient in a large number of cases. However, recurrences of pyometra are possible at following estrus.

Sub-clinical endometritis as a cause of infertility or embryonic resorption in the bitch

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Infertility is a growing problem in veterinary practice, due to the interest in breeding pure-bred dogs.

Preliminary studies performed in Australia (Watts and Wright 1995, Watts et al.1997) tended to show that uterine sub-clinical diseases may be at the origin of infertility problems in the bitch. As endometritis is considered by several authors as representing one of the major cause of infertility in mares (LeBlanc and Causey 2009), we made the hypothesis that it could be the same in bitches and we underwent a series of experiments to confirm/infirm it. Using the same tools and the same cytological criteria used by Watts and Wright (1995), we investigated uterine and cytology by cannulating the cervix on a vaginoscopic approach from 26 bitches suffering from infertility (Fontaine et al. 2009). Thirty-eight percent (10 ⁄ 26) bitches were suffering from endometritis. Bitches were examined in anoestrus (4), prooestrus (1) and dioestrus (21). Among them, 70% (7 ⁄ 10) showed bacteria in heavy growth inside the uterus. In order to confirm if these bacteria were really intra-uterine or if they were a contamination in the vagina, in a second study in our laboratory (Mir et al.2013), we investigated 21 bitches: 14 bitches with unexplained infertility (group 1) and 7 bitches that had experienced unexplained pregnancy loss (group 2). This time, samplings were performed by surgical uterine biopsies by laparotomy.16/21 bitches were in dioestrus at the time of biopsy. In group 1, 4/14 showed sub-clinical endometritis. In group 2, 3/7 bitches suffered from sub-clinical endometritis. Research of infectious agents was performed in 20 of 21 cases. Surprisingly, no bacteria were isolated from the uterine lumen in any of the cases.

The high incidence of endometritis in sub-fertile bitches was confirmed by another study (Gifford et al. 2014) on a much higher number of cases, in which the authors found endometritis in 170/399 bitches (42.6% of the cases).

What remains unknown is the origin of sub-clinical endometritis as a potential cause of sub-fertility in bitches. In a recent retrospective study in our laboratory (Borges et al.2014), 15 subfertile bitches were treated with anti-cox2 NSAID carprofen (n=11) or meloxicam (n=4) in the 5 days that followed insemination, without any antibiotics. All bitches conceived , with a mean litter size of 7.7 puppies (4 to 14). However, in another study, England et al. (2012) had found that post-mating administration of systemic antibiotics increased the pregnancy rates in bitches with endometrial hyperplasia.

All these studies show that subclinical endometritis seem to be a common cause of subfertility in the bitch and that further studies are needed to better understand the pathogenicity and the treatment of this problem.

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ENDOMETRIAL FACTORS FAVORING PYOMETRA ONSET IN DIESTRUS

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Pyometra is the most common inflammatory disease of the canine uterus, and may progress from an existing disease (like cystic endometrial and mucometra), or originate from uterine contamination following parturition or oestrus (endometritis-metritis), the latter mainly in young females. Any way, pyometra is typically a diestrus disease, which exact aetiology remains rather unclear. The involvement of ovarian steroids was suggested, mainly the prolonged repeated progesterone stimuli typical of the canine cycle. Moreover, it is commonly believed that the inflammatory condition is initially silent, for an unquantified period, preceding the onset of clinical signs of pyometra. This report pretends to discuss available information on cellular and molecular changes in canine endometrium during diestrus (DI) that may foster the development of pyometra.

Endometrial immunity relies on cell-mediated mechanisms as well as in molecular pathways. Recently, we shown that the number of resident T-lymphocytes and macrophages markedly decreased in progesterone stages (1). This may in part be compensated by a slight increase in neovascularization mediated by VEGF, particularly in early dioestrus, in healthy endometrium (unpublished data); however, in some conditions, like in CEH, a reduction in the number of endometrial vessels was reported (2). We also found a decrease in the strength of the E-cad/□-catenin complex in DI, at the level of surface and the superficial glandular epithelia, suggestive of a weakening of the epithelial barrier that favour the endometrial invasion by the embryo but also by pathogens (unpublished data). It was also shown that in DI the production of endometrial mucin is decreased, which was associated with increased bacteria adherence in the canine uterus (3). Little is unveiled on the local cyclic changes in endometrial interleukins but it was shown that TNF, a pleiotropic cytokine, shows a decrease in the dioestrus (4) as well as all the three TGF-β isoforms (5). Therefore, cellular and molecular changes in the canine endometrium in DI may facilitate pyometra.

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Changes in ovaries and uterus after aglepristone administration in the third week of luteal phase of non-pregnant bitches

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Aglepristone (RU534) is a progesterone receptor (PR) antagonist used for endometritis pyometra complex (EPC) treatment, termination of pregnancy and for delivery induction. However, the exact physiologic consequence of blocking of nuclear PR in uterus and ovaries in diestrus phase remains unclear.

The aim of this study was to describe the mechanism by which aglepristone influences ovaries and uterus and to measure the levels of steroid sex hormones in non-pregnant bitches.

Fourteen bitches assigned to a study (n=9) and control (n=5) group were given aglepristone and saline solution, respectively, on the 19th and 20th day after LH peak. On the 26th day after LH peak an ovariohysterectomy was performed. Blood samples were screened for estradiol and progesterone concentrations. Ovaries and uterine horns and bodies were isolated for histological and morphometrical diagnosis and immunohistochemistry analysis of α-estrogen and progesterone receptor expression.

A decrease of progesterone ($p<0.01$) and no differences in total estrogen level in the study group were observed. There were no significant differences either in the histomorphometry or α-estrogen and progesterone receptors expression in ovaries.

Increase in expression of progesterone receptors in endometrium without surface epithelium of horns ($p<0.05$), endometrial surface epithelium ($p<0.05$), myometrium of uterine body ($p<0.01$) and estrogen receptors in endometrium without surface epithelium of horns ($p<0.05$) was observed. Elevated estrogen receptors probably increased sensitivity of tissues to estrogens in the bloodstream and led to notable inflammation, haemorrhages, and hyperplasia in endometrium with mononuclear immune cell infiltration.

The myometrium of horns and endometrium of uterine body of study bitches were significantly thicker than in the control group ($p<0.05$ and $p<0.01$). Furthermore myometrium of uterine body was thicker than myometrium of horns ($p<0.001$) and expression of progesterone receptors was higher in uterine body ($p<0.01$). No differences were observed between endometrium of horns and body within groups.

To the knowledge of the authors this is the first study, which describes the inflammatory effect developing in uterus in response to aglepristone administration, and attempts to elucidate its mechanisms.

Microarray assay as a fingerprint in endometritis – pyometra in bitches.

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Canine pyometra is defined as a complex disease associated with activation and proliferation of immune specific cells, B and T cells, as well as synthesis and activation of immune and pro-inflammatory molecules. Although all of these mechanisms are well recognized in several human immune diseases and cancers, the possible role or dysfunction of these molecules in dogs with pyometra still require investigations. The objective of the study was to evaluate the gene expression profile in dogs with pyometra compared with those that were clinically normal. The study included uteri from 87 mongrel bitches (47 with pyometra, 40 clinically healthy). RNA used for the microarray study was pooled to four separated vials for control and pyometra. Altogether, 17.138 different transcripts were analyzed in the uteri of female dogs with pyometra and of healthy controls. From 264 inflammatory response-related transcripts, we found 23 transcripts that revealed 10- to 77-fold increased expression. Thereby, expression of interleukin 8 (IL8), interleukin-1-beta (IL1B), interleukin 18 receptor (IL18RAP), interleukin 1-alpha (IL1A), interleukin receptor antagonist (IL1RN) and interleukin 6 (IL6) increased 77-, 20-, 17-, 13-, 13- and 11-fold, respectively. Furthermore, expression of calcium binding proteins S100A8 was 44-fold higher, and that of S100A12 and S100A9 37-times, respectively, in the uteri of canines with pyometra compared with that of the controls. Moreover, expression of transcripts of toll-like receptors (TLR8 and TLR2), integrin beta 2 (ITGB2), chemokine ligand 3 (CCL3), semaphorin 7A (SEMA7A), CD14 and prostaglandin-endoperoxide synthase 2 (PTGS2) was increased between 10- and 18-fold. Furthermore, after using RT-qPCR we found an increased expression of AOAH, IL1A, IL8, CCL3, IL1RN and SERPINE 1 mRNAs, which can also serve as markers of the occurrence of pyometra in domestic bitches. It is suggested that increased expression of B cell-specific immune response molecules may be associated with recruitment of immunologically specific cells in bitches with pyometra as well as with activation of pro-inflammatory proteins as a consequence of exposure to foreign antigens due to the bacterial infection.

Tissue Kynurenic Acid in Bitches with Pyometra

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Pyometra is a common reproductive disorder of dogs that mostly affects older bitches at the metoestrus stage of the estrus cycle. Although the etiology of pyometra is complex and still not fully understood, it is commonly accepted that both hormonal and infective factors contribute to its development. Hormonal imbalance cause cystic hypertrophy of the uterine mucous membrane glands leading to their hyperactivity, which favors bacterial infection. The inflammatory processes occurring in the uterus result in changes in concentrations of numerous tissues biomarkers e.g. kynurenic acid (KYNA). Kynurenine is formed from tryptophan by tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO). IDO is activated by inflammatory stimuli. Kynurenic acid (KYNA) is produced from L-kynurenine by kynurenine aminotransferases. KYNA is a ligand of G protein-coupled receptor GPR35, which is highly expressed in the immune system and has numerous functions such as protecting nerve cells exposed to hypoxia during parturition, bactericidal activity, promoting proper digestion and also acts as a prognostic factor in neoplastic diseases. The aim of the present study was to determine KYNA concentrations in endometrium in healthy bitches (n=10) and those with pyometra (n=10). Immediately after surgery, the uterine endometrium biopsies were taken. The endometrium section, 1.0×1.0 cm, was excised from the central right horn of the uterus, placed in the plastic Eppendorf tubes and frozen at −80 °C. KYNA was determined by means of the high performance liquid chromatography with fluorescence detection. The mean level of KYNA noticed in endometrium in bitches with pyometra and in healthy ones was 559.3±530.3 pmol/g and 189.1±155.2 pmol/g, respectively. Our results strongly suggest an increase of KYNA concentration in the endometrium of bitches with pyometra however, the difference did not reach statistical significance probably due to high variability between the results and limited number of investigated subjects.

How members of innate immunological response in the feline endometrium may differ dependently on the stage of the estrous cycle, pyometra or medroxyprogesterone acetate treatment

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Uterine response to infection is regulated by the opposite action of estrogen and progesterone. Bacterial cell wall components binding to the respective germ-line-encoding pattern recognition receptors, including Toll-like receptors (TLRs), initiate an early non-specific immune response. Activation of TLRs promotes a downstream signaling cascade of intracellular reactions. Administration of synthetic progestin like medroxyprogesterone acetate (MPA) mimics luteal phase by promoting gland growth and increased secretion of mucus and, thereby, creating ideal condition for bacterial growth.

The aims of this study were (i) to examine whether mRNA levels of *TNFα/TNFR1* and *TLR2/4* (Real Time PCR) as well as (ii) immunolocalizations of TNFα/TNFR1 and TLR2/4 (immunohistochemistry) differ in cats during estrus, diestrus, following MPA treatment and suffering from pyometra. Uteri from thirty-five queens, with age ranging from 7 months to 11 years (average 2.67 years) old were collected during ovariohysterectomy with animals' owners request and consent. Queens were grouped depending on the stage of the estrous cycle, MPA-treatment or clinical presence of pyometra.

The mRNA level of *TLR2* was higher in pyometric endometrium compared with remaining groups ($P<0.001$). *TLR4* gene expression was also the highest in pyometra compared to anestrus, estrus and short-term treated with MPA ($P<0.001$), as well as late diestrus and long-term treated with MPA ($P<0.05$). The level of *TNF* mRNA was higher in mid diestrus compare to anestrus, and MPA-treated animals ($P<0.05$). Levels of *TNFR1* mRNA in the feline endometrium did not differ significantly. Low mRNA levels of *TLR2, TLR4* and *TNF* in endometrium of cats treated with MPA might enable the development of pyometra. Immunolocalisation of all examined proteins was confirmed in each experimental group. Interestingly, markedly visualized signals were observed in endometrial glands and in superficial epithelia, whereas in stroma the staining was weak or not visible. We observed strong signals against TLR2/4 and TNFα during estrus in both surface and glandular epithelia. In inflamed uteri the distinct staining against TLR2 was observed in surface epithelium, against TNFα in endometrial glands and against TNFR1 in both epithelia. In MPA-treated cats moderate signal against TLR2 was observed in both epithelia and stroma and diversification of staining against TNFR1 was seen in the surface epithelium.

Exogenously administered P_4 suppress leukocyte function and inhibits cytokine production, what might be related with low levels of *TNF* and *TLR4* mRNA observed in feline endometrium during MPA treatment. Changes in immunostaining intensity regarding MPA-treatment are localized mainly in endometrial glands.

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In vivo knocking down of *BMP15* affects ovarian functions in transgenic pigs

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Bone morphogenetic protein 15 (BMP15) has been proved to play an important role in regulating ovarian functions in animals. Natural occurring point mutations in single allele of *BMP15* could improve the ovulation rate and litter size in sheep, however, homozygous mutations lead to infertility. Improving litter size in pigs has attractive economic importance for pig industry. However, up to date, a natural occurring mutation in porcine *BMP15* for causing increased litter size has not yet been identified. Here, we introduce our transgenic (TG) pigs generated with an integrated shRNA expression vector in attempt to knock down the expression level *BMP15* to a half. Unexpectedly, high-throughput sequencing on RNA isolated from transgenic cumulus oocyte complex (COC) showed that *BMP15* expression level was *in vivo* knocked down to 1.25% of that of wild type (WT) gilts. The robust *in vivo* interfering of *BMP15* caused a disrupted oestrus cycle, and pregnancy failure in transgenic gilts after artificial insemination. Quantification of peripheral concentration of oestradiol (E2) and progesterone indicated a lack of a clear E2 surge and decrease of progesterone before ovulation in TG gilts as compared with WT gilts. To our surprise, the size of ovaries collected from sacrificed TG gilts on day 140, 170, 200 and 365 were remarkably reduced as compared to WT gilts. Dissection of the ovaries revealed TG ovaries contain significantly less follicles, but substantially more follicles with diameter larger than 5 mm. Histological analysis of ovaries showed abnormal morphology of follicles in TG ovary, most obviously, several oocytes co-located within single follicle. Quantitative PCR analysis of the expression level of BMP15 receptor BMPR2, progesterone receptor PGR, and E2 receptor ESR1 and ESR2 in different size of follicles (diameter:1-3mm, 3-5mm, >5mm) revealed that BMPR2, and PGR was down-regulated in TG follicles, but ESR1 and ESR2 expression level were not significantly different from that of WT follicles. Unexpectedly, we found that the expression level of the receptor of follicle stimulating hormone (FSHR) in TG follicles was significantly lower than that of WT follicles, which is inconsistent to the commonly accepted concept that BMP15 inhibits the expression of the FSHR during follicle development. We speculated that the low level of *BMP15* in TG gilts may cause a defective follicular granular cell and theca cell process, resulting in insufficient expression of FSHR and disrupted synthesis of ovarian hormones. Therefore, *in vivo* knocking down of *BMP15* heavily affects the ovarian functions and lead to an abnormal estrus cycle in gilts.

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Ovarian activity, endocrine profiles and superovulatory responses in cyclic ewes receiving Folltropin®-V after pre-treatment with a single dose of estradiol-17β and synthetic progestin (medroxyprogesterone acetate) or natural progesterone

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Follicular wave status at the beginning of exogenous follicle-stimulating hormone (FSH) administration is an important contributor to variability in superovulatory responses in ruminants. Studies in ewes have shown a decrease in the number of ovulations when superovulation is initiated in the presence of ostensibly ovulatory-sized ovarian follicles. Hormonal ablation of large antral follicles with the progestin–estradiol (E_2-17β) treatment significantly reduces this variability in superovulated anestrous ewes, but the effects of the treatment in cycling ewes have not yet been assessed. Sixteen Rideau Arcott x Polled Dorset ewes (November-December) received either medroxyprogesterone acetate (MAP)-releasing intravaginal sponges (60 mg) or controlled internal drug release (CIDR) devices (containing 300 mg of natural progesterone-P_4) for 14 days (Days 0-14), with a single i.m. injection of 350 μg of E_2-17β on Day 6. The superovulatory treatment consisted of six injections of porcine FSH (Folltropin®-V) given twice daily, followed by a bolus GnRH injection (50 μg i.m.) on Day 15. There were no differences ($P<0.05$) in the ovulatory responses and embryo yields between the two groups of ewes. In both subsets of animals, the next follicular wave emerged ~2.5 days after an E_2-17β injection ($P>0.05$). A decline in maximum follicle size following an E_2-17β injection was more abrupt in CIDR-compared with MAP-treated animals and the ewes pre-treated with exogenous P_4 had significantly more 3-mm follicles at the start of the superovulatory treatment. The metabolic clearance rate of exogenous E_2-17β appeared to be greater in MAP-treated ewes but circulating concentrations of porcine FSH failed to increase significantly after each Folltropin®-V injection in CIDR-treated animals. The CIDR-treated ewes exceeded ($P<0.05$) their MAP-treated counterparts in serum E_2-17β concentrations during superovulation. In spite of differences in antral follicle numbers and endocrine profiles between MAP- and CIDR-treated cyclic ewes receiving E_2-17β prior to ovarian superstimulation, there were no differences in superovulatory responses.

Gene expressions of enzymes involved in testosterone synthesis in the bovine corpus luteum

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Luteolysis is characterized by a reduction in progesterone (P4) production, tissue degeneration and cell death. Testosterone (T) is secreted by the adrenal gland, ovary and placenta in cow. T is also known to be secreted by the bovine corpus luteum (CL). The T concentration in peripheral blood is known to increase during luteolysis in cattle and T is known to decrease P4 secretion by cultured bovine luteal cells. T is synthesized by two pathways: the pregnenolone (PREG) → P4 → T pathway and the PREG → dehydroepiandrosterone (DHEA) → T pathway. Previous studies have suggested that T is a local luteolytic factor. However, it is unclear how T is synthesized in the bovine CL. In the present study, we used quantitative RT-PCR to investigate changes of the mRNA expressions of four enzymes involved in the PREG → DHEA → T pathway in the bovine CL: cholesterol side-chain cleavage enzyme (P450scc), cytochrome P450 (CYP17A1) and two hydroxysteroid dehydrogenases (HSD17B1, HSD3B). In Exp. 1, we examined the spontaneous changes during the estrous cycle and in Exp. 2, we determined the changes during a 24 h period following administering prostaglandin F2α (PGF2α) to cows at the mid luteal stage (day 10 after ovulation). In Exp. 1 CL tissues were collected at the early (Days 2-3 after ovulation), developing (Days 5-6), mid (Days 8-12), late (Days 15-17) and regressed (Days 19-21) luteal stages, and in Exp. 2 they were collected at 0, 2, 4, 12 and 24 h after PGF2α administration to cows. In Exp. 1, *HSD3B* mRNA expression was lower at the regressed luteal stage than at the late luteal stage. *P450scc*, *CYP17A1* and *HSD17B1* mRNA expression did not change throughout the estrous cycle. In Exp. 2, *P450scc* mRNA expression was lower at 4 and 12 h than 0 h after PGF2α administration. *HSD3B* mRNA expression was lower at 12 and 24 h than 0 h. *CYP17A1* and *HSD17B1* mRNA expression did not change. The overall findings suggest that the PREG → DHEA → T pathway is not involved in the increase of T production during luteolysis in cattle. Further studies are needed to determine whether the PREG → P4 → T pathway is related to luteolysis.

miRNA-mRNA interaction analysis in theca cells of bovine ovarian follicles

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It is known that microRNAs (miRNAs) regulate gene expression through mRNA degradation or silencing translation, however, this relationship between miRNAs and mRNAs in ovarian follicle development is not well understood. Recent research has identified numerous genes as well as sets of miRNAs that are associated with cellular proliferation and apoptosis in ovarian follicles. Using data obtained from previous studies, the aim of this study was to analyse the interaction between miRNAs and mRNAs coding for transcription factors that were differentially expressed between dominant and subordinate follicles in theca cells. *In silico* analysis was performed with DIANA microT v. 5.0 software (http://www.microrna.gr/microT-CDS) which uses an algorithm to find transcripts regulated by the uploaded miRNAs. Target prediction score (miTG) was set at 0.8 (ranges from 0 to 1; higher value -> higher accuracy of prediction). 92 miRNAs (Zielak-Steciwko et al. 2014; Physiol Genomics, 46:735-45) and 8 mRNAs coding for transcription factors (Zielak et al. 2008; Mol Reprod Dev, 75:904-14) were chosen for analysis. Putative interaction between mRNAs coding transcription factors whose expression was lower in dominant than in subordinate follicles and selected miRNAs whose expression was higher in dominant than subordinate follicles was analysed. Such interactions were demonstrated between several pairs, including: FKHRL1 mRNA and miR-597 (miTG=0.94) and miR-1182 (miTG=0.91) and miR-122-5p (miTG=0.83) and miR-543 (miTG=0.83) and miR-20a-3p (miTG=0.81); SRF mRNA and miR-122-5p (miTG=0.86) and miR-4311 (miTG=0.8). An opposite interaction (mRNA with lower expression in subordinate than dominant and miRNAs with higher expression in subordinate than dominant follicles) was also analysed. An interaction between NCOR1 mRNA and miR-629-3p (miTG=0.99) and miR-200b-3p (miTG=0.9) and miR-29b-1-5p (miTG=0.9) was demonstrated. These findings describe associations among interacting miRNA and mRNA pairs and imply that miRNAs negatively regulate the levels of mRNAs that code for transcription factors in ovarian follicles cells. We suggest that these findings indicate that the analysed miRNAs are involved in survival or apoptosis of granulosa cells in ovarian follicles.

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Estimation of influence of synthetic beta-carotene on the sexual cycle of dogs

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The leading role in the development of infertility belongs to the disorders of the reproductive system, especially ovarian dysfunction. The purpose of this work is studying of ovarian dysfunction of bitches for developing a method of its correction with using "Carofertin". The research was performed in two stages. In the first experience we had 25 bitches, which were divided into 5 groups, from which we have taken the material in the various stages of the sexual cycle using the method ovariogistereoktomiya. Then we've examined it histologically. For the second stage we've taken 90 bitches, and divided them into 3 groups. In the first experimental group was used "Carofertin", in the second -"Trivit". Also, all the test animals have been injected HCG one time. After that was made the clinical examine. According to the results of the first phase we've known that due to the fact that the waves follicular growth continues, new tissues are formed. Dystrophic tech and granulosis change the metabolism of the ovaries, so there is a transformation of new tertiary follicles in the cyst. At the beginning of folliculogenesis the key role played by the internal mechanisms of regulation. Most important is estradiol. Granulosis hyperplastic follicular cysts synthesizes estradiol during pathological processes in the ovaries. The influence of the hypothalamic-pituitary system on the functional state of the ovary is disturbed. In the regulation of growth and development of follicles the leading place belongs to granulosa hyperplastic follicular cysts. In the second experiment we received data showing that in the first test group 90% the time of anoestrus has stabilized - 7,23 ± 1,31 months, and at 10% of the females - 3 34 ± 0,47 months. ($p < 0,05$). In the second case we've got the next results: the time of anoestrus normalized in only 70% - 7,38 ± 1,21 months, in 30% - 3,34 ± 0,47 months. ($p < 0,05$). In the control group only 53% of the dogs was stabilized and the time of anoestrus was 7,06 ± 1,14 months. And 47% - 3,36 ± 0,48 months. ($p < 0,05$). So "Carofertin" is a highly effective method of treatment for infertility of dogs.

E.coli lipopolysaccharide (LPS) adversely affects bovine luteal endothelial cell network formation and survival *in vitro* but has no effect on progesterone production

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In dairy cows, post-partum endometritis caused by Gram negative bacteria (e.g. *E. coli)* has a negative impact on follicular function and causes subfertility. Intriguingly, lipopolysaccharide (LPS) is detected in follicular fluid (0.004-0.88μg/ml) and *in vivo* treatment with LPS decreased circulatory progesterone concentrations. Recently, we showed that LPS dose-dependently decreased the total area of endothelial cell (EC) networks *in vitro* and this was even detectably lower at 0.01μg/ml LPS. Furthermore, this suppression occurred in the presence of pro-angiogenic factors, fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor A (VEGFA). The hypothesis tested was that LPS decreases luteal EC network formation by increasing EC apoptosis and inhibiting EC proliferation. The effect of LPS of progesterone production was also investigated. Luteal cells (EC, steroidogenic cells and pericytes) were enzymatically dispersed from abattoir-derived bovine corpora lutea (early luteal phase) and cultured in specialised EC media (n=3-4 cultures). From day 1 of culture, cells were treated with LPS (0, 0.01 or 1μg/ml) under angiogenic-stimulated conditions (1ng/ml FGF2; 1ng/ml VEGFA). Spent media was analysed for progesterone by ELISA and replaced every 2 days. On day 5, ECs were immunostained for von Willebrand factor while apoptotic and proliferation indices of ECs were quantified using caspase-3 and Ki67 dual immunofluorescence staining, respectively. The effect of LPS was determined using randomised block one-way ANOVA. On day 5, ECs formed large island of cells with >50 cells in each. LPS treatment reduced ($P<0.001$) the number of ECs by three (0.01μg/ml) and six-fold (1μg/ml). Both doses of LPS reduced proliferation index of luteal ECs by approximately 20-30% ($P<0.05$). The proliferation index of other cell types was evident. Simultaneously, the luteal EC apoptotic index was increased by nearly 3-fold (both doses; $P<0.001$). Progesterone production increased 3-fold from day 3 to 5 ($P<0.001$), however, LPS had no effect on progesterone concentrations *in vitro*. In conclusion, LPS dramatic inhibition of *in vitro* luteal EC network formation appears to be due to decreased EC proliferation and increased apoptosis, while LPS has minimal effects on luteal steroidogenic cell function.

Rapid effects of estradiol-17β on the expressions of regulators of smooth muscle activity in bovine oviductal epithelial cells

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Estradiol-17β (E2) plays roles in reproductive organs. Biological effect of E2 is exerted by binding with estrogen receptors (ERs). ERα and ERβ which are members of nuclear receptor family slowly mediate gene transcription. Recently, G protein-coupled estrogen receptor (GPER) was identified as an estrogen receptor that is expressed on the plasma membrane and induces rapid signal transduction. Although the expressions of ERα and ERβ in bovine oviduct are already detected, that of GPER is not unclear. At the time of ovulation, follicular fluid with an oocyte containing a high concentration of E2 enters the oviduct, and then the oocyte is rapidly transported to the ampulla where fertilization occurs. Since E2 is known as a regulator of oviductal contraction, we hypothesized that E2 derived from follicular fluid regulates acute contraction of oviductal smooth muscle via GPER. To test the above hypothesis, we investigated the expression and localization of GPER in oviductal tissues obtained from cattle at days 0-1 after ovulation, and acute effects (0.5, 1, 2 h) of E2 on mRNA expressions of cyclooxygenase-2 (*COX-2*), endothelin (*ET*)-*1*, *ET-2*, *ET-3* and inducible nitric oxide synthase (*iNOS*), which are genes of related peptides that regulate oviductal contraction, in cultured oviductal epithelial cells. GPER mRNA and protein were detected in the oviductal tissues and their expressions were highest in the isthmus. GPER was immunolocalized in epithelial cells. E2 stimulated the mRNA expression of *ET-2*, which is known as a smooth muscle constrictor, at 0.5 and 1 h after treatment, whereas E2 suppressed the mRNA expression of *iNOS*, a mediator of oviductal relaxation, via the synthesis of nitric oxide, at 1 h after treatment in isthmic epithelial cells. By contrast, E2 did not affect the mRNA expressions of *ET-2* or *iNOS* in ampullary epithelial cells. E2 did not affect the expressions of the other genes in either ampullary or isthmic epithelial cells. The overall findings suggest that E2 derived from follicular fluid quickly induces contraction of the isthmus after ovulation via GPER by mediating ET-2 production and iNOS suppression, keeping the oocyte within the ampulla to achieve fertilization.

Regulation of oviductal functions by mitosis and apoptosis of ciliated and secretory epithelial cells in cattle

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Two types of oviductal epithelial cells, ciliated and secretory, play crucial roles in the first days of pregnancy. Secretory cells produce oviductal fluid that is rich in amino acids and various molecules that are required for an optimal micro-environment for embryo survival and development. Beating of motile cilia produces a stream of oviductal fluid, which transports an ovulated cumulus-oocyte complex to the ampulla of the oviduct where fertilization occurs. The stream also transports the embryo to the uterus. Since the proportions the two cell types change during the estrous cycle, oviductal epithelial functions are possible to depend on epithelial morphological changes. In the present study, we examined proportion of the proliferating and apoptotic cells to clarify the regulatory mechanisms of oviductal epithelial functions in cattle. The proportion of cells being positive for MKI67 (a mitosis marker) or cleaved caspase-3 (CCP3; an apoptotic cellular marker) in epithelial cells during the estrous cycle were immunohistochemically examined. MKI67 was double-stained with FOXJ1 (a ciliated cell marker). MKI67 and CCP3-positive cells in the ampulla were observed during the estrous cycle. The proportion of MKI67-positive cells was highest at the day of ovulation (Day 0) and Days 19-21, whereas that of CCP3-positive cells was highest at Days 8-12 in the ampulla. The proportions of both MKI67 and CCP3-positive cells were highest at Day 0 and Days 2-3, and MKI67-positive cells were not observed at Days 8-12 in the isthmus. All the MKI67-positive cells were secretory cells in the ampulla and isthmus. These findings suggest that luminal epithelium of the oviduct is remodeled by cell mitosis and apoptosis during estrous cycle. Furthermore, our result that MKI67 protein was localized only in secretory cells, implies that the remodeling of the epithelium is regulated by differentiation and/or proliferation of secretory cells.

Antioxidant effect of Dihydroquercetin on reproductive ability in cows at hot time

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High milk production often causes infertility in dairy cows. Cows with high milk production sometimes don't show the signs of the estrus and they have reduced estrus period. All of these complicates accurate determination of the cows in heat. One of the factors that have a negative impact on the reproductive function and productivity in dairy cattle is seasonal temperature changes. This is especially true for high temperature and humidity. Increased temperature changes in the summer is a stress factor that leads to growth of lipid peroxidation and disruption of the physiological processes at the subcellular level. Violation in estrus cycle, maturation of gametes, reduced fertility increase in dairy cattle especially, in cows with high milk. Antioxidants reduce the negative effect of stress factors and development of peroxidation. One of these antioxidants is Dihydroquercetin (Taxifolin). The aim of the study was an attempt to reduce the impact of hot time stress on cows by feeding of Dihydroquercetin.The study was conducted in July. Air temperature varied from 20^0 to 33^0C.The temperature was kept at $30 – 33^0$ for 16 days. 90 Holstein dairy cows of first lactation with average milk yield 8895 ± 77.5 were used. The animals were divided into 2 groups: control (C) and experimental (G). Animals of gr(G) was given Dihydroquercetin (1mg per 1 kg of body weight)daily. AI was performed after heat detection by Heatime HR. Plasma malondialdehyde (MDA) level was taken as an indicator of lipid peroxidation in this study. MDA was 2,81 ± 0,08 mM / l in control (C) with is significantly ($p < 0.001$) higher than in the experiment (G) (1,62 ± 0,10 mM / l). First insemination pregnancy rate in the G group was 25% , in C group - only 9% . Total pregnancy rate - 50 ± 6,7%, and 38 ± 8,3% respectively. Open days period cows was 127 ± 13,2 days in G group and 163 ± 25,5 days in the C group. Thus, Dihydroquercetin provides antioxidant protection and helps to reduce negative effect on the reproductive ability in dairy cows under high temperature stress.

The influence of azaperone treatment at weaning on reproductive performance of sows: Altering effects of season and parity

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Azaperone treatment can control aggression and decrease chronic stress caused by re-grouping and hierarchical fighting of sows, but the effects of this sedative administered at weaning on reproductive parameters are poorly characterized. In this year-long study, a total of 619 cross-bred sows (Polish Large White x Polish Landrace) kept on a commercial farm received an i.m. injection of azaperone (Stresnil®; 2 mg/kg b.w.) just prior to weaning and were artificially inseminated during the following estrus with 3 x 10^9 spermatozoa per dose of semen collected from boars on farm; 1180 sows served as untreated controls. Immediately after weaning, the sows were moved to 4 pens of 7-9 animals each; primiparous sows were kept separately. A teaser boar was used twice daily to check for estrus and sows were bred at heat detection. All sows stayed in individual stalls until pregnancy testing on Day 30 post-AI and were then re-grouped until farrowing. The proportion of pigs that were in estrus before Day 7 post-weaning was significantly lower in azaperone-treated groups of animals than in controls (71.4% vs. 84.2%). Overall, the azaperone-treated sows had a significantly longer weaning-to-estrus interval (WEI; 8.73±10.12 vs. 6.25±8.13 days) and a significantly larger litter size (11.78±2.97 vs.11.33±3.17; treated vs. control sows). Treatment of the winter-farrowing sows was associated with a 2.89 more piglets/sow/year compared with controls but it hindered the weaning-to-effective-service interval (WESI; $P<0.05$). In the summer months, a significantly increased duration of WEIs was accompanied by an increase in the litter size only in the on-time sows (bred before Day 7 post weaning). On-time Parity II and late estrus Parity >IX sows had the greatest increase in the number of piglets born along with prolonged WEIs ($P<0.05$). In all, an application of azaperone at weaning increased piglet productivity in winter months, and in Parity II and >IX sows. However, the extra cost and labor due to delayed onset of behavioral estrus may nullify the reproductive benefits of azaperone treatment.

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Complex evaluation of equine semen after freezing

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Semen freezing is a multi-step process that includes several stages - diluting, cooling, freezing and thawing. Each stage has a detrimental effect on sperm, reducing the number of viable fertile cells. Dissolution, freezing and thawing affect certain areas of the head, tail, the plasma membrane, mitochondria, and the acrosome of the sperm. Thus, methods to evaluate the integrity of the cells that are more objective than the determination of % motile sperm can be developed. The goal was to assess the safety of acrosome, membrane permeability and respiratory activity of equine sperm after freezing. Sperm from 30 stallions was frozen. The acrosomal status was assessed by phase-contrast microscopy; membrane permeability was determined by fluorescent microscopy with ethidium bromide. Respiration was assessed by the polarographic method. Phase-contrast microscopy showed the damage in acrosomes, the neck and in the tail of sperm, which increased after cryopreservation(in tail, neck from 19,2 ± 1,53 to 22,5 ± 1,81 and in the acrosome from 10,9 ± 0,75 to 14.7 ± 0,73). Violations of membrane permeability were observed in fresh sperm. The number of injured membranes increased after thawing. The intensity of energy metabolism is very important criterion to assess sperm quality. ATP consumed during movement of sperm is kept constant by glycolysis and respiration. Respiration is more important than glycolysis in equine. Respiration rate of fresh sperm was 291 ± 60,4 nAO2/min. We detected stimulation of respiration (in 1,2 ± 0,06 times) after addition of potassium succinate, which shows a little violation in membrane permeability. After freezing we observed a greater increase in respiratory stimulation by succinate (1,7 ± 0,09), indicating that further damage of membranes. Further we studied conjugation respiration and phosphorylation , which evaluated by the reaction of respiration on adding 2,4- dinitrophenol (2,4 - DNP) . In fresh semen respiratory stimulation by 2,4 -DNP was 1,8 ± 0,14, indicating a good pairing respiration and phosphorylation. After thawing we observed decrease respiratory stimulation by 2,4 -DNP (1,5 ± 0,11), which indicates the presence of damage in mitochondrial respiratory chain on the part of the sperm. Thus the most sensitive departments of equine spermatozoa are neck and tail where there are mitochondria and fibrillar structures.

Liquid storage of boar semen at 17°C in extenders prepared with deionized water or nanowater

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The aim of this study was to evaluate the usefulness of nanowater (water declusterized using exposure to cold plasma) as a solvent for boar semen extender on the progressive changes in sperm motility and morphology during the 15-day liquid storage at 17°C. Ejaculates collected from 8 boars were initially subjected to standard evaluation and then diluted in commercially available semen extender (CRONOS®, Medichimica, Italy) prepared with deionized water (DW) or nanowater (NW). Sperm motility was assessed daily and histopathological evaluations were done on days 2, 5, 10 and 15. After the first day of storage, the percentage of abnormal spermatozoa decreased significantly from initial 15.0±8.0% to 6.1±2.7% in NW and numerically (to 11.2±8.2%) in DW group due mainly to reduction in the number of detectable tail defects. Subsequently, the proportion of abnormal spermatozoa increased gradually to day 5 (18.8±10.6% and 11.3±4.2%) and day 10 (22.8±16.6% and 18.6±11.7%), and then more rapidly to day 15 (41.8±26.4% and 34.8±25.4%; DW and NW groups, respectively); on each day, the difference between the two groups was significant. Sperm progressive motility decreased ($P<0.05$) over the first 2 days of storage from 77.5±3.8% to 68.8±9.9% in NW group. The percentage of motile spermatozoa decreased gradually but significantly ($P<0.05$) in both groups until day 10, and then rapidly ($P<0.01$) to day 13. The rate of decline in sperm motility was greater ($P<0.05$) in DW compared with NW group between days 4 and 6. The decrease in sperm progressive motility below 40% was noted on day 8 in DW and on day 10 in NW. On day 15, sperm motility was ≤5% in all samples tested. To summarize, the use of nanowater as a semen extender diluent appeared to exert cytoprotective effects on boar spermatozoa and, despite a significant initial drop, to delay a decline in sperm progressive motility by ~2 days. It would now be interesting and justified to corroborate the present findings with a fertility/IVF trial to determine the influence of storage time in extenders containing nanowater on fertilizing ability of boar semen.

Differential endometrial mucin gene expression in repeat breeder cows

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Mucins (MUC) are anti-adhesive glycoproteins covering epithelial surfaces to protect from bacterial infections. Before implantation, MUC down-regulation is required for blastocyst attachment in many species. Detailed knowledge is missing about the contribution of MUC to subfertility in cows.
The objective of this study was to analyse endometrial mRNA expression of selected MUC (*MUC1*, *MUC4*, *MUC5A*, *MUC6*, *MUC12*, *MUC15* and *MUC16*) in subfertile repeat breeder cows (RBC) and control heifers.

Cytobrush samples were collected from 91 RBC (cows with ≥3 unsuccessful artificial inseminations without signs of clinical endometritis) independent of the stage of the oestrous cycle and from 11 synchronized healthy heifers during oestrus (CON). In addition, the plasma progesterone (P4) and estradiol (E2) concentration was measured and an ultrasonographic examination of the ovaries was performed on the day of sample collection. A proportion of ≥5% polymorphonuclear neutrophils detected at cytological examination was used for the diagnosis of subclinical endometritis (SE). Total RNA was isolated from cytobrush samples and subjected to quantitative PCR.

Ultrasound examination of the ovaries as well as E2 and P4 concentrations revealed undisturbed ovarian activity in RBC. RBC showed a significantly higher *MUC4* (fivefold) and *MUC12* (14-fold) mRNA expression compared with CON, whereas the other MUC were not differently expressed between these groups. RBC were further subdivided in cows with and without SE (RBC-SE; n = 13 and RBC-noSE; n = 78). Significantly higher (fivefold) *MUC4* transcript levels were noticed in RBC-noSE compared with CON. *MUC12* mRNA expression was ninefold and 14-fold higher in RBC-SE and RBC-noSE compared with CON, respectively. RBC in the luteal phase (P4 >1ng/ml) showed significantly lower *MUC1*, *MUC5A* and *MUC16* transcript levels than RBC with P4 ≤1ng/ml. In contrast, *MUC12* expression was significantly up-regulated (threefold) in RBC with P4 >1ng/ml.

The results of the study suggest that *MUC4* and *MUC12* up-regulation might contribute to subfertility in RBC independently from the occurrence of SE. Furthermore, this study confirms the already known inhibitory effect of progesterone on *MUC* gene expression. The underlying mechanism of elevated *MUC12* expression in RBC during the luteal phase needs to be elucidated in future studies.

Defining the inflammatory gene signature associated with endometritis in bovine endometrial epithelial cells

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Endometritis is one of the leading causes of infertility in dairy cattle. Following parturition, bacterial contamination of the uterus occurs in over 90% of cows and can result in severe inflammation. Endometrial epithelial cells orchestrate the immune response by detecting incoming pathogens and producing pro-inflammatory factors. Previous work by our group has uncovered a prolonged inflammatory gene signature in endometrial biopsies which is associated with the development of endometritis. This included the consistent expression of inflammatory markers such as IL1 and IL8 in endometritic cows at 7 and 21 days postpartum (DPP), whereas healthy cows showed a decline in inflammatory mediator expression by 21 DPP. Fewer genes were differentially expressed between healthy and endometritic cows at 7 DPP, although higher expression of CD27, CD69, CD79A and CD79B (involved in B-cell activation) was present in cows that later developed clinical endometritis (CE). The aim of the current study was to investigate this profile in more detail and in a larger panel of postpartum cows of differing uterine health status (healthy, subclinical endometritis or clinical endometritis). Duplicate cytobrush samples were used to collect endometrial epithelial cells from the uterus at 7 and 21 days postpartum, from a total of 139 cows. Cytological assessment classified 45% (n=60) of the animals with clinical endometritis. Total RNA was extracted from n=10 cows per group (total n=30) and relative gene expression analysis is currently being performed using qPCR. Gene targets will include key components of the innate immune signalling pathways, such as toll-like receptors, cytokines, chemokines and antimicrobial peptides. A deeper understanding of the immune response in the postpartum cow and perturbations that contribute to disease will aid the development of future intervention strategies for endometritis.

Demonstration of bacteria in endometrial biopsies from postpartum cows by Fluorescence *In Situ* Hybridization (FISH)

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The study objective was to determine the location of bacteria in endometrial biopsies from postpartum cows. The endometrial biopsies were taken at three time points, I; 4-12 days in milk (DIM), II; 23-32 DIM, III; 46-53 DIM. The specimens were fixed in formalin, embedded in paraffin, sectioned at 3 µm for FISH using an oligonucleotide probe targeting 16S ribosomal RNA specific for domain bacteria, *Fusobacterium necrophorum*, *Porphyromonas levii*, *Trueperella pyogenes* and *Escherichia coli*. Fluorescence microscopy showed that bacteria were located on the epithelial surface, intraepithelially, and/or in lamina propria of the endometrium. Bacteria were demonstrated in 78.9%, 83.9% and 88.9% of the biopsies at time point I, II and III, respectively. In 35.1% (I), 11.1% (II) and 26.2% (III) of the biopsies bacteria were present intraepithelially, and/or in lamina propria as well as on the luminal surface, whereas the bacteria were present only on the surface, in the remaining biopsies. F. *necrophorum* and P. *levii* were observed on the epithelial surface but also intraepithelially, and/or in lamina propria, while T. *pyogenes* and E.*coli* only were visualized on the epithelial surface. In conclusion, FISH can be used to visualize bacteria in endometrial biopsies, and T. *pyogenes* and E.*coli* are located only on the epithelial surface while other bacteria, amongst these F. *necrophorum* and P. *levii*, are located also intraepithelially, and/or in lamina propria.

Accuracy and efficacy of histopathology and cytology methods to diagnose subclinical endometritis in dairy cows

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The present study was performed to explore the accuracy and efficacy of different methods to diagnose subclinical endometritis (SCE) in dairy cows. Endometrial samples for cytology examinations (CY) were collected from Holstein-Friesian cows (n=32) before slaughtering at 315±173 days in milk. Half of the CY samples was obtained using the CytoBrush (CB) technique while the other half by Low Volume Lavage (LVL). After slaughtering, reproductive tracts were collected, and the endometrium was sampled at eight predefined locations evenly spread over the opened uterus. At each location, both a tissue sample for histopathologic examination (HP) as well as a local CY sample (CY_L) was taken using a cytobrush. Histopathology samples were stained with Naphtol-AS-D-chloracetate-esterase (CIAE), while CY and CY_L samples were stained with Diff-Quick®. In the HP samples, polymorphonuclears (PMNs) in 5 HPF (High Power Fields) at 400X were calculated and averaged. To assess the PMN% in CY and CY_L samples, the HPF100 (400X) counting technique was performed. The threshold for SCE was set at 3 PMN/HPF for HP and 3% PMN for CY and CY_L. In the 32 CY samples, the prevalence of SCE was assessed at 18.75% (n=6), being 6.2% (n=2) of positive CB samples and 12.5% (n=4) of positive LVL samples. Prevalence of SCE in all HP samples (n=256) was 37.1% (n=95), while in the CY_L this was 25% (n=64). Pearson correlation was strong for CB (r=0.80; P=<.0001) and moderate for LVL (r=0.52; P=<.0001) when compared to each CY_L. The agreement was found very good (k=0.96) and substantial (k=0.65) for CB and LVL, respectively. When HP and CY_L were compared at each predefined location, the correlation was strong (r=0.60; P=<.0001) and the agreement moderate (k=0.50). To conclude, CY is a good diagnostic tool to diagnose SCE in dairy cows. Although both CB and LVL are acceptable techniques to diagnose SCE, CB seems to be more reliable and accurate than LVL.

Effect of intrauterine $PGf_{2\alpha}$ infusion on reproduction performance in dairy cow with mild endometritis

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The present study investigates therapeutic effect of intrauterine prostaglandin$f_{2\alpha}$ infusion if any on fertility and reproduction performance of dairy cows with mild grade 1 endometritis. A total of 64 lactating Holstein Friesian cows (first to five lactation) were selected between 28 to 35 days postpartum based on endometritis detection by trans rectal uterine ultrasonography and uterovaginal dicharge (Clear or translucent mucus containing flecks of white pus) regardless of the presence or absence of CL. The animals were divided into two groups (control and treatment). Treatment group animals (N=32) were infused intrauterine with 250 mcg cloprostenol using 100 ml normal saline and animals in control group (N=32) were received an intrauterine infusion of 100 ml normal saline as soon as uterine infection were diagnosed. Calving to first insemination interval, numbers of inseminations, conception rate at first insemination and Calving to conception were recorded and data were statistically analyzed with spss software (version20). Calving to first insemination interval were 69.48 and 78.9 days for treatment and control group respectively which were not significantly different (P≥ 0/05). Conception rate in first insemination was 45.16percent in the treatment group and was 32.25percent in the control group (P≤ 0/05). Calving to conception was 116.90 days in the treatment group and was 160.80 in the control group (p≤ 0.05). Intrauterine administration of $PGF_{2\alpha}$ cause significant difference in the numbers of insemination (2.03 in the treatment group and 3.22 in the Control group (P≤ 0/05). Result of the study showed that intrauterine administration of 250 mcg cloprostenol 28 to 35 days postpartum may affect reproduction performance in dairy cows with mild endometritis.

Effects of intrauterine Infusion of $PGF_{2\alpha}$ on reproduction performance in cows with calving and puerperal traits

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The purpose of this study was to investigate the effects of intrauterine Infusion of $PGF_{2\alpha}$ on reproduction performance of Holstein cows with calving and puerperal traits (dystocia metritis-stillbirth-retained placenta-twin birth). A total of 70 lactating Holstein Friesian cows (first to five lactation) were selected based on presence of at least one of calving and puerperal traits regardless of the presence or absence of CL and were divided into two groups (control and treatment).Treatment group were infused intrauterine with 250 mcg cloprostenol by100 ml normal saline 30 days postpartum. Animals in Control group received only 100 ml saline solution intrauterine 30 days postpartum. Calving to first insemination interval- number of inseminations – conception rate in first insemination and Calving to conception were recorded and data were statistically analyzed with spss software (version20). Intrauterine administration of $PGF_{2\alpha}$ didn't cause significant difference in the numbers of insemination (1/97 in the treatment group and 2/02 in the Control group ($P\geq 0/05$). Calving to first insemination interval were 68/55 days in the treatment group and this value was 68/85 in the control group ($P\geq 0/05$). Conception rate in first insemination was 37/14 percent in the treatment group and was 42/85 percent in the control group ($P\geq 0/05$). Calving to conception was 109/97 days in the treatment group and was 119/25 in the control group ($p\geq 0.05$). Result of the study showed that intrauterine administration of 250 mcg cloprostenol 30 days postpartum did not affect reproduction performance in dairy cows with calving traits.

Mycoplasma infection provokes endometritis in cows and mares

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Bovine uterine infections are the most important cause of economic losses in cattle industry. Uterine disease often causes infertility in horses as well. According to our research, mycoplasma is often found in animals with endometritis. Mycoplasma is latent pathogenic infection, however, it begins to develop actively and accumulate in the female genital tract when immunity decreases. This is typical for high-producing cows, and high-performance horses. Mycoplasma is an intracellular parasite. It grows and multiplies in the cells of the mucous membranes of the genital tract. Waste products and bacterial toxins accumulat and change biochemical properties of the mucous membranes of the genital tract and reduce their protective function. Reducing the protective function of the mucous membranes allows other pathogenic infection to get into the genital tract. There are no symptoms in animals for a long time after infection by mycoplasma. It is difficult to identify the disease in early stages. To identify mycoplasma is still quite difficult at the later stages, since many infections have similar symptoms. Currently, the most accurate, fast and effective method is polymerase chain reaction (PCR). PCR allows to reveal the presence 100% of mycoplasma in biological material. It can detect the disease at its earliest stages, when the number of microorganisms in the female genital tract is extremely small. At this stage, the number of mycoplasmas is not sufficient to trigger the development of endometriosis. Timely detection of animal-carriers allow to carry out more effective treatment and preventive measures to improve the reproductive function. We tested 40 cows and 16 mares from farms in Leningrad Region, Russia. Almost all the animals with symptoms of endometritis and problems with reproduction were with mycoplasma. Mycoplasma was also found in several animals without any symptoms of diseases. This is the earliest stage of the disease. Thus, molecular genetic techniques can detect infected animals very early and predict endometritis in cows and mares.

Serum concentration of zinc, copper and selenium in post-partum clinical endometritis in buffaloes (Bubalus bubalis)

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Endometritis is a common health event in cows and buffaloes and responsible for high costs and decrease welfare of animals. Trace elements, in particular zinc, copper and selenium have become of particular interest for several authors in relation to this health event. The aim of this study was to investigate whether there is an association between incidence of endometritis and the serum levels of some trace elements. Blood samples were collected from ten normal post-partum buffaloes and other ten samples were from buffaloes suffering from post-partum clinical endometritis. Zinc, copper and selenium were determined by flame emission atomic absorption spectrophotometer. Results showed that serum zinc and copper were significantly ($P<0.01$) decreased in endometritis (24.6±2.5, 36.5±8.9 Vs 88.5±5.7 and 65.8±6.8 µg/dl in normal animals, respectively). There was no difference between serum selenium levels in the two groups (25.5±1.8 and 23.8±1.4 µg/dl). It was concluded that zinc and copper concentrations were decreased in buffaloes suffering from post-partum endometritis. Supplementation of these elements before parturition might decrease the incidence of endometritis in buffaloes.

Endometrial release of immunoreactive arachidonate metabolites and tumor necrosis factor during endometritis is enhanced in cyclic versus anestrous heavy draft mares

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Endometritis is one of the main causes of subfertility or infertility in draft mares and it seriously reduces reproductive efficiency, leading to economic losses in equine reproduction. During the course of clinical infection, fluid accumulation in the uterus is often present, together with a vulvar discharge. By contrast, in subclinical endometritis the lack of obvious clinical signs renders this condition difficult to be diagnosed and treated. Recognition of pathogen components by host receptors, including Toll Like Receptors, leads to exacerbation of inflammation by activation of innate immune mechanisms, including increased secretion of proinflammatory cytokines like Tumor Necrosis Factor α (TNF) or immunoreactive arachidonate metabolites, that are known to regulate the inflammatory process but are also involved in the regulation of mares´ estrous cycle. The main effect of enhanced TNF secretion is an increase in cytotoxicity and phagocytotic activity of immune cells, as well as chemotaxis of immune cells towards the site of inflammation. Polymorphonuclear cells (PMNs) are the first line of cells infiltrating the equine endometrium after mating and their infiltrating ability is positively correlated with 17β-estradiol concentration.

Therefore, we hypothesized that endometrial secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a metabolite of prostacyclin I_2 (6-keto$PGF_{1\alpha}$), leukotriene B_4 and C_4 (LTB_4 and LTC_4) and TNF is enhanced in the inflamed endometrium of mares at estrus or shortly after ovulation compared with mares in seasonal anestrus. A total of 43 draft mares were used in this study. Mares were classified as follows: an anestrous group with no fluid in the uterus and no polymorphonuclear cell (PMNs) infiltration observed in the endometrial luminal epithelium and the stratum compactum (n=13); an anestrous group with fluid in the uterus and infiltration of the endometrial tissue by PMNs (n=9, clinical endometritis); mares in estrus/early diestrus with no fluid in the uterus and infiltration of the endometrial tissue by PMNs (n=10, subclinical endometritis); and mares in estrus/early diestrus with fluid in the uterus and infiltration of the endometrial tissue by PMNs (n=11, clinical endometritis). In the present study, the strikingly higher secretion of $PGF_{2\alpha}$ and TNF was observed in clinical cases of endometritis in mares at estrus/early diestrus compared to the others. The secretion of $PGF_{2\alpha}$ and LTB4 ($P<0.01$), as well as LTC4 and TNF ($P<0.001$) was distinctly elevated in cyclic clinical endometritis mares compared to cyclic subclinical endometritis mares. None of the arachidonate metabolites or TNF endometrial secretion was increased in anestrous clinical endometritis mares in comparison with anestrous genitally normal mares. The profile of LTC_4 secretion was almost identical with that observed for $PGF_{2\alpha}$ and TNF, thus we concluded that secretion of these factors in the course of endometritis might be linked with stage of the estrous cycle.

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The effect of prostaglandins (PGs) and lysophosphatidic acid (LPA) on myometrial motor function and PG and LPA receptors mRNA transcription: preliminary study on the impact of pregnancy and endometrial fibrosis

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Myometrial contractions play very important role in reproductive physiology. The aim of present study was to clarify the effect of prostaglandins (PGs) and lysophospatidic acid (LPA) on equine myometrial contractility during the early pregnancy and in the course of endometrial fibrosis. In the Exp. 1 we investigated the myometrial mRNA transcription of PGE receptors (EP) 1-4, PGF receptor (FPr) and LPAR 1-6 using Real-time PCR. In the Exp. 2 the effects of PGE_2 (0.01 – 1.0 μM), 0.01 – 1.0 μM) or LPA (0.01 – 1.0 μM) on myometrial contractility was determined. Uteri were collected from early luteal stage (n=12) and early pregnancy (Day 26-28; n=4). Myometria (early luteal stage) were obtained from uterus which endometrium were classified to Category I (no degenerative changes: n=4), Category II (moderate and mild fibrosis: n=4) or Category III (severe fibrosis: n=4) according to Kenney's classification system.

In Exp. 1, the *FPr* mRNA transcription was up-regulated in myometria assigned to Category I and II compared to Category III ($P<0.05$). Although *EP2* and *4* mRNA transcription were up-regulated in myometrium assigned to Category I, *EP3* mRNA was down-regulated in myometria assigned to Category I and Category III ($P<0.05$). *LPAR4* and *5* mRNA transcription were up-regulated in myometrium assigned to Category III compared to other categories ($P<0.05$).

In Exp. 2, PGE_2 stimulated myometrial contractility at the early luteal stage (assigned to Category I; $P<0.05$), but did not affect on myometrial contractility in the early pregnancy ($P>0.05$). In turn, PGF and LPA stimulated myometrial contractility in the early luteal stage (assigned to Category I; $P<0.05$) and in the early pregnancy ($P<0.05$). Furthermore, OT as well PGs and LPA did not affect myometrial contractility in moderate and severe fibrosis (assigned to Category II and Category III; $P>0.05$).

Presented data showed that myometrial mRNA transcription of PG and LPA receptors and the myometrial contractility is disturbed in the course of endometrial fibrosis. These findings suggest that fibrosis may effect on physiological events occurring in equine uterus by alteration in myometrial contraction.

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Changes in the endometrium of mares after uterine lavage

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Uterine lavage is one of many methods of treatment the endometritis. It be required after breeding or insemination and postpartum, and it is used to remove debris, infectious organisms and fluid from the uterine lumen.

The aim of the study was to evaluate the changes in the endometrium of mares after uterine lavage. The research was conducted on 17 fertile mares, Icelandic horses, in oestrus cycles during one season. All mares were clinically healthy and had no clinical signs of endometritis (no discharge, no fluid in the uterus). In all mares a uterine lavage with 1L 0,9% NaCl heated to 38ºC, was performed, and two endometrial biopsies were taken: once before the flushing and then just after the flushing. The endometrial biopsies were fixed in 4% formalin and examined for the presence of PMNs within the luminal epithelium, stratum compactum and stratum spongiosum as well as for the Kenney-Doing score. Infiltration of PMNs in the biopsies was assessed on the basis of two criteria: more than one and more than three PMNs per five fields of high magnification (x 400). The results of biopsies obtained before the uterine lavage were compared to the results obtained after flushing.

We found changes in some samples in both biopsies taken from the same mares. In some cases we did not observe the influence of changes in the endometrium biopsies after the uterine lavage.

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Gene expression of prostacyclin synthase and receptor in the inflamed porcine uterus

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Uterine inflammation (metritis/endometritis) is the most frequent reproductive disorder in livestock with consequences ranging from no effect on reproductive performance to permanent sterility. Our earlier studies revealed that inflammation of the porcine uterus upregulates prostacyclin (PGI_2) synthesis and that PGI_2 increases contractile activity, however, the gene expression profiles of PGI synthase (PTGIS) and receptor (PTGIR) remain unknown. Therefore, the goal of the study was to determine the expression of PTGIS and PTGIR mRNA in the inflamed porcine uterus. On Day 3 of the estrous cycle (Day 0 of the study), either 50 ml of saline or *Escherichia coli (E. coli*) suspension (10^9 colony-forming units/ml) were infused into each uterine horn of the gilts. The uteri were collected on Days 8 and 16 following treatment, and on Day 0 without any experimental procedures. Acute endometritis developed in all bacteria-inoculated gilts, however on Day 8 of the study a severe form of acute endometritis was noted more often than on Day 16. The levels of PTGIS mRNA expression in the endometrium (Day 16) and myometrium (Day 8) of the *E. coli*-injected uteri were greater ($P<0.05$) than in the saline-treated ones. The expression of this gene in the endometrium of saline-(Day 16; $P<0.05$) and *E. coli* (Days 8 and 16; $P<0.01$)-injected uteri was higher as compared to the control tissues (Day 0). On Day 16 after treatment with saline ($P<0.01$) and bacteria ($P<0.05$), the myometrial mRNA contents of mRNA PTGIS were lower than on Day 0. The expression of PTGIR mRNA increased ($P<0.01$) in the inflamed endometrium on Day 16 as compared to the saline-treated uteri. Comparing to the control tissues, the amounts of the PTGIR mRNA in the endometrium of the saline-($P<0.05$, $P<0.01$) and *E. coli* ($P<0.01$)-injected organs on Days 8 and 16 were increased. A similar situation ($P<0.01$) was observed in relation to the mRNA PTGIR level in the myometrium of the inflamed uteri. Our studies show that an increase in the gene expression profile of PTGIS might be significant for PGI_2 synthesis in the inflamed porcine uterus, while the elevated level of PTGIR mRNA may be important for the impact of PGI_2 on the function of pathologically-changed organs. In general, the reported changes may be essential for the course/consequences of uterine inflammation.

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ENDOMETRIAL FACTORS FAVORING PYOMETRA ONSET IN DIESTRUS

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Pyometra is the most frequent disease of the canine uterus. Being a clinical entity *per se* it may conceal various pre-existing conditions that foster inflammation and the accumulation of exudate in the uterus. In the female dog, it is rather difficult to detect the exact moment when the inflammatory endometrial conditions begin, as often they evolves silently for a variable amount of time. It is possible that infertility would account for a frequent symptom, but in bitches not intent to breed this sign may be disregarded. The present work report a retrospective cohort case series study on the prevalence of inflammatory uterine diseases in OVH excisions received at the histopathology laboratory at UTAD (LHAP/UTAD), in the past 7-years (January 2008 to December 2014), for a total of 397 canine ovariohysterectomy specimens. Uterine pathology was diagnosed in 52% (207/397) of the specimens submitted to analysis. Histopathological classification[1] grouped the lesions as: non-inflammatory [32%(66/207); which included congenital abnormalities and endometrial atrophy, displasia, mucometra and cystic hyperplasia grade1] and inflammatory endometrial conditions (68%; 141/207). Inflammatory conditions of the endometrium were frequently associated with cystic endometrial hyperplasia (CEH[2]), in particular CEH grade4/pyometra (25%; 63/141), CEH grade2 (9%; 23/141) displaying a chronically inflammation of the endometrium, and CEH grade3 (6%; 16/141). Other inflammatory conditions of the endometrium evolved isolated: endometritis (8%; 18/141), metritis (4%; 11/141) and pyometra (4%; 9/141). Though the majority of the inflammatory endometrial diseases were associated with various form of CEH (72%; 102/141), and therefore developing in older females, there are close to 28% (39/141) of cases emerging in the absence of co-existing uterine pathology and independently of the female´s age. Retrospective data gathered in this study shows that endometrial inflammation is an important entity in female dogs. The fact that it may evolve sub-clinically for a while before originating a severe life-threatening disease endorses the need for additional studies pursuing the development of precocious detection methods for the clinical diagnosis of inflammatory uterine diseases.

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Inflammatory conditions in feline uterus

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Pyometra is a clinically relevant problem in female cats. In clinical practice, feline pyometra most often evolves in cyclic mature females in diestrus, but contrasting to dogs, it is less commonly found in association with cystic endometrial hyperplasia (Payan-Carreira, unpublished data). Besides the occurrence of ovulation (1) and breeding, progestagen contraceptives are pointed as major risk factors for this clinical syndrome (2). Diagnosed pyometra in cats often leads to surgery for OVH, but young breeding females are commonly subject to medical treatment. Using the archives from the histopathology laboratory at UTAD (LHAP/UTAD), for the past 7-years period (January 2008 to December 2014), this is retrospective cohort case series study on the prevalence of inflammatory uterine diseases in feline OVH excisions and the concurrent uterine lesions that might be found in pyometra specimens.

From a total of 313 ovariohysterectomy specimens, uterine pathology was diagnosed in 47.3% of the cases (148/313). Histopathological examination revealed that 69.6% (103/148) of the cases represented inflammatory conditions involving the cat uterus. The inflammatory conditions evolved independent from other uterine disease in only 14.6% (15/103) of the situations, in the form of pyometra (7.8%; 8/103) or endometritis (6.8%; 7/103). In all the remainder situations, the inflammatory conditions were diagnosed as coexisting with: 1/ cystic endometrial hyperplasia (54.4%; 56/103), being more frequently accompanied of endometritis than of pyometra (48.6% *vs.* 5.8%); 2/ feline endometrial adenocarcinoma (15.5%; 16/103), which more frequently coexisted with pyometra than with endometritis (12.6% *vs.* 2.9%). Cystic endometrial disease and endometrial carcinoma developed isolated in 38.8% (40/103) and 30.1% (31/103) of the cases, respectively.

Data gathered in this report show that in cats, alike in dogs, inflammatory conditions of the uterus coexists with other pathologies, particularly at the endometrium. This supports the need for the study of the changes associated with disturbance on the endometrial homeostasis and/or morphology on the immune competence of the organ and on the etiopathogenesis of particular diseases.

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