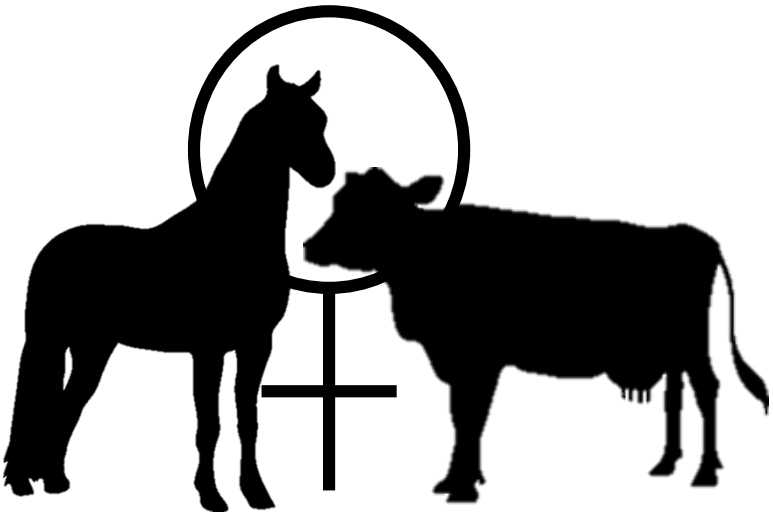
"4th International Conference on Endometritis in Cows and Mares"

and

joint Polish-Japanese Seminar "Cutting edge of Reproductive Physiology - Key processes for birth of a new life"

9th-11th of September 2019, Warsaw, Poland



POLSKA AKADEMIA NAUK

Institute of Animal Reproduction and Food Research • PAS •

Krajowy Naukowy Ośrodek Wiodący

Konsorcjum Naukowe "Zdrowe Zwierzę - Bezpieczna Żywność"
Leading National Research Centre
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http://endometritis.pan.olsztyn.pl/

We are grateful to our sponsors for generous support of the Confernce:

- KNOW Consortium
- joint JSPS-PAS project,
- PAS International Cooperation Department
- Institute of Animal Reproduction and Food Research of Polish Academy of Sciences
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Welcome to the Conference

Dear Colleagues,

We are pleased to welcome everyone to Warsaw for the International Conference "ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS". It is fourth edition of our Conference and we hope not last.

The first edition was held in Olsztyn in 2013 under special EU program Regpot – project Refresh that was realized to increase research standards of Institute and integration with the European Research Area and regional development.

The second one, in Gdansk, in 2015 was established as a main part of bigger international Conference on **"Biology and Pathology of Reproduction in Domestic Animals".**

The third Conference, held again in Olsztyn in 2017, was established under our next project KNOW: Leading National Research Center in Veterinary Sciences: "Healthy Animal – Safe Food".

This year, we are meeting in Warsaw, again with our collages from Japan. The fourth "Endometritis" conference is held together with the **joint Polish-Japanese Seminar "Cutting edge of Reproductive Physiology - Key processes for birth of a new life". More than 60** participants from Poland, Germany, Austria, Portugal, Switzerland, Finland, Belgium, Ireland, UK, Israel, Japan, Iran, Egypt (**researchers, students and practitioners)** are attending this year Conference.

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

We want to thank all of our invited speakers who have accepted our invitation to come to Warsaw. We are very pleased to have them with us in our meeting and look forward to hearing about the interesting research that they carry out.

Finally, we want to thank organizing committee for their hard work. We are grateful to you for your work and support. Especially I want to thank:

Anna Szóstek-Mioduchowska, Karolina Łukasik, Paweł Kordowitzki, Beenu Moza Jalali from the Department of Reproductive Immunology and Pathology, IAR&FR PAS and Wojtek Baranski from the Department of Animal Reproduction, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn

We wish all of you many fruitful discussions, meeting old friends, making new partnerships and having a pleasant stay in Warsaw.

The Conference is organized under KNOW Consortium support and funds of PAS: joint JSPS-PAS project, BWZ and DUN.

Tomasz Janowski

Dariusz J. Skarzynski

GENERAL INFORMATIONS

The Conference Venue will be the Aramis Hotel, talks will be held in the conference room at lobby floor.

FOOD SERVICE

Meals

Lunches will be provided with a voucher in Aramis Hotel Restaurant according to the schedule of the meeting.

Coffee Breaks

Coffee and cookies will be available on the corridor close to the presentation room.

SOCIAL EVENTS

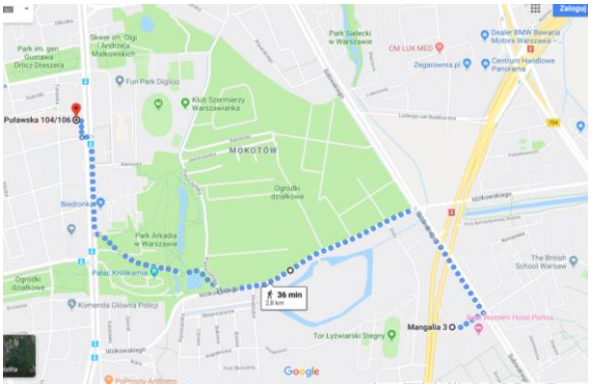
Welcome

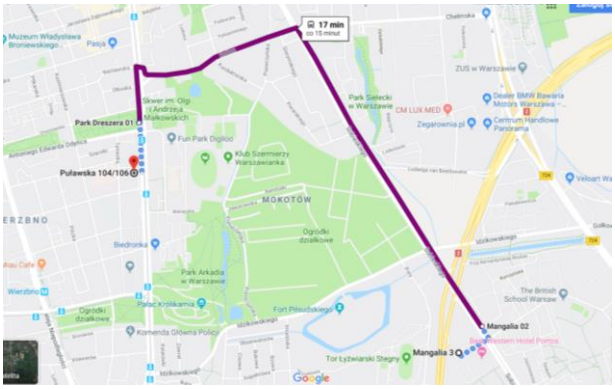
Sunday, 8. September, 6:00- 8:00 pm (Free for registrants), dinner at Atos Hotel Restaurant

Gala Dinner

Monday, 9. September, 8:00- 11:30 pm (Free for registrants). The below named Restaurant is located in walking distance from the Venue Hotel. You can also take the bus number 172 (5 stops):

Restauracja Stary Dom
ul. Puławska 104 / 106
02-620 Warszawa





Sunday, September 8th

18:00-20:00 - Welcome reception & Registration – Atos Hotel restaurant

Monday, September 9th

8:00-8:45 - Registration

8:45-9:00 - Opening ceremony - Kyoshi OKUDA & Dariusz SKARŻYŃSKI & Tomasz JANOWSKI

9:00-9:45 - **Plenary lecture I –** ***Tolerance and Innate Immunity Shape the Development of Endometriti****s,* **Martin SHELDON**

9:45-10:00 - Coffee break

10:00-12:00 Session I: Endometritis and Endometrosis and Placentitis in mares. Moderators: Ch. AURICH; I. CANISSO

10:00-10:30 - *Update on diagnostic procedures and therapeutic approaches for endometritis in the horse*; Christine AURICH,

10:30-11:00 - *Recent developments in the pathogenesis, diagnosis, and treatment of placentitis in mares*; Igor F. CANISSO,

11.00-11:20 - *Changes in secretion of anti-inflammatory cytokines and acute-phase proteins in the uterus after artificial insemination in the mare*; Roland KOZDROWSKI

11:20-11:40 - *Does epigenetics regulate MMP2 and MMP9 transcripts in equine endometrosis?*; Graca FERREIRA - DIAS

11:40-12:00 - *The role of mediators of inflammation in the development of mare endometrosis*; Anna SZÓSTEK – MIODUCHOWSKA

12:00-12:10 - *Comparative study of steroids receptor expression in mare endometrial fibroblast and myofibroblast culture*; Natalia LECIEJEWSKA

12:10-13:00 Lunch

13:00-13:45 Plenary lecture II - ***Bovine endometritis and bacteriology of uterus****,* **Takeshi OSAWA**

13:45-14:00 Coffee break

14:00-16:00 Session II: Endometritis in cows. Moderators: M. DRILICH; R. de La SOTA

14:00-14:30 - *Current concepts of endometritis in cattle*; Marc DRILICH

14:30-15:00 - *Metritis and endometritis in grazing dairy cows: risk factors, diagnosis, treatment and reproductive performance*; Rodolfo Luzbel de La SOTA

15:00-15:20 - *Influence of intrauterine administration of Lactobacillus buchneri DSM 32407 on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis*; Christoph GABLER

15:20-15:40 - *Inhibin, folliculostatin and peristatin as new markers for uterine health and future pregnancy*; Dawid TOBOLSKI

15:40-16:00 - *The effect of clinical endometritis on the ovarian activity in high yielding dairy cows during post-partum period*; Zeravan MOHAMMED

16:00-16:15 Coffee break

16:15-17:35 Session II: Endometritis in cows. Moderators: M. DRILICH; R. de La SOTA

16.15-16.35 - *Cytological endometritis in primiparous and multiparous dairy cows - an analytic approach for diagnosis based on endometrial polymorphonuclear cells cytology threshold;* Tal RAZ

16:35-16:50 - *Uterine bacterial metagenomics and bacterial load in dairy cows with metritis and cytological endometritis*; Ron SICSIC

16.50-17:05 - *Exploring the role of Streptococcus uberis in bovine endometritis*; Panagiotis BALLAS

17:05-17:20 - *Investigation of the reproductive tract health in cows with different degrees of pneumovagina*; E. Sinem ÖZDEMİR SALCI

17:20-17:35 - *Interleukin 8 mRNA was higher expressed in the endometrium at the time of artificial insemination in cows that did not conceive compared with their fertile counterparts*; Karen WAGENER

20:00-23:30 - **Gala Dinner - Stary Dom Restaurant**

Tuesday, September 10th

8:30-9:00 - Registration

Endometritis and Polish-Japanese Seminar JOINT SESSION

9:00-9:45 Plenary lecture III - *MicroRNAs: small but potent molecules in animal reproduction,* Monika KACZMAREK

9:45-10:15 Coffee break

10:15-12:30 Session III: Immune-endocrine function of uterus. Moderators: J. SCHÖN; B. JALALI

10:15-10:45 - *Current approaches and future perspectives for modeling the female reproductive tract in vitro*; Jennifer SCHÖN

10:45-11:05 - *Remodeling of porcine endometrium during Peri-implantation period: molecular changes*; Beenu MOZA JALALI

11:05-11:25 - *Understanding the uterine environment in subfertile cattle*; Shuichi MATSUYAMA

11:25-11:45 - *The influence of Heat Stress on the Endocrine Function of Bovine Endometrium*; Koji KIMURA

11:45-12:00 - *Heat stress alters innate immune responses in bovine endometrial cell*; Sunsuke SAKAI

12:00-12:15 - *Bisphenol a and its analogs affects contractile activity of the porcine uterine smooth muscle*; Aleksandra ZYGMUNTOWICZ

12:15-12:30 - *Obesity Alters Leptin Signalling in Mouse Uterus: Putative Link to Epigenetic Regulation During Decidualisation*; Edyta WALEWSKA

12:30-13:30 Lunch

Polish-Japanese Seminar: Cutting edge of Reproductive Physiology - Key processes for birth of a new life

13:30-14:55 OVARIAN FUNCTIONS – FOLLICLE/CORPUS LUTEUM. Moderators: A. BLITEK, R. NISHIMURA

13:35-13.55 - *Role of prostacyclin in the corpus luteum of the pig*; Agnieszka BLITEK

13.55-14:15 - *Role of glycoconjugates and lectins in the corpus luteum*; Junko NIO-KOBAYASHI

14:15-14:35 - *VASPIN – new adipokine in the ovarian physiology: expression and direct effect on signaling pathways, steroidogenesis, proliferation and apoptosis. In vitro studies on the porcine model*; Agnieszka RAK

14:35-14:55 - *Multiple roles of hypoxia in bovine corpus luteum function*; Ryo NISHIMURA

14:55-15:25 Coffee break

15:25-16:30 OVARIAN FUNCTIONS – CORPUS LUTEUM. Moderators: A. BLITEK, R.NISHIMURA

15:25-15:45 - *The action of transcription factor Gata4 on gonadal promoter regulation is modulated via PKA and ERK1/2 pathway in steroidogenic cells*; Hiroaki TANIGUCHI

15:45-16:05 - *The effect of PGF2α on synthesis and release of progesterone through peroxisome proliferator-activated receptors in the bovine corpus luteum*; Barbara SOCHA

16:05-16:30 - *Progesterone receptor isoforms in bovine corpus luteum*; Robert RĘKAWIECKI

Wednesday, September 11th

9:00-11:00 OVIDUCT FUNCTIONS and GAMETS AND EARLY EMBRYO DEVELOPMENT. Moderators: Y. YAMAMOTO; A. ANDRONOWSKA

09:00-09:20 - *Generating mechanisms for spntanieus rhythmic contraction of bovine oviduct*; Yuki YAMAMOTO

09:20-09:40 - *The effect of lysophosphatidic acid (LPA) on ovarian follicle function, oocyte fertilization and early embryo development in cows*; Izabela WOCŁAWEK – POTOCKA

09:40-10:00 - *Multi-step process of ciliogenesis in bovine oviductal epithelium during the estrous cycle*; Sayaka ITO

10:00-10:20 - *Function of the porcine oviduct under different physiological and hormonal conditions*; Aneta ANDRONOWSKA

10:20-10:40 - *The novel techniques of in vitro maturation of porcine oocytes using 3D culture systems*; Gabriela GORCZYCA

10:40-11:00 - *The biology of telomeres and TERRA in bovine oocytes- two aspects of reproductive ageing*; Paweł KORDOWITZKI

11:00-11:30 Coffee break

11:30-12:30 CURRENT ASPECTS OF HORSE BREEDING IN POLAND AND JAPAN AND WILD ANIMALS. Moderators: K. OKUDA; D. SKARŻYŃSKI

11:30-11:50 - *The history and current view of polish konik horse*; Marta SIEMIENIUCH

11:50-12:10 - *Status and prospect of Horse breeding and reproductive treatment in Japan*; Yasuo NAMBO

12:10-12:30 - *The relationship between size of sebaceous glands in back skin and plasma testosterone concentration in male brown bear*; Jumpei TOMIYASU

12:30-12:40 Closing ceremony - Kyoshi OKUDA & Dariusz SKARŻYŃSKI & Tomasz JANOWSKI

12:40 Lunch

Plenary lecture I

Tolerance and Innate Immunity Shape the Development of Endometritis

I. Martin Sheldon

Institute of Life Science, Swansea University Medical School, Swansea University, Singleton Park, Swansea, SA2 8PP, UK. E-mail i.m.sheldon@swansea.ac.uk

Pathogenic bacteria are always present in the uterus of cattle after parturition. Maintaining uterine health depends on a robust innate immune response to resist the pathogens, and the ability to tolerate the damage that pathogens cause. Unfortunately, up to 40% of modern dairy cattle develop some form of uterine disease, and this is important because uterine disease impairs fertility. Here, I argue that uterine disease is shaped by the innate immune response to the pathogens in the endometrium, and the damage pathogens cause to endometrial cells.

Innate immunity depends on receptors on host cells binding to pathogen-associated molecular patterns, such as bacterial lipopeptides and lipopolysaccharide. Activation of innate immunity leads to the secretion of inflammatory mediators, such as chemokines and cytokines. These inflammatory mediators attract and regulate the neutrophils and macrophages that clear pathogens and damaged cells from the endometrium, and these cells form the pus that is the cardinal sign of uterine disease.

The innate immune response is scaled to match the severity of the pathogen challenge and tissue damage. However, metabolism is a key regulator of innate immunity. Innate immunity is metabolically demanding; glucose and glutamine fuel the innate immune response in the endometrium. Conversely, metabolic stress, caused by limiting glucose and glutamine, limits the ability to mount robust inflammatory responses.

Damage to the endometrium is caused by trauma and by pore-forming toxins secreted by pathogenic bacteria. For example, pyolysin is a cholesterol-dependent cytolysin that forms pores in the plasma membrane of cells, particularly leading to the death of endometrial stromal cells. However, metabolism can influence the sensitivity of cells to pyolysin, and manipulating cellular metabolic pathways alters the ability of stromal cells to tolerate pyolysin.

In conclusion, postpartum uterine disease is a consequence of failure to tolerate pathogens and/or compromised resistance. The severity of disease is shaped by the inflammatory response to pathogenic bacteria and the damage pathogens cause. Enhancing the ability of animals to tolerate pathogens, and controlling metabolic stresses that impair immunity and tolerance, may help counter uterine disease.

Session I: Endometritis and Endometrosis and Placentitis in Mares

Update on diagnostic procedures and therapeutic approaches for endometritis in the horse

Christine Aurich

Artificial insemination and Embryo Transfer, Vetmeduni Vienna, Austria

Persistent endometritis is one of the major causes for subfertility in horses. Mares that suffer from subclinical persistent endometritis may be mated but will not conceive, loose their embryo or develop placentitis. Because optimization of antibiotic treatment is a keystone in the fight against increasing antimicrobial resistance, the precise diagnosis of endometritis and associated pathogens in mares intended for breeding is more important than ever.

Pathogenic organisms responsible for development of persistent endometritis in mares are either introduced at breeding or ascend into the genital tract. Susceptibility of mares to infectious endometritis is, however, mostly a result of impaired mechanical uterine clearance mechanisms. Escherichia coli and Streptococcus equi subsp. zooepidemicus (S. zooepidemicus) are the most common pathogens involved. Infectious endometritis in mares caused by S. zooepidemicus has recently been recognized as an infection with specifically adapted endometrial pathogenic strains and is no longer considered a result of random contamination (Doolewert-Rasmussen et al. 2013, Vet Res 44:26). S. zooepidemicus isolates from mares with endometritis often show a high genetic relation, i.e. they belong to a genetically distinct subpopulation. Some strains of S. zooepidemicus are able to reside deep in equine endometrial tissue in chronically infected mares. In this dormant stage, they evade the host immune response, persist in the uterus without being associated with cytological evidence of inflammation and are not detectable by standard bacterial culture techniques. Other bacteria, e.g. Pseudomonas aeruginosa produce biofilm, a common persistence strategy among bacteria for survival. Biofilms may be persistent to the host immune response as well as treatment with antibiotics. In such cases, endometrial areas free of tissue-adherent bacteria exist besides specific focal sites of infection. An inflammatory endometrial response, however, is present throughout the endometrial tissue (Ferris et al. 2017, Infect Immun 85:e00332-17).

Diagnostic approaches in mares with a history of subfertility should go beyond sampling of endometrial bacterial swabs but include a careful examination of the genital tract and collection of more invasive samples for bacterial culture like low-volume uterine lavage or endometrial biopsy. In addition, endometrial tissue samples should undergo histological assessment for signs of inflammation. Unexplained inflammation may suggest focal infection and require further diagnostic measures. Treatment of endometritis should exclusively include effective antibiotics. Intrauterine application with optimal dosage regimens is most appropriate. For treatment of endometrial infections with dormant S. zooepidemicus, their activation (Petersen et al. 2015, Vet Microbiol 179:119-125) may be necessary. Alternative approaches to support antibiotic treatment have to be included. They aim at enhancement of mechanical uterine clearance, physical removal of bacteria, biofilm disruption as well as anti-inflammation.

Recent developments in the Pathogenesis, Diagnosis, and Treatment of Placentitis in Mares

Igor F. Canisso

Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, USA.

Bacterial placentitis is in important cause of abortion, stillbirth, and neonatal death in horses. Four morphologic types have been described, namely ascending, focal mucoid (nocardioform) diffuse (hematogenous) and multifocal. Overall, ascending placentitis is the most prevalent type of placentitis. Common bacteria causing placentitis include β-hemolytic streptococci (Streptococcus equi spp. zooepidemicus and Streptococcus equisimilis), Crossiella equi, Amycolatopsis ssp, Leptospira, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa, of which β-hemolytic streptococci predominate. Premature mammary gland development and vulvar discharge are commonly associated with ascending placentitis, but not with the other types. Pathogenesis of placentitis has not been fully elucidated, but it has been suggested that bacteria infects the fetoplacental unit via migration through the cervix, or hematogenous routes, or during gynecological procedures. Once the infection of the chorioallantois is established, bacteria disseminate to the fetoplacental unit. Placental infection coupled with the massive fetal exposure to pro-inflammatory cytokines by the fetal membranes are thought to play a pivotal role in the chronic fetal stress, in-utero growth retardation, and premature delivery. Infected chorioallantois secretes pro-inflammatory cytokines and prostaglandins. Acute placentitis results in a downregulation of the progesterone receptor in the myometrium and reduction in the progesterone metabolizing enzymes. Fetoplacental unit steroids have been measured in plasma of mares suffering from placentitis. Mares with chronic placentitis have an increase in peripheral progestogens; however, mares acutely infected will display a reduction in peripheral concentrations of progestogens. Estradiol-17β (free- and conjugated forms) are drastically reduced in plasma of mares with placentitis. Recently proteins present in the fetoplacental unit have also been measured in plasma of mares with placentitis and alpha-fetoprotein appears to be a suitable diagnostic and prognostic marker for this disease. Acute-phase proteins, particularly serum amyloid A, are increased in plasma of placentitis mares, this increase is due to endometrial secretions, and minimally from the chorioallantois and fetus. Treatment for placentitis is based on preventing widespread of bacteria through the fetoplacental unit by administering antibiotics, reducing inflammation of the chorioallantois and endometrium by administering non-steroidal anti-inflammatories, and steroids to promote uterine quiescence (progestins) and uterine blood flow (estrogens). Recently, administration of estrogen has been shown to be an effective approach to prolong the time from treatment to delivery and fetal growth. Correction of the reproductive barriers (i.e., vulva and cervix), may prevent air aspiration and infection of the fetoplacental unit. Vaccination of late pregnant mares may aid prevention of leptospirosis, however, this has not been critically assessed. This presentation aimed to review recent discoveries regarding placentitis in mares.

Changes in secretion of anti-inflammatory cytokines and acute-phase proteins in the uterus after artificial insemination in the mare

Wojtysiak K.[1], Ryszka W.[2], Stefaniak T.[3], Kozdrowski R.[1]

[1]Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Plac Grunwaldzki 49, 50-366 Wroclaw, Poland;
[2]Equi Salus, Glinno 65, 64-300 Nowy Tomyśl, Poland;
[3]Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, C. K. Norwida 31, 50-375 Wroclaw, Poland.

The mechanisms responsible for development of persistent breeding-induced endometritis (PBIE) are not fully understood. The objective of the study was: 1) evaluation of the concentrations of anti-inflammatory cytokines: interleukin-1 receptor antagonist (IL-1RA) and interleukin-10 (IL-10) in uterine lavage fluid before and after artificial insemination (AI); 2) evaluation of the concentrations of acute-phase proteins: serum amyloid A (SAA) and haptoglobin (Hp) in uterine lavage fluid before and after AI; and 3) determination of the importance of fluid accumulation in the uterine lumen during estrus in the etiopathogenesis of PBIE. Based on ultrasound examination mares were divided into three groups: group 1 (n=9) served as a control group. In this group, no fluid was detected in the uterus during estrus and 7 h after AI. In group 2 (n=8), no fluid was detected in the uterus during estrus but 7 h after AI a depth of more than 2 cm of fluid was detected in the uterus. In group 3 (n=8), more than 2 cm of fluid was detected in the uterus during estrus and also 7 h after AI. In all groups of mares 7 h after AI a significant increase in polymorphonuclear cells (PMN) was recorded, however, no differences were found among particular groups of mares before and after AI. The concentration of IL-1RA before AI was significantly higher in group 3 mares compared to group 1, while the concentration of SAA before AI was significantly lower in group 1 compared to group 2. After AI, a significant increase in IL-1RA and SAA was observed in all three groups of mares, however no significant differences were detected among the groups of mares at this time. No differences were found in the concentration of IL-10 and Hp among particular groups of mares before and after AI. Modulation of the inflammatory process in response to AI is a part of the innate immune defense mechanism and based on our results we suggest that inflammatory response and anti-inflammatory processes occurring 7 h after semen deposition are similar in mares resistant and susceptible to PBIE. At this time, independent from the status of the mare before AI, the endometrial response characterized by PMN influx, and SAA, Hp, IL-1RA and IL-10 production, is similar. The presence of intrauterine fluid during estrus is not connected with PMN influx but can impact uterine IL-1RA production at this time, and also can reduce pregnancy rates.

Does epigenetics regulate MMP2 and MMP9 transcripts in equine endometrosis?

Alpoim-Moreira J.[1], Fernandes C.[1], Pimenta J.[2,3], Bliebernicht M.[4], Rebordão M.R.[1,5], Skarzynski D.J.[6], Ferreira-Dias G.[1]

[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal;
[2]Reproduction and Development Laboratory, Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal;
[3]Genetic Resources and Biotechnology Unit, National Institute of Agrarian and Veterinarian Research, Santarém, Portugal;
[4] Embriovet, Muge, Portugal;
[5]Coimbra College of Agriculture, Polytechnic Institute of Coimbra, Coimbra, Portugal;
[6]Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland

Endometrosis is still a puzzle regarding pathogenesis and treatment of this fibrotic degenerative condition in mare endometrium. Collagen (COL) deposition is associated with the advance of mare endometrial fibrosis. Collagen 1 is usually predominant in Kenney´s category III endometrium (severe fibrosis), and COL3 in category I healthy endometrium. *Matrix metalloproteinases* (MMPs) are responsible for the remodelling of the extracellular matrix. When their transcription is inhibited there is an increase of COL in the tissues, as COL degradation is lower than its production. Epigenetics have been involved with endometrial fibrosis, as demonstrated in our previous studies. One way to evaluate epigenetics mechanisms is through DNA methylation by DNA methyltransferases (DNMT1, DNMT3A and DNMT3B). Therefore, the aim of this study was to assess whether epigenetics mechanisms (expression of DNA methyltransferases) correlate with *MMP2* and *MMP9* transcription in the endometrium with different degrees of fibrosis. Endometrium biopsies were obtained from cyclic mares (n=26) and classified according to Kenney's grading system (category I, n=8; category II =12; category III, n=6). Transcripts of *COL1A2, COL3A1, DNMTs, MMP2* and *MMP9* were assessed by qPCR. Protein from COL1 and COL3 was evaluated by enzyme immunoassay. Data were analysed by one-way analysis of variance (ANOVA), Mann-Whitney test, T-Test and Pearson correlation. Transcripts of *MMP9* were lower in category III than in category I endometrium ($P<0.05$), and transcripts of *MMP2* also fell in category III, but in relation to category II ($P<0.05$). Regarding *COL1A2* mRNA, the levels decreased in category III endometrium compared to category II ($p<0.05$). There were no significant differences in *COL3A1* mRNA levels between endometrium categories. However, COL1 protein levels in mare endometrium were higher in category III than in categories I and II ($P<0.05$), while COL3 protein was increased in category I, with respect to categories II and III ($P<0.05$). Transcripts of *MMP2* were correlated with *COL1A2* ($P<0.01$), *COL3A1* ($P<0.01$) and *DNMT3B* ($P<0.001$) mRNA levels, and also with COL3 protein levels ($P<0.05$). Regarding *MMP9* transcripts the only correlation found was with COL3 protein levels ($P<0.05$). Levels of *DNMT3B* mRNA increased with fibrosis ($P<0.05$), indicating a higher methylation, which in turn may lead to gene repression. Lower transcription levels of *MMP2* have been associated with higher collagen transcription levels of *COL1A2* and *COL3A1* in mare endometrium. Although this was not observed in our study, the COL1 protein levels were higher as endometrial fibrosis increased. As hypermethylation usually results in gene repression, our data suggest that, as fibrosis increases, such high methylation might be repressing *MMP2*, *MMP9* and ultimately *COL1A2* transcription. In the category III endometrium, the high COL1 protein levels might be ascribed not only to a decrease in its degradation, but also to its synthesis.
Funding: UID/CVT/00276/2019; PTDC-CVT-REP-4202-2014.

The role of mediators of inflammation in the development of mare endometrosis

Szóstek-Mioduchowska A.Z.[1], Baclawska A.[1], Ferreira-Dias G.[2], Okuda K.[3,4]
Skarzynski D.J.[1]

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Endometrosis is a degenerative chronic condition in the equine endometrium, defined as an active or inactive fibrosis around the endometrial glands and/or in the stroma, often associated with pathological changes in the endometrial glands within fibrotic foci [1-2]. This condition is characterized by excessive deposition of extracellular matrix (ECM) components, as collagen type I (COL1) and fibronectin around the endometrial glands and stroma that leads to the destruction of tissue architecture and impairment of endometrial function [1-3]. Equine endometrosis causes not only alterations in the uterine morphology, but also in functions, ultimately leading to changes in uterine microenvironment, ovarian cycle and early pregnancy dysfunction [1; 3; 5-7]. The pathogenesis of equine endometrosis is not yet well known. However, it has been hypothesized that this condition occurs as a consequence of repeated, chronic inflammation due to insults associated with breeding, foaling and veterinary intervention [1; 8-9]. Inflammation seems to be associated with development of fibrosis in a paracrine way by the secretion of profibrotic chemokines, cytokines and other factors from injured tissue and inflammatory cells. Inflammatory mediators could act on endometrial, as well immune resident cells, such as fibroblasts, monocytes and macrophages, and affect fibrogenesis and ECM remodeling [10-11]. This process could include quantitative and qualitative changes in the ECM, mediated by enzymes that are responsible for ECM degradation, such as matrix metallopeptidase (MMPs). Their activity is low in physiological conditions but elevated during repair or remodeling processes [12]. Our study showed that the level of MMP-1, -2, -9 was up-regulated in the course of endometrosis compared to category I endometrium in follicular phase of estrous cycle ($P<0.05$). In turn, the level of MMP-3 was down-regulated in category III endometrium compared to category 1 endometrium in mid luteal phase of estrous cycle ($P<0.05$). Our findings suggest that in endometrial degeneration the level of endometrial MMPs and TIMPs is altered. Although MMP are important factors in the process of fibrosis, the knowledge about their regulation in equine endometrosis is still limited. The aim of our study was to investigated the effect of prostaglandin (PG)E_2, $PGF_{2\alpha}$, interleukin (IL)-1β, IL-6 and transforming growth factor (TGF)-β1 on MMPs and their inhibitors as well as on COL1 and COL3 in equine endometrial cells and tissue. Prostaglandin E_2 treatment increased MMP-2 and MMP-9 secretion, and decreased MMP-13 secretion in a time and dose-dependent manner in equine endometrial fibroblasts ($P<0.05$). In turn, $PGF_{2\alpha}$ treatment increased MMP-2, MMP-13 and COL1 secretion and decreased MMP-1 secretion in time and dose-dependent in equine endometrial fibroblasts ($P<0.05$). IL-1β treatment up-regulated secretion of COL1, MMP-2, TIMP1, and TIMP2 in category I endometrial fibrosis tissues ($P<0.05$). IL-6 treatment up-regulated secretion of ECM, MMP-2, and MMP-3 and down-regulated secretion of MMP-9 in category I endometrium ($P<0.05$). Transforming growth factor-β1 up-regulated MMP-1, -3, -9, TIMP-2, COL1 and COL3 secretion but down-regulated MMP-

3 secretion from fibroblast cells in time-dependent manner (P<0.05). In epithelial cells, TGF-β1 up-regulated MMP-1, -9, -13 and TIMP-1, -2 in time-dependent manner (P<0.05). Our results showed that PGs, ILs and TGF-β1regulated expression of MMPs and their inhibitors (TIMPs) and COL what strengthen the hypothesis that mediators of inflammation participate in development of mare endometrosis by effect on ECM remodeling.

References:

[1]Kenney RM. Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. J Am Vet Med Assoc. 1978 ;172(3):241-62.
[2] Kenney RM, and Doig PA. 1986. *Equine Endometrial Biopsy. In Morrow, D.A. (Hrsg.): Current Therapy in Theriogenology 2.* Philadelphia: W.B. Saunders, Comp.
[3]Hoffmann C, Ellenberger C, Mattos RC, Aupperle H, Dhein S, Stief B, Schoon HA.The equine *endometrosis*: new insights into the pathogenesis. Anim Reprod Sci. 2009;111:261-78.
[4]Hanada M, Maeda Y, Oikawa MA. Histopathological characteristics of endometrosis in thoroughbred mares in Japan: results from 50 necropsy cases. J Equine Sci. 2014;25:45-52.
[5]Lehmann J, Ellenberger C, Hoffmann C, Bazer FW, Klug J, Allen WR, Sieme H, Schoon HA. Morpho-functional studies regarding the fertility prognosis of mares suffering from equine endometrosis. Theriogenology 2011;76:1326-36.
[6]Szóstek AZ, Lukasik K, Galvão AM, Ferreira-Dias GM, Skarzynski DJ. Impairment of the interleukin system in equine endometrium during the course of *endometrosis*. Biol Reprod. 2013;89:79
[7]Szóstek AZ, Siemieniuch MJ, Lukasik K, Galvão AM, Ferreira-Dias GM, Skarzynski DJ. mRNA transcription of prostaglandin synthases and their products in the equine endometrium in the course of fibrosis. Theriogenology 2012;78:768-76.
[8]LeBlanc MM, Causey RC. Clinical and subclinical endometritis in the mare: both threats to fertility. Reprod Domest Anim. 2009 Sep;44 Suppl 3:10-22.
[9]Rebordão MR, Galvão A, Szóstek A, Amaral A, Mateus L, Skarzynski DJ, Ferreira-Dias G. Physiopathologic mechanisms involved in mare endometrosis. Reprod Domest Anim. 2014;49:82-7.
[10]Liu Y. Cellular and molecular mechanisms of renal fibrosis. 2011. Nature Rev Nephrol. 7, 684-696.
[11]Zeisberg M, Kalluri R. Cellular Mechanisms of Tissue Fibrosis . 1. Common and organ-specific mechanisms associated with tissue fibrosis. Am J Physiol Cell Physiol. 2013 304:C216-25.
[12]Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15:786-801.

Comparative study of steroids receptor expression in mare endometrial fibroblast and myofibroblast culture

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Endometrosis is a degenerative chronic condition in the equine uterus. It is one of the most common cause of mare infertility. Endometrosis is characterized by increased endometrial collagen deposition, periglandular nets formation and excessive myofibroblast activation. Myofibroblasts arising during the disease are one of the factors that cause disturbances in the proper functioning of the organ. Endometrium with fibrotic changes shows a distinctly disturbed expression of sex steroid hormone receptors in comparison to the health endometrium. As a result, cells are unable to react to cyclic endocrine changes and become independent of hormonal control mechanisms in the endometrium. The mechanism involved in endometrosis development is still not known. Obtaining the cellular model of the endometrosis in vitro would facilitate the search for common mechanisms responsible for the development of the disease. The aim of study was to compare the expression of steroid receptors in equine endometrial fibroblasts (EEF) and myofibroblasts differentiated from endometrial fibroblasts. The horse serum was used to differentiate fibroblasts into myofibroblasts which make it possible to obtain a myofibroblast cellular model. Fibroblasts were isolated and cultured in Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F12 without phenol red medium. Fibroblasts were cultured in the presence of 10% addition of fetal calf serum (FCS) or 10 % addition of horse serum (HS) for differentiation fibroblasts into myofibroblasts. Cells were cultured during 7 days and the medium was changed every second day. The mRNA transcription of steroid receptors: estrogen receptors 1 (ESR1) and 2 (ESR2), progesterone (PGR) and androgen (AR) receptor have been studied using Real-time PCR. There were differences in steroid receptor expression in fibroblasts compared to myofibroblasts. Myofibroblasts showed a lower expression of ESR1, ESR2, PGR and AR compared to fibroblasts ($P<0.05$). Disorders in endocrine regulation of the endometrium may be associated with an increasing amount of myofibroblasts in the affected tissue. These cells show reduced expression of steroid receptors, which negatively affects the functioning of the organ. Our results suggest that myofibroblasts obtained during the culture in the presence of 10% HS may mimic typical changes occurring in the course of endometrosis. Although more analyses are needed, the myofibroblasts can become an cellular model in the study of endometrosis.

Plenary Lecture II

Bovine endometritis and bacteriology of uterus

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Heavy bacterial colonization in the genital tract subsequent to trauma, dystocia, or poor hygiene and poor uterine defense mechanisms can lead to establishment of puerperal uterine infection. In cattle, nonpathogenic bacteria in the uterus disappear more quickly after a difficult calving than after normal parturition, and pathogenic isolates persist longer in dystocia-affected animals. Although prevalence of subclinical endometritis in beef cows is usually much lower than dairy cows, suckled cows had higher percentage of neutrophils in the endometrium (PMN%) than early weaned cows during early postpartum (pp) period in Japanese Black cows [1]. Poor nutritious status that resulted from energy consumption and fat mobilization by suckling may be involved in the delay not only in the resumption of ovarian cyclicity but also restoration of the endometrium in the process of uterine involution.

Escherichia coli and Trueperella pyogenes are known to be pathogenic bacteria causing endometritis. In our study, pathogenic bacterial detection rates were higher in the cows with endometritis at week 3 pp (W3) and W5 than those at W7 [2]. We analyzed blood metabolite concentrations with persistent bacterial uterine infection, that caused by T. pyogenes and anaerobic bacteria, uterine bacteriological swabs were collected from lactating Holstein cows at W5 and W7, and PMN% was evaluated [3]. Glucose concentrations prepartum were negatively correlated with persistent bacterial infection postpartum ($P < 0.01$). Decreased prepartum blood glucose concentrations might be an important risk factor for persistent pp uterine infection. We also investigated the relationship between the persistence of uterine bacterial infections with endometritis and ovarian function [4]. A positive correlation ($P < 0.001$) was noted between the severity of endometritis and the persistence of infection. Cows with persistent infections had a significantly prolonged luteal phase compared with cows without infection ($P < 0.01$).

Mycoplasma and ureaplasma species are believed to be not part of the normal vaginal flora. M. bovigenitalium and U. diversum have been detected in the genital tract of normal and repeat breeder cows, which has led to speculation concerning its role as a pathogen [5,6]. We investigated the incidence of mycoplasma infection in the uterus of pp dairy cows and its relationship to the occurrence of endometritis. Intrauterine samples of the dairy cows at W5 and W7 were placed in mycoplasma culture broth as well as specific agar plates and cultured. A rapid PCR was used to detect seven mycoplasma species. Of the seven mycoplasma species, only M. bovigenitalium was detected in 7.4% of the samples. The incidence of dystocia was higher ($P < 0.001$) in mycoplasma positive (29%) compared with mycoplasma negative (2%) cows. In addition, the incidence of cytological endometritis was higher ($P < 0.05$) in mycoplasma positive (50%) than mycoplasma negative (24%) cows at W7. M. bovigenitalium infection in the uterus may be associated with recent dystocia and with endometritis in pp dairy cows.

Recent studies have reported that Lactobacillus spp. are present in the uterus of the cow during uterine restoration period in pp. Co-culture experiment with L. ruminis and endometrial epithelial cells in vitro confirmed that Lactobacillus spp. not causing endometrial epithelial cells

damage, conversely, revealing immunomodulatory properties [7]. We evaluated the influence of Lactobacillus spp. on uterine environment in pp dairy cows. The results suggested that Lactobacillus spp. reduce PMN% in cows with endometritis, may inhibit the growth of T. pyogenes, and accelerate the clearance in the uterus in the medium stage of pp. Conversely, absence of Lactobacillus spp. with low PMN% during the last stage of pp implies that uterus is completely involuted. Therefore, timing of the presence of Lactobacillus spp. during pp may have a diagnostic significance. Lactobacillus spp. can improve health status of the reproductive tract and help accelerate uterine involution in pp dairy cows.

In an attempt to eliminate endometrial pathogens, bovine practitioners have used antibiotics. However, the drawbacks are residues in meat and milk and potential development of bacterial resistance. Therefore, it is important to find effective therapies without such negative consequences. Use of probiotics such as Lactobacillus spp. or other type of antimicrobial agents such as povidone-iodine may be an option for therapeutic or preventive measures to control bovine endometritis.

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References:

[1] Kozuka N, Uematsu M, Kitahara G, Osawa T. 2018. Effects of early weaning on postpartum endometrial environment in beef cows. Proc XXX World Buiatrics Congress, Sapporo, Japan, pp. 138-9.
[2] Nazhat S, Kitahara G, Kozuka N, Mido S, Sadawy M, Ali H, Osawa T. 2018. Associations of periparturient plasma biochemical parameters, endometrial leukocyte esterase and myeloperoxidase, and bacterial detection with clinical and subclinical endometritis in postpartum dairy cows. J Vet Med Sci 80: 302-10.
[3] Ghanem ME, Tezuka E, Sasaki K, Takahashi M, Yamagishi N, Izaike Y, Osawa T. 2016. Correlation of blood metabolite concentrations and body condition scores with persistent postpartum uterine bacterial infection in dairy cows. J Reprod Dev 62: 457-63.
[4] Ghanem ME, Tezuka, E, Devkota B, Izaike Y, Osawa T. 2014. Persistence of uterine bacterial infection, and its associations with endometritis and ovarian function in postpartum dairy cows. J Reprod Dev 61: 54–60.
[5] Ghanem ME, Higuchi H, Tezuka E, Ito H, Devkota B, Izaike Y, Osawa T. 2013. Mycoplasma infection in the uterus of early postpartum dairy cows and its relation to dystocia and endometritis. Theriogenology 79: 180-5.
[6] Díaz JM, Prieto A, López G, Díaz P, López C, Quintela LÁ, Morrondo P, Fernández G. 2019. Association of Ureaplasma diversum with reproductive disease in cattle. N Z Vet J 67: 249-56.
[7] Gärtner MA, Bondzio A, Braun N, Jung M, Einspanier R, Gabler C. 2015. Detection and characterisation of Lactobacillus spp. in the bovine uterus and their influence on bovine endometrial epithelial cells in vitro. PLoS One. 10: e0119793.

Session II: Endometritis in cows.

Current concepts of endometritis in cattle.

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It is generally accepted that bovine endometritis has a negative effect on fertility and reproductive performance in cattle and causes high economic losses. However, there are also studies that could not confirm e.g. decreased conception rates and prolonged days open in affected cows or on a herd level. Beside questions of definitions and diagnostic techniques, this putative contradiction can be solved when endometritis is not regarded as an inevitable result of a host-pathogen interaction but as a factorial disease.

Whereas in former times identifying pathogens for uterine diseases seemed to the key for understanding the infection, inflammation and, as a consequence, for choosing an adequate treatment, recent research has shown that identifying the pathogen itself, e.g. Escherichia coli or Trueperella pyogenes, is only one aspect of the endometritis story. Understanding the mechanism of infection and inflammatory response made tremendous progress with a detailed description of, e.g. bacteria-associated toxins and virulence factors, which are not necessarily expressed by all strains of the pathogens. Beside known uterine pathogens, such as E. coli and T. pyogenes, the role of opportunistic or minor pathogens, such as S. uberis or B. pumilus is not fully understood, yet. Furthermore, recent research has shown that the intrauterine microbiota is very complex and changes with time and with inflammatory status of the uterus (and vice versa). It requires further research to understand the interaction between the different bacterial species and under which conditions a “physiological” microbiome turns into a “pathological”.

On the host site, recent studies from several working groups have increased our knowledge about the cows’ innate uterine defense mechanism, immunity, and mechanism of inflammation. This inflammatory response and the capability to control an infection are, among others, depending on the metabolic status of the animal. Thus, endometritis can be regraded to some extent as a management related disease. Finally, there are some models to explain the link between uterine diseases in the postpartum period and a reduced fertility later in lactation.

In conclusion, our understanding of (endo)metritis in dairy cattle as a complex disease with several pathogen- and host-specific aspects has increased a lot in the recent past, but further research is required to answer some of the open questions.

Metritis and endometritis in grazing dairy cows: risk factors, diagnosis, treatment and reproductive performance

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Puerperal metritis (PM) and clinical endometritis (CE) are two diseases that affect dairy cows early in lactation. The overall occurrence of metritis in grazing dairy cows is around 39.3%. However, most of the cases are PM (29.7%), and only a few of them are CM (9.6%). Cows with abnormal parturition (dystocia, retained fetal membranes) have 2.58 (OR; CI, 1.19-5.56, P<0.01) more chances of having PM compare to cows with normal parturition (65.1 vs. 34.6%). If cows are in negative energy balance and have clinical or subclinical ketosis, they are at higher risk of having PM; and multiparous cows tended to have a lower chance of having PM compared to primiparous cows (0.65, P<0.09). β-hydroxy-butyrate concertation's during the first week of lactation increase much earlier in PM cows than in normal cows (>450 µM, P<0.05). Diagnosis of PM is based upon the type of vaginal discharge examination (0-3; fetid watery red-brown [PM3]), signs of systemic illness and fever (≥39.5°C). Cows with PM3 produce 3.6 kg/d of milk during the first 120 days in milk (DIM) and have 0.20 chances of becoming pregnant at 100 DIM than normal cows (13,3 vs. 37.5%; P<0.01). Furthermore, cows with PM3 were open 50 d more than normal cows (164 vs. 114, P<0.01). The total cure rate (Score 3 to Score0) and the partial cure rate (Score 3 to Score 2&1) at 21 DIM of cows with PM3 treated with sodic ceftiofur hydrochloride (CEFT; 2.2 mg/kg/d, 3d) was like the untreated control cows (14.3 vs. 15.9%, P>0.46; 53.6 vs. 54.5, P>0.45). However, cows treated with CEFT had 8.3 fewer chances of being culled from the herd compared to untreated control cow (1.8 vs 13.6, P<0.05). The total cure rate was 15, 31, and 77 % at 21, 31, and 41 DIM respectively, or about 2% daily (1.96, 1.15-3.32, P<0.01). The cure rate was affected by body condition score (BCS), cows with high BCS had more chances of having a total cure compared to cows with low BCS. Cows that are not systemically ill but have purulent vaginal discharge within 21 DIM have clinical metritis (CM). The occurrence of CM is <10% and cows with CM3 have twice the cure rate of cows with PM3. Cows with CM3 have 18% higher pregnancy rate (31,0 vs. 13.3%; P<0.05) and are open 43 day shorter than PM3 cows (121 vs. 164 DIM, P<0.05).

The occurrence of CE in grazing dairy cows is of 20%, being higher in primiparous than in multiparous cows (37.0 vs. 24.3, P<0.05) and in cows with abnormal parturition compared to normal parturition (42.3 vs. 25.75%, P<0.05). Cows that had PM during the first 7 DIM had 2.2 more chances of having CE compared to normal cows (1.07-4.59, P<0.03; 22.2 vs. 11.4%, P<0.05; 2). Diagnosis of CE is based upon type of vaginal discharge examination (0-3; 50% mucus/50% pus [CE2]; >50% pus, fetid [CE3]). Early diagnosis of CE (CE1-CE3) yields false-positive cows that then spontaneously resolved to bacterial contamination and became cleared (CE0). In fact, only 42% of cows were diagnosed CE0 between 14-20 DIM, but >80% of cows were diagnosed CE0 after 35 DIM (P<0.05). Cows with CE had 10 less chances of being pregnant at 100 DIM compared to healthy cows (0.10, 0.02-0.43, P<0.01; 4% vs. 44%, P<0.01) and had 3 more chances of being open at 200 DIM compared to healthy cows (2.87, 1.27-6.45, P<0.02; 17.0% vs. 35.6%, P<0.01). In fact, CE cows had 70 days open more than health

cows (184 vs. 115, P<0.01). Prostaglandins (PGF) have proven to be not successful for the treatment of CE1 and CE2 cases from 21 to 50 DIM. Untreated control cows had a similar recovery rate, and pregnancy rate to PGF treated cows.

The occurrence of subclinical endometritis SCE in grazing dairy cows is less than 20%. Using a cut-off point of 5% neutrophils at cytobrush examination between 21 and 50 DIM. Cows with SCE had an 18.2% lower 1st service pregnancy rate (32.1 vs. 50.3%, P<0.05) 27.8% lower pregnancy rate at 100 DIM (34.5 vs. 62.3%, P<0.05), 27 more days open (130 vs. 103 d, P<0.05) compared to healthy cows. Cephapirin benzathine (CEPH) has proven to be not successful for the treatment of SCE cases from 21 to 50 DIM. Untreated control cows had a similar recovery rate, and pregnancy rate to CEPH treated cows.

In summary, it seems that dairy cows that calve with metabolic stress have a flowed immune response to bacterial contamination during parturition. Some cows may have a lesser immune response and have PM, CM, or CE; whereas others have a higher immune response and have SCE. Whereas in the first group, the spontaneous cure rate may be high, and response to treatment seem high, very few studies have negatives control to access the true effectiveness of antibiotic treatments. It seems that management practices that minimize the metabolic stress at parturition may improve spontaneous recovery of PM, CM, and CE cases. Treatment with CEPT is usually recommended only in cows with fetid watery red-brown (PM3), signs of systemic illness and fever. Similarly, treatment with CEPH is usually recommended only in cows with vaginal discharge examination >50% pus and fetid odor (CE3). Conversely, the treatment of cows' SCE with CEPH is not recommended because of the lack of consistent results. Change in management practices could be a key factor to reduce the occurrence of SCE.

Influence of intrauterine administration of Lactobacillus buchneri DSM 32407 on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis

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For the therapy of bovine uterine inflammatory diseases, mainly antibiotics and the hormone PGF2α are applied. However, there are disadvantages to both strategies, such as possible residues in animal products and the potential development of bacterial resistances or a questionable efficiency, respectively. This explains the ongoing search for alternatives, which includes research about Lactobacillus (L.) spp. as potential probiotics for the bovine reproductive tract. Therefore, the aim of the present study was to analyse the influence of an intrauterine administration of the Lactobacillus buchneri DSM 32407 on reproductive performance, uterine health status, and endometrial mRNA expression of pro-inflammatory factors of cows with signs of subclinical endometritis (SCE). In addition, the impact of L. buchneri DSM 32407 on the histopathology of endometrial biopsy samples of clinically healthy cows taken at various days postpartum (pp) was evaluated. L. buchneri DSM 32407 (n=56; [LAC]) or a placebo (n=60; [PLA]) was administered on day 24-30 pp to cows with signs of SCE and healthy cows, because detection of SCE could be only done after administration. Endometrial cytobrush samples of cows with SCE were taken before the administration and at three following weeks (n=16 cows each for LAC/SCE and PLA/SCE). A higher proportion of cows of the LAC and LAC/SCE group was pregnant after the first service compared with the PLA and PLA/SCE group, respectively. Clinically healthy cows treated with L. buchneri DSM 32407 had significantly shorter median days to conception compared with cows treated with a placebo (103 vs. 133 median days to conception; P = 0.035). This difference was even more significant when only cows with SCE were observed (74 vs. 164 median days to conception; P = 0.001). Three weeks after the administration, the endometrial mRNA expression of CXCL1/2, CXCL3, CXCR2, IL1B, IL8 and PTPRC was lower in the LAC/SCE group compared with the PLA/SCE group. The histopathological evaluation showed an infiltration of immune cells into the endometrium one week after the intrauterine administration of L. buchneri DSM 32407. This study showed a significantly higher number of endometrial samples positive for Histophilus somni on days 31-37 pp in the SCE group that received a placebo in comparison to the whole group that received L. buchneri DSM 32407. The present study showed that an intrauterine administration of L. buchneri DSM 32407 first had an immunostimulatory effect with a subsequent downregulation of the endometrial immune system on days 45-51 pp. These findings suggest that the presence of L. buchneri DSM 32407 contributes to a uterine environment that results in a better reproductive performance. This study was supported by DFG (GA 1077/5-1).

Inhibin, folliculostatin and periostin as new markers for uterine health and future pregnancy

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The study aimed to analyze the mRNA expression of Inhibin Subunit Beta A (INHBA), Inhibin Subunit Alpha (INHA), Follistatin (FST), Periostin (POSTN) in uterine biopsy samples by qPCR. Cows were examined in fourth and sixth week postpartum. Vaginoscopy and cytology in 4th week were performed to assign cows to three endometritis groups: clinical endometritis (C) - > 50% of pus in the vagina (n=9, sampled only in 4th week), subclinical endometritis (S) - no signs of clinical endometritis and more than 5% of neutrophils in cytobrush cytology (n=27), healthy cows (H) - no signs of clinical endometritis and less than 5% of neutrophils in cytobrush cytology (n=11). Groups C, S, and H were compared at week 4 using the Kruskal-Wallis test while at week 6 after delivery the groups S and H were compared using Mann–Whitney U test. Animals from groups S and H were examined for pregnancy up to 200 days after delivery and based on this test assigned to the groups: pregnant animals up to 100 days as a pregnant group (P), pregnant between 100 and 200 days pp as not-pregnant (NP) and other animals as culled (not analyzed). Collected data were analyzed using Python 3.7 programming language (Python Software Foundation 2018) and R 3.6.0 (R Core Team, 2017). INHBA expression at week 4 was higher ($p = 0.015$) in group S than in H while expression in group C did not differ from H and S. FST expression at week 4 differed between all endometritis groups ($p = 0.003$). The highest was in group H and the lowest in group C. Comparing S and H groups POSTN expression differed statistically ($p=0.019$) at 4th week. INHBA, INHA, FST, POSTN expressions at week 6 did not differ between endometritis groups. Comparing pregnancy groups, the P group had higher INHA expression ($p=0.003$) and lower INHBA expression ($p=0.018$) than the NP group. In conclusions, INHBA, FST, POSTN expression can be used as indicator genes for detection of subclinical endometritis at 4th week postpartum. In turn expression of INHA and INHBA at 6th week after delivery can be used as indicators of completed endometrial recovery allowing for next pregnancy.

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The effect of clinical endometritis on the ovarian activity in high yielding dairy cows during post-partum period

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Clinical endometritis in high yielding dairy cows is regarded as a major factor adversely affecting on post-partum reproductive performance and fertility. The aim of this study was to quantify the impact of clinical endometritis (as defined by the presence of any abnormal vaginal discharge after 21 days post-partum)on post-partum ovarian cyclicity in high yielding dairy cows. Milk samples were collected three times weekly from 170 dairy cows across three different commercial herds. Progesterone concentrations in the milk were determined by commercial ELISA kit (Ridgeway Science). Cows were examined for discharge from the vulva at twice a week after calving and subsequently grouped into healthy cows (n=98, no signs of vulval discharge) and cows with clinical endometritis (n=72, presence of vulval discharge >3 weeks after calving). The relation between the occurrence of clinical endometritis and the incidence risk of atypical ovarian cycle profiles during the calving to conception period were determined by binominal logistic regression with all statistical analyses were performed using GenStat (17thEdition, Hemel Hempstead, UK).The incidence odds of atypical ovarian cycles($P < 0.05$) increased in cows with clinical endometritis especially prolonged luteal phase (OR=4.60 [95%CI; 1.53-13.84]; $P < 0.05$). In addition, cows with clinical endometritis had prolonged time (3 days) to onset of luteal activity after parturition ($P < 0.05$) compared to healthy cows. This study showed that a relatively low incidence (30%) for reproductive cycle problems in healthy cows compared to cows with clinical endometritis (75%) during the calving to conception period. However, the incidence odds of atypical ovarian profiles, in particular prolonged luteal phase, were high(38.9%; $P < 0.05$)in cows with clinical endometritis, which would have significantly impaired reproductive function.This study showed that an abnormal vaginal discharge score (VDS 2) increased the odds of atypical ovarian profiles (OR=2.44 [95% CI: 1.29–4.61]; $P<0.05$), which numerically equated to 81% of cows having atypical ovarian profiles. In addition, there was a significantly increased odds of prolonged luteal activity in cows with VDS2 (OR = 4.66 [95% CI: 1.48–14.6]; $P<0.05$).In conclusion, this study established that clinical endometritis had a negative influence on the ovarian cyclicity in Holstein dairy cows during post-partum period.

Cytological endometritis in primiparous and multiparous dairy cows - an analytic approach for diagnosis based on endometrial polymorphonuclear cells cytology threshold

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Cytological endometritis (CEM) is usually diagnosed based on proportion of polymorphonuclear cells (PMN) in endometrial cytology. Incidence of CEM varies greatly among studies, ranging from 12 to 45%, presumably reflecting diversity among herds, but potentially due to diagnostic criteria, including parity, threshold of PMN proportion, as well as the timing of cytology sample collection. Our objective was to examine whether primiparous and multiparous cows should be diagnosed for CEM by different criteria and timing, using a combination of selected reproductive performance outcomes (interval to first service and to pregnancy, pregnancy rate at 180 days-in-milk, first service conception rate, and number of AIs to pregnancy). Two endometrial cytobrush cytology samples were collected from Holstein dairy cows (n=213; 133 multiparous, 80 primiparous), at 30-40 and at 60-70 days-in-milk (DIM); slides prepared (Diff-Quick staining) and PMN proportions evaluated blindly. PMN proportion thresholds were set at ≥1, ≥2, and up to ≥10%, ≥15%, and ≥20%, for diagnosis of CEM. Data were analyzed by Cox's proportional hazard model, Logistic regression, or Wilcoxon signed-rank tests, controlling for the effect of farm. Cytological endometritis in primiparous cows were best identified at 30- 40DIM, whereas in multiparous cows, CEM was best diagnosed at 60-70DIM. In primiparous cows, a threshold of ≥7% at 30-40DIM was associated with significant reductions in the majority of reproductive performance parameters analyzed (time to first service and to pregnancy; pregnancy rate at 180DIM; first service conception rate; number of AIs to pregnancy). However, at 60-70DIM, none of the PMN thresholds were associated with reduced reproductive performance in primiparous cows with CEM. In contrast, in multiparous cows, a threshold of ≥3% at 60-70DIM was associated with significant reductions in the majority of the reproductive performance parameters analyzed. However, at 30-40DIM, none of the PMN thresholds were associated with reduced reproductive performance in multiparous cows with CEM. Differences between primiparous and multiparous cows could be related to metabolism, immune function, and uterine involution. In conclusion, diagnosis of CEM in primiparous and multiparous cows should be made using different PMN threshold criteria and at different intervals after calving.

Uterine bacterial metagenomics and bacterial load in dairy cows with metritis and cytological endometritis

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Metritis and cytological endometritis (CEM) are common in dairy herds worldwide, and are thought to have a bacterial etiology. Our objective was to compare uterine bacterial community composition and bacterial load between healthy cows and cows diagnosed with metritis and CEM. Dairy cows (n=129) were sampled three times by endometrial cytobrush, at 5-12, 30-40 and 60-70 days-in-milk (DIM). Analyses included bacterial load qPCR quantification and metagenomic multivariate bacterial community analysis based on 16s-rRNA Sequencing. Cows were divided to groups as follows: healthy/metritis at 5-12DIM by clinical examination; and healthy/CEM at 30-40 and 60-70DIM, based on endometrial cytology with a Polymorphonuclear threshold of >5% at 30-40DIM and >3% at 60-70DIM. Bacterial load did not differ in each time point based on the diagnosis of uterine disease (Wicoxon-Rank-Sum Test). Metagenomic analysis revealed that at 5-12DIM bacterial communities in metritis cows formed a distinct group, characterized by a typical community dominated the genera Bacteroides, Porphyromonas and Fusobacterium. These genera belong to the phyla Bacteroidetes and Fusobacteria that showed mean relative abundance of 9.1±1.6% and 19.0±2.8% (respectively) in healthy cows, vs. 28.1±2.9% and 28.3±2.4 in metritis cows ($P<0.05$). In contrast, healthy cows at 5-12DIM had more diverse community compositions, with a much higher mean relative abundance of the Proteobacteria phylum (17.6±2.9% vs. 3.6±0.8%, $P<0.05$). Interestingly, bacterial communities did not differ between healthy cows and cows diagnosed with CEM at 30-40DIM and 60-70DIM.

In conclusion, metritis seems to be a disease of polymicrobial etiology, dependent mainly on bacterial community composition and not on bacterial load. In contrast, CEM at 30-40DIM and 60-70DIM cannot be associated with bacterial community composition or bacterial load. Future research of CEM should focus on cow factors driving uterine inflammation by methods such as gene expression analysis, and on specific candidates suspected as bacterial pathogens rather than on community analysis.

Exploring the role of *Streptococcus uberis* in bovine endometritis.

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Streptococcus uberis is an important, well studied pathogen in bovine mastitis but there is a lack of information regarding its effect on bovine endometrium and its involvement in endometritis, although it has been found to be one of the predominant bacteria in cows with endometritis [1]. Thus, we aim to elucidate the role of S. uberis in the development of endometritis by using bovine endometrial epithelial cells as an in vitro infection model.

53 isolates, identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), as S. uberis were screened for virulence genes. Eight strains harboring different sets of virulence genes were subjected to a cell viability assay. Therefore, the strains were co-cultured with endometrial epithelial cells by using four multiplicity of infection (MOI) schemes, and the cellular viability was assessed after 24, 48 and 72 hours. Out of the eight strains, three strains with different virulence factor profiles were used for evaluating the mRNA expression of selected cellular pro-inflammatory factors.

Genes encoding for virulence factors, such as plasminogen activator (pauA) and surface lipoprotein (slp) were found in all the strains, whereas hyaluron capsule gene hasC and S. uberis adhesion molecule gene sua were found in 83 % and 93 % of the total number of strains. In cells infected with S. uberis (n =3), an immediate effect on cellular viability was observed. After 24 hours of incubation at MOI 1, cellular viability was significantly decreased ($p<0.05$), with some strains being able to destroy up to 60 % of the cells. 48 hours after infection, the effect was even stronger with cellular viability reaching less than 25 % ($p<0.001$). No cellular viability was detected 72 hours post-infection. When the cellular inflammatory response was measured, it was found that infection with S. uberis provokes an upregulation of Toll-like receptor genes (TLR4), Chemokine ligands genes (CXCL2) and also Prostaglandin-Endoperoxide Synthase 2 (PTGS-2) and Interleukine (IL-8) genes, with expressions differing significantly from the uninfected controls ($p<0.05$).

In summary, our study provides the first information about the effect of S. uberis on uterine cells. Its effect on cellular viability and on upregulation of pro-inflammatory factor genes leads to the assumption that S. uberis could play a role as endometritis pathogen.

Investigation of the reproductive tract health in cows with different degrees of pneumovagina

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The aim of this study was to investigate the cytologic, microbiological and histopathological findings of the reproductive tract of cows with different degrees of pneumovagina (PV).A total of 16 cows, 4 of which were normal and the others were affected with PV, were used as material for the study. PV grading of the cows was made by considering the vulval angle (VA), vulva-archus ischiadicus length (VAI), depth of the anus (DA) and perineum length (PU). Groups were defined as Group I (PV negative, control, VA = 0-10 °, VAI = 1-2 cm, DA <4 cm, PU> 4 cm) (n = 4); Group II (PV suspected, VA = 10-30 °, VAI = 2-3cm, DA> 4-6 cm, PU <4 cm,) (n = 4); Group III (PV positive, VA = 30-55 °, VAI = 3-4 cm, DA = 6-8 cm, PU <4 cm,) (n = 4) and Group IV (Severe PV, VA> 55 °, VAI> 5 cm, ANG> 8 cm, PU <4 cm) (n = 4). Sterile swab samples were taken from vagina, cervix and uterus for microbiological examination. Samples were subjected to bacteriological culture and identification. For histopathological examination, samples were taken from vagina, cervix and uterus and the preparations were stained with hematoxylin-eosin. In cytological examination, smears from vagina, cervix and uterus were stained with Diff-Quick. Histopathological and cytological examinations were performed under light microscope. Microbiologically, E. coli (17.65%), Enterococcus spp. (17.65%), Citrobacter sedlakii (11.76%) and Staphylococcus spp. (11.76%), while E. coli (40.9%) and Serratia spp. (27.27%). The most common bacteria in Group III and Group IV were E. coli (50%). Histopathologically, in Group I, mild degree cervicitis was present in one cow and one cow had vaginitis, cervicitis and endometritis. In Group II, all cows had endometritis and one cow had vaginitis. In Group III, one cow had vaginitis and one cow had endometritis and one cow had endometritis and cervicitis. In Group IV, one cow had vaginitis, one cow had endometritis and one cow had vaginitis, cervicitis and endometritis. Cytologically, one cow in Group I had vaginitis, cervicitis and endometritis, whereas in Group IV, one cow had vaginitis and one cow had endometritis. As a result, it is concluded that the presented method for the grading of PV in cows is reliable and there is a relationship between the severity of PV and the reproductive tract flora and pathology in cows with PV graded according to this method.

Interleukin 8 mRNA was higher expressed in the endometrium at the time of artificial insemination in cows that did not conceive compared with their fertile counterparts

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Impaired reproductive performance of cows represents a substantial problem for dairy cattle farmers because longer calving intervals and resulting increased herd culling rates contribute to enormous financial losses. The reason for subfertility is multi-factorial and still not understood in detail. It is suggested that inflammatory processes may be the cause for failure to conceive. Therefore, the objective of this study was to assess the mRNA expression of selected pro-inflammatory factors obtained from the endometrium by cytobrush technique from cows at time of artificial insemination (AI).

A total of 66 dairy cows without a history of clinical and subclinical endometritis in the postpartum period were included in the study and subjected to an Ovsynch protocol. Cows were bred by AI between 80 and 100 days in milk. Directly after AI, cytobrush samples were taken to determine the proportion of polymorphonuclear cells (PMN) as well as for RNA isolation. Extracted total RNA was subjected to RT-qPCR for selected pro-inflammatory factors interleukin (IL) 1A, IL1B, IL8, prostaglandin-endoperoxide synthase 2 (PTGS2), tracheal antimicrobial peptide (TAP), chemokine CXL ligand (CXCL) 3 and CXCL5 and mucins (MUC4 and MUC16). Pregnancy diagnosis was performed 39 days after AI by ultrasound.

From 66 animals, 35 were pregnant after AI (PREG-POS; 53%) whereas 31 remained non-pregnant (PREG-NEG; 47%). At the time of AI, none of the cows had a PMN content of ≥ 5% in the cytobrush samples. In 17 PREG-POS samples and 13 PREG-NEG samples no PMN were detected at all. IL8 mRNA expression in PREG-NEG cows was significantly higher (two-fold) compared with cows of the PREG-POS group. The MUC16 mRNA expression showed a tendency (P=0.153) towards a higher expression in samples obtained from PREG-NEG cows compared with PREG-POS cows. No differences were found between groups for the other selected factors IL1A, IL1B, CXCL3, CXCL5, TAP and MUC4.

The findings that the cows in this study showed no signs of inflammation at the time of insemination indicates that present inflammatory processes are not the main reason for not conceiving after AI. However, lower mRNA expression of IL8 in PREG-POS cows indicates that the down-regulation of this factor seems to be important for conceiving. On the other hand, a higher IL8 mRNA expression in PREG-NEG cows is maybe a result of a former inflammation.

Plenary lecture III

MicroRNAs: small but potent molecules in animal reproduction

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MicroRNAs (miRNAs) are small, approximately ~22-nt, non-coding, endogenous and mostly evolutionarily conserved RNAs that mediate post-transcriptional gene repression [1]. These molecules control a wide range of biological processes, including metabolism, cell proliferation, apoptosis, and differentiation in almost all cell types across the animal kingdom. Today, a total of 2654 mature miRNAs have been identified in *Homo sapiens* (miRBase: Release 22.1: Oct 2018). In other animals, numbers of mature miRNAs were identified, *e.g.*, 1978 in *Mus musculus*, 1025 in *Bos taurus*, and 457 in *Sus scrofa*. Bound with an Argonaute protein to form a silencing complex (RISC), miRNAs function as sequence-specific guides, directing the silencing complex to transcript, primarily through Watson-Crick pairing between the miRNA seed sequence (nucleotides 2–7) and complementary sites located preferentially within the 3′untranslated regions (3′UTRs) of target RNAs [2,3]. The miRNAs conserved to fish have been grouped into 87 families, each with a unique seed region. On average, each of these families has hundreds of conserved targeting interactions, and together these interactions involve most mammalian mRNAs [4]. Interestingly, a large proportion of miRNAs are localized as clusters in the genome, transcribed together from physically adjacent miRNAs and show similar expression profiles [5].

To date, hundreds or even thousands of molecules have been investigated in mammals during crucial reproductive events, but their function still remains unknown in most of the cases. Recently, miRNAs have been suggested to play an important role in control of oocyte maturation, folliculogenesis, corpus luteum function, implantation, and early embryonic development. For example, knockout of Dicer, the cytoplasmic enzyme that cleaves the pre-miRNA to its mature form, results in early embryonic lethality in several animal models, *e.g.*, mouse [6]. miRNAs were shown to be involved in processes associated with establishment and maintenance of pregnancy, including preparation of the *endometrium* for implantation [7] or control of genes linked to inflammatory responses [8]. Studies performed in our laboratory indicated that both the conceptus and the *endometrium* can be a source of miRNAs involved in the embryo-maternal dialogue during early pregnancy in pigs [9,10]. Our recent results have shown that unique sets of miRNAs can already be observed in circulation of pigs during the first weeks of pregnancy, as a result of initiated embryo-maternal communication [11]. In addition, miRNAs are currently being examined as potent modulators of cell-to-cell communication at the embryo-maternal interface, as well as events supporting conceptus development and maintenance of luteal function during early pregnancy in the pig.

Collectively, it has become clear that miRNAs serve as key regulators of gene expression, and our understanding of their functional relevance in reproductive events is exponentially growing. However, much work remains to be done to understand the precise roles of miRNA-mRNA interactions in mammalian reproduction.

References:

[1] Bartel DP. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function Cell 116:281-297.

[2] Lewis BP, Burge CB, Bartel DP. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15-20.

[3] Bartel DP. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136:215-233.

[4] Friedman RC, Farh KK, Burge CB, Bartel DP. (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19:92-105.

[5] Kabekkodu SP, Shukla V, Varghese VK, D' Souza J, Chakrabarty S, Satyamoorthy K. (2018) Clustered miRNAs and their role in biological functions and diseases. Biol Rev Camb Philos Soc 93:1955-1986.

[6] Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. (2003) Dicer is essential for mouse development. Nat Genet 35:215-217.

[7] Pan Q, Chegini N. (2008) MicroRNA signature and regulatory functions in the endometrium during normal and disease states. Semin Reprod Med 26:479-493.

[8] Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H, Dey SK. (2007) MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proc Natl Acad Sci USA 104:15144-1514.

[9] Krawczynski K, Najmula J, Bauersachs S, Kaczmarek MM. (2015). MicroRNAome of porcine embryos and trophoblasts: expression profile of microRNAs and their potential to regulate genes crucial for establishment of pregnancy. Biol Reprod 92, 21.

[10] Krawczynski K, Bauersachs S, Reliszko ZP, Graf A, Kaczmarek MM. (2015). Expression of microRNAs and isomiRs in the porcine endometrium: implications for genes regulation at the maternal-conceptus interface. BMC Genomics 16, 906.

[11] Reliszko ZP, Gajewski Z, Kaczmarek MM. (2017) Signs of embryo-maternal communication: miRNAs in the maternal serum of pregnant pigs. Reproduction 154, 217-228.

Current approaches and future perspectives for modelling the female reproductive tract *in vitro*

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The female reproductive tract represents a complex immune-endocrine interface, protecting the maternal system from pathogens while allowing conception and development of the conceptus. Modelling the three dimensional (3D) structures and their dynamic hormonal regulation within the female reproductive tract is a challenging endeavor. In vitro models have to balance functionality with reduction of complexity and applicability. This presentation gives an overview on different strategies to model female reproductive tract tissues with a special focus on epithelia. Current challenges in the development of more in vivo-like models are discussed and some perspectives are presented on how these problems could be overcome through integration of rising technologies as 3D bioprinting, introduction of extracellular matrix components, and microfluidic devices.

Remodeling of porcine endometrium during peri-implantation period: molecular changes

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Implantation and establishment of pregnancy in mammals, including pigs, require effective interactions between a competent blastocyst and the receptive uterus. Towards this goal, uterine endometrium during the peri-implantation period undergoes morphological and molecular transformations to support the embryo development and implantation. These changes, called “plasma membrane transformations” are induced by the ovarian steroids and later modified by the conceptus-secreted factors to regulate the 1) para-cellular permeability across the epithelium and 2) increase the adhesion between trophoblast and endometrial epithelial cells. The plasma membrane transformations during early pregnancy period are associated with a partial loss in polarity of epithelial cells that is regulated by tight junction, adherens junction, polarity protein complexes and actin binding proteins. Using techniques such as qPCR, western blotting and immunofluorescence, we present the evidence that, in pigs, depending upon the stage of estrous cycle and reproductive status of the animals (between Days 10-16 of cycle and oregnancy), the endometrial epithelial cells undergo change in the expression or distribution of i) tight junction (TJ) proteins such as occludin (OCLN), claudin1 (CLDN1) and zona occludens-1 (ZO-1), ii) adherens junction (AJ) proteins cadherin (CDH1) and β-catenin (CTNNB), iii) partition defective (PAR) complex – PAR3/PAR6/aPKC/CDC42 proteins and iv) actin binding proteins such as cofilin (CFL), vinculin (VCL) and gelsolin (GSN). The TJ proteins OCLN and CLDN1 were concentrated on the lateral plasma membrane in pregnant endometrium between Days 13 and 16 as compared to corresponding days of cycle. On the contrary, ZO-1 abundance was decreased in endometrium of pregnant animals on Day 16 with it translocation to the basal side that results in change in epithelial polarity. A strong reactivity of AJ proteins, CDH1 and CTNNB was observed in the lateral plasma membrane of pregnant animals during implantation period. As observed by co-localization and co-immunoprecipitation results, CDH1 and CTNNB associated with each other in lateral plasma membrane only on Day 16 of cycle and pregnancy. We speculate that there is a possibility that lateral CDH1 distribution and its interaction with CTNNB during implantation period might be a possible way adopted by porcine endometrium to limit attachment of conceptus trophoblast to the apical epithelium. The PAR complex proteins were expressed in the porcine endometrium with little to no change in their expression during days of estrous cycle or pregnancy. However, a significant decrease in PAR6 expression was observed on Day 16 of pregnancy as compared to corresponding day of estrous cycle. On Day 16 of pregnancy, whereas, a redistribution of aPKC to lateral plasma membrane from its apical position on Days 10 and 13 was observed, CDC42 protein was more concentrated towards the apical pole of luminal epithelium on Days 13 and 16 of pregnancy. The tight junction proteins are known to interact with the actin cytoskeleton. Along with the changes in the distribution of junction and polarity proteins, we observed that among the actin binding proteins investigated; VCL abundance was significantly higher in Day 16 of pregnant endometrium as compared to other days of cycle of pregnancy. A further investigation of VCL function in cell adhesion revealed that it facilitates conceptus attachment to endometrial epithelial cells through focal adhesion kinase.

In summary, porcine endometrial epithelium undergoes transformations during peri-implantation period that alters its paracellular permeability to allow the transport of molecules from uterine stroma to the conceptuses and change the adhesive property of epithelial cells to facilitate trophoblast adhesion.

Understanding the uterine environment in subfertile cattle

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Reproductive failure in an individual is caused by various external and internal factors. Moreover, the coexistence of multiple elements makes it difficult to delineate the effects of each individual factor on reproductive function. Previous studies have shown that fertilization rates following artificial insemination (AI) of cattle exceed 90% [1], suggesting that embryonic death is responsible for the majority of reproductive failures. Several findings indicate that most embryonic deaths occur before and just after the pregnancy recognition period. Previously, we showed that embryos transferred into the uterus died immediately, prior to reaching the pregnancy recognition period [2], suggesting the uterine environment during embryo transfer strongly influences embryo viability. The gene expression pattern in the endometrium of cattle has been associated with an optimal uterine environment. Forde et al. revealed that circulating progesterone concentrations in the first few days after estrus may influence the endometrial gene expressions in cyclic heifers [3] and, ultimately, the ability of the uterus to support conceptus development. Therefore, we hypothesized that a functional disorder of the endometrium could lead to early embryonic death and infertility.

In our study, global gene expression analysis revealed that endometrial gene expression pattern was different between fertile and subfertile cattle. Interestingly, a larger number of genes were more highly expressed in subfertile cattle than in fertile cattle- genes encoding ribosomal proteins and mitochondrial oxidative phosphorylation in particular were remarkably highly expressed. These results indicated that cellular senescence was induced in the endometrium of subfertile cattle. Cellular senescence is characterized by irreversible cell cycle arrest and one of the cellular pathways contributing to aging. It has been reported that aging suppresses mitochondrial biogenesis and autophagy [4]. The age-related decline in autophagic activity might cause accumulation of damaged mitochondria [5], then it might lead organs dysfunctions. SIRT1 and Bcl2L13 are the genes which are involved in the regulation of mitochondrial biogenesis and mitophagy, respectively. Quantitative RT-PCR analysis demonstrated that SIRT1 and Bcl2L13 mRNA expressions were significantly lower in subfertile cattle than in fertile cattle. In addition, the mitochondrial DNA copy number was significantly higher, whereas the ATP content did not differ between fertile and subfertile cattle. Based on these results, the malfunctions of mitochondrial biogenesis and mitophagy would accumulate damaged mitochondria in the endometrium of subfertile cattle.

Resveratrol, a small polyphenolic antioxidant compound, has been shown to accelerate mitophagy [6]. In our study, Bcl2L13 mRNA expression slightly increased and the mitochondrial DNA copy number slightly decreased in subfertile cattle following resveratrol administration—each reaching a point between the values observed for fertile and subfertile cattle—though the effects were not significant. From these results, we speculate that resveratrol increased mitophagy in subfertile cattle, but not to the level seen in fertile cattle. Additionally, two out of five subfertile animals treated with resveratrol—but none of the five subfertile cattle who received the vehicle—conceived after embryo transfer. Considering resveratrol's effect, it is possible that mitophagy malfunction in the endometrium is a cause of both an inadequate uterine environment for embryonic survival and subfertility in cattle.

Collectively, some mitochondrial dysfunctions (i.e. mitophagy disorder) associated with cellular senescence would be occurred in the endometrium of subfertile cattle and they might contribute to infertility.

References:

[1] Diskin, M. G. & Sreenan, J. M. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod. Fertil. 59, 463–468 (1980).

[2] Matsuyama, S., Kojima, T., Kato, S. & Kimura, K. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. Reprod. Biol. Endocrinol.: RB&E 10, 21, doi:10.1186/1477-7827-10-21 (2012).

[3] Forde, N. et al. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. Biol. Reprod. 84, 266–278 (2011).

[4] Peterson, CM. et al. Skeletal muscle mitochondria and aging: a review. J. Aging Res. 2012: 194821 (2012).

[5] Kume, S. et al. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. J. Clin. Invest. 120(4), 1043-1055 (2010).

[6] Wu, Y. et al. Resveratrol-activated AMPK/SIRT1/autophagy in cellular models of Parkinson's disease. Neurosignals 19, 163–174, doi:10.1159/000328516 (2011).

The Influence of Heat Stress on the Endocrine Function of Bovine Endometrium

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The impact of global warming on the livestock industry is a critical issue, particularly that heat stress decreases fertility in cows. This decrease is caused by elevated body temperature which directly or indirectly affects hormone secretions from the hypothalamus and pituitary, ovarian function, estrous behavior, folliculogenesis, oocyte competence and embryonic development. Moreover, it has been indicated that heat stress also alters uterine function and, consequently, the environment for embryo development. The aims of the present study are to investigate the influence of heat stress on the endocrine function of bovine endometrial cells.

In the first experiment, the effects of heat stress on the production of prostaglandin (PG)E2 and PGF2α in cultured bovine endometrial epithelial and stromal cells were examined separately. Epithelial and stromal cells from endometrium derived from local slaughterhouses were enzymatically collected, and then cultured at 38.5℃(control) or 40.5℃ (HS). After treatment, PGE2 and PGF2α levels were measured by enzyme immunoassay (EIA) and mRNA expressions of enzymes involved in PG synthesis were examined via quantitative reverse transcription polymerase chain reaction. HS did not influence the production of PGE2 or PGF2α in the epithelial cells, however, HS significantly enhanced the production of both PGE2 and PGF2α in the stromal cells ($p < 0.05$). In addition, HS significantly increased *phospholipase A2 (PLA2), cyclooxygenase 2 (COX2), prostaglandin F synthase (PGFS), prostaglandin E synthase (PGES), and carbonyl reductase 1 (CBR1)* mRNA expression in the stromal cells ($p < 0.05$).

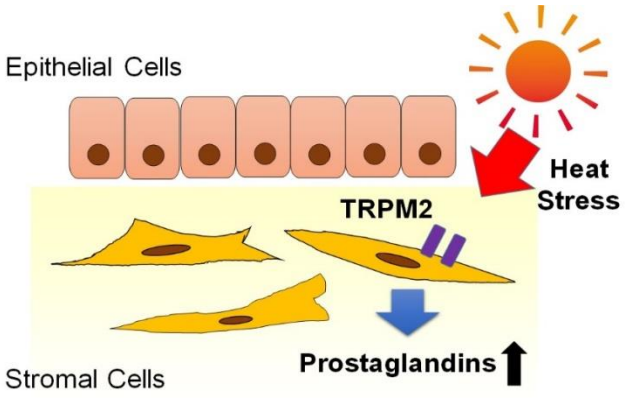

Figure 1. Mechanism of sensing and response to heat stress in bovine endometrium

Each cell in the body has several signaling pathways and cascades to sense and react to various environmental stimuli such as osmolality, temperature, and mechanical stimuli. Transient receptor potential (TRP) channels are ion channels located on plasma membranes, some of which are involved in temperature sensing. The second experiment was conducted to investigate the role of TRP channel-mediated temperature sensing on the enhancement of PG secretion from bovine endometrial cells under HS conditions. Firstly, we investigated the endometrial location of temperature-sensing TRP channels (TRPV3, V4, and M2), which can sense bovine body temperature, by immunohistochemistry. Slaughterhouse derived uteri classified at the late luteal stage (day 14–17) were used for the experiment. Uterine horns ipsilateral to corpus luteum were fixed and sliced, and each section was immunostained with antibodies against TRPV3, TRPV4, or TRPM2.

While TRPV3 was not clearly observed in the bovine endometrium, TRPV4 was slightly detected in luminal epithelial cells. TRPM2 was observed in each region of the uterine tissues, especially in luminal and glandular epithelial cells, which presented stronger signals than stromal cells, myometrium, and endothelial cells.

Next we investigated the effect of TRP channels inhibition on PGs secretion by HS endometrial stromal cells. As mentioned above, HS did not influence PGs secretion by epithelial cells; therefore, only stromal cells were used in the present experiment. Bovine endometrial stromal cells were collected and cultured in the presence of each of the antagonists for TRPV3, V4, and M2 ($n = 5$, 5, and 9, respectively) for 34 hours, at either 38.5℃ (control) or 40.5℃ (HS). After incubation, the PGs concentrations in the culture media were measured by EIA. When stromal cells were cultured in the presence of TRPV3 or TRPV4 antagonists, HS significantly increased the secretion of PGs ($p < 0.05$) compared to the controls, suggesting that inhibition of TRPV3 and TRPV4 does not reduce the effect of HS on PG secretion by bovine stromal cells. On the contrary, when the TRPM2 antagonist was added to the culture medium, PGs secretion significantly decreased ($P < 0.05$) under HS condition. No significant difference was observed in the PGs secretion of HS and control at higher concentrations of TRPM2 antagonist. Moreover, this effect was not detected in controls.

Taken together, the present study suggests that bovine endometrial stromal cells can sense heat stress via TRP channels and then increase the production of prostaglandins (Figure 1).

Heat stress alters innate immune responses in bovine endometrial cells

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After parturition, cows frequently develop uterine bacterial infections, resulting in the onset of endometritis. To eliminate the bacteria, bovine endometrial cells release cytokines, such as interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP1), which recruit macrophages (MΦ) to the epithelial layer. Although these immune responses support recovery from endometritis, the symptoms last longer in the summer than in any other season. Based on these findings, we hypothesized that the immune responses in the bovine uterus would be suppressed by heat stress (HS) and performed the following three experiments. First, cultures of endometrial epithelial and stromal cells with/without lipopolysaccharides (LPS) were analyzed at 38.5°C (control) and 40.5°C (HS), and the mRNA expression of MCP1 and IL-6 in these cells was measured using quantitative RT-PCR. In the endometrial epithelial and stromal cells cultured at the control condition, LPS significantly ($P<0.05$) induced the mRNA expression of MCP1 and IL-6 (n=7). In the endometrial epithelial cells cultured at the HS condition, this LPS-induced mRNA expression was significantly ($P<0.05$) suppressed (n=7). In contrast, HS significantly ($P<0.05$) enhanced the LPS-stimulated mRNA expression of MCP1 and IL-6 in the endometrial stromal cells (n=7). Next, MCP1 and IL-6 production was measured using EIA. In the endometrial epithelial and stromal cells cultured at the control condition, LPS significantly ($P<0.05$) stimulated IL-6 production (n=6). At the HS condition, this LPS-stimulated IL-6 production was significantly ($P<0.05$) suppressed in the endometrial epithelial cells and significantly ($P<0.05$) enhanced in the endometrial stromal cells (n=6). Nevertheless the presence of LPS, the production of MCP1 was not detected in the endometrial epithelial cells (n=3). On the other hand, in the endometrial stromal cells cultured at the HS condition, LPS-stimulated MCP1 production was significantly ($P<0.05$) enhanced (n=6). Finally, to determine whether HS directly affected the MΦ chemotaxis, migration assay was conducted at the HS condition and the mRNA expression of CCR2 (MCP1 receptor) and IL-6R was quantified using qRT-PCR. In MΦ, HS did not affect the mRNA expression of CCR2 and IL-6R (n=6). In addition, MΦ chemotaxis was not influenced by HS (n=4).

In conclusion, although HS did not affect MΦ chemotaxis, it affected the cytokine production of the endometrial epithelial and stromal cells. In particular, HS enhanced MCP1 and IL-6 production in the endometrial stromal cells and suppressed IL-6 production in the endometrial epithelial cells. During summer, MΦ might not be recruited to the endometrial epithelial layer and accumulate in the endometrial stromal layer, resulting in prolonged endometritis symptoms in cows.

Effects of bisphenol A and its analogue on the uterine contractility in immature pigs

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Bisphenol-A (BPA) is a widespread industrial compound, which has been shown to alter the normal function of the endocrine system, and so is classified as an endocrine disruptor chemical. The estrogenic activity of BPA may lead to reproductive disorders in both females and males. Currently BPA is gradually substituted by its analogues, whose effects have been poorly researched. Therefore, the aim of the study was to investigate the effect of bisphenol A and its selected analogue, bisphenol S (BPS), on the contractile activity of the uterine smooth muscles in immature and cyclic pigs.

Segments of the uterine horns (about 1.5 cm in length), collected from the middle part of the horns were transferred to ice, moved to the laboratory and immediately processed for examination of contractility. Strips of the myometrium (MYO) were suspended in 5 ml of water bath (Schuler Organ bath type 809; Hugo Sachs Electronic, Germany), containing Krebs-Ringer solution which consists of (mmol/L): NaCl, 120.3; KCl, 5.9; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 15.5; glucose, 11.5; pH 7.4, continuously saturated with carbogen (95% O2 , 5% CO2). After 60-90 min. of preincubation, the strips were exposed to BPA and BPS at concentrations of 10-10 - 10 -1 M. The smooth muscle contractility was determined with a Hugo Sachs Elektronik equipment for measuring isometric contractions.

BPA caused significant a decrease in the amplitude, frequency and tension in concentrations 10-6 - 10-1 M ($P < 0.001$) and 10-5 - 10-1 M ($P < 0.001$), 10-5 - 10-1 M ($P < 0.01$). BPS caused a decrease in the amplitude in concentrations 10-4 - 10-1 M ($P < 0.001$) and frequency in concentrations 10-4 - 10-1 M ($P < 0.001$) and tension in concentrations 10-4 - 10-1 M ($P < 0.05$).

The obtained results indicate that BPA exerts stronger effect on the contractile activity of the porcine uterine smooth muscles than its analogue the BPS.

Obesity Alters Leptin Signalling in Mouse Uterus: Putative Link to Epigenetic Regulation During Decidualisation

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Obesity is a major risk factor for reproductive success and can be associated with disruption of endometrial receptivity, decidualisation and placentation by increased levels of estradiol (E2) or progesterone (P4). In our study we hypothesise that obesity leads to impaired leptin signalling in the uterus, with further impact on epigenetic regulation during decidualisation. First, we conducted an in vivo study in which C57BL/6J mice were subjected to a diet-induced obesity (DIO) protocol for 4 and 16 wks. We characterised the expression level of oestradiol receptor (ER) a; Er b and progesterone receptor (PR) b; Pr a+b ($P<0.05$) by real-time PCR as well as leptin signalling components in the uterus and confirmed the increase of Suppressor of cytokine signalling 3 (SOCS3) levels by real-time PCR, Western blotting, immunohistochemistry (IHC) and immunofluorescence (IF). Next, we stained for 5 methyl-cytosine (5mC) and 5 hydroxymethyl-cytosine (5hmC) in endometrial stroma of our DIO model and also an in vivo pharmacological hyperleptinemic mouse model (LEPT) as well as genetically obese mouse Leprdb/db , revealing decrease in 5mC and 5hmC in endometrial stromal cells after 16 wks HFD (DIO) ($P>0,01$; $P>0,0001$), in leptin treated animals (L) ($P>0,06$), and the genetic obese mouse db/db ($P>0,0001$). We link here changes in leptin signalling in the obese endometrium to levels of 5mC and 5hmC in the stroma. We are now analysing the DNA methylome of decidua from our protocols to further explore the relationship between obesity, leptin and methylation in the maternal primordia of the future placenta.

AG was supported by Marie S Curie Individual Fellowship; Work funded by Polish National Science Centre programmes (2014/15/D/NZ4/01152) and (2016/23/B/NZ4/03737), UK Medical Research Council (MR/K011332/1) and Biotechnology and Biological Sciences Research Council (BBS/E/B/000C0423).

Role of prostacyclin in the corpus luteum of the pig

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Corpus luteum (CL) is a transient endocrine gland that develops from the postovulatory follicle. The principal function of the CL is to produce progesterone (P4) that is necessary for the establishment and maintenance of pregnancy. In all mammalian species, the lifespan of the CL determines the length of the estrous cycle [1]. CL is a highly vascularized tissue; thus, local hemodynamic changes within the blood vessels play a pivotal role in the regulation of CL development and activity. Since prostacyclin (prostaglandin I2; PGI2) is a well-known modulator of a vascular function, its role in luteal tissue seems to be crucial. PGI2 is a derivative of arachidonic acid and the terminal enzyme in its biosynthesis is prostacyclin synthase (PTGIS). PTGIS enzyme is abundant in endothelial and smooth muscle cells, but is also present in other cell types. PGI2 is chemically unstable in biological fluids and rapidly undergoes spontaneous transformation to its inactive form, 6-keto PGF1α, which thus directly reflects PGI2 concentration [2]. In the vascular system, PGI2 is a potent vasodilator and inhibitor of platelet aggregation [3]. The biological actions of PGI2 are mediated by a membrane G-protein-coupled receptor (PTGIR), whose activation leads to increased cAMP formation. PGI2 may also signal via nuclear peroxisome proliferator-activated receptors (PPARs), and PPARD isoform has been identified as a biological target for endogenous PGI2. Several synthetic PGI2 analogs have been demonstrated to directly interact with both PTGIR and PPARD isoform [4].

To clarify the role of PGI2 in the porcine CL we analyzed profiles of 1) PTGIS mRNA and protein expression, 2) PGI2 metabolite concentration, and 3) PTGIR and PPARD expression in luteal tissue on days 2 to 16 of the estrous cycle and days 10 to 30 of pregnancy. Moreover, we examined the in vitro effect of PGI2 analogs on 4) P4 synthesis by luteal cells and 5) the proliferation and proangiogenic gene expression in endothelial cells of the pig CL.

In cyclic gilts, decreased PTGIS mRNA expression was detected on days 15-16 compared to days 2-4. In contrast to mRNA, PTGIS protein level gradually increased during the studied period of the estrous cycle and was greater on day 12 compared with days 2-4. In pregnant animals, greater concentration of PTGIS transcripts was observed on day 30 compared to days 12, 15-16, 21, and 25-26 and was accompanied by elevated PTGIS protein level. However, the content of 6-keto PGF1α did not change in the luteal tissue of both cyclic and pregnant animals. A transient decrease in PTGIR mRNA expression was observed on days 5-7 of the estrous cycle. PTGIR protein level did not vary in the luteal tissue of cyclic animals, but increased on days 21 to 30 compared to day 10 in pregnant gilts [5]. PPARD mRNA expression also showed dynamic changes in the CL of both cyclic and pregnant gilts (M. Szymanska, A. Blitek, unpublished). The treatment of cultured luteal cells with stable analogs of PGI2, iloprost and carbaprostacyclin, increased HSD3B1 mRNA expression and P4 secretion. Moreover, the addition of PTGIR or PPARD antagonists abolished a PGI2-stimulated P4 synthesis in luteal cells indicating that activation of PGI2 receptors has a luteotropic action in the pig CL [5]. In vitro proliferation of endothelial cells was stimulated by iloprost and this effect was mediated through the activation of PI3K signaling pathway.

Moreover, iloprost increased VEGF and ANGPT1 mRNA expression in cultured endothelial cells (M. Szymanska, A. Blitek, unpublished).

Summarizing, we reported dynamic changes of PTGIS and PGI2 receptor expression in porcine CL during the estrous cycle and early pregnancy. Moreover, our results indicate a possible luteotropic role of PGI2 in the pig. The supportive action of PGI2 on the CL development and function includes the stimulatory effect on P4 synthesis by luteal cells and proangiogenic activity in endothelial cells.

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References:

[1] Spencer TE, Johnson GA, Burghardt RC, Bazer FW. Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biol Reprod 2004;71:2-10.

[2] Helliwell RJA, Adams LF, Mitchell MD. Prostaglandin synthases: recent development and a novel hypothesis. Prostaglandins Leukot Essent Fatty Acids 2004;70:101-113.

[3] Gryglewski RJ. Prostacyclin among prostanoids. Pharmacol Rep 2008;60:3-11.

[4] Wise H. Multiple signaling options for prostacyclin. Acta Pharmacol Sin 2003;24:625-630.

[5] Szymanska M, Blitek A. Prostacyclin synthesis and prostacyclin receptor expression in the porcine corpus luteum: evidence for a luteotropic role in vitro. Biol Reprod 2019;100:162-174.

Role of glycoconjugates and lectins in the corpus luteum

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Glycosylation is important for the regulation of cell function including development, differentiation, apoptosis, and signal transduction. Lectins are sugar-binding proteins, and endogenous animal lectins are classified into more than 10 families according to the sugar-binding affinity. Galectins are β-galactoside-binding animal lectins and 15 members are identified in mammals so far. Galectins recognize *N*-acetyllactosamine (LacNAc; Galβ1-3/4GlcNAc) and broadly distribute in mammalian body, modulating various cell functions. In the female reproductive system, galectin-1 and galectin-3 are major subtypes and their expression changes during estrous/menstrual cycle. In the mouse ovary, the regressing corpus luteum (CL) expresses both galectins. In cows and women, galectin-1 is expressed in the healthy functional CL whereas the galectin-3 expression elevates in the old regressing CL, suggesting subtype-specific function of galectins. We have been investigating the role of galectins in the human CL, and found that α 2,6-sialylation and glycosphingolipid contents are important for the regulation of the CL function.

Both galectin-1 and galectin-3 can bind to LacNAc motif; however α2,6-sialylation of terminal galactose blocks the binding of galectin-1 (Fig. 1). When we analyzed the expression of a α 2,6-sialyltransferase (ST6GAL1) in the human CL, the expression of *ST6GAL1* was high in the regressing CL like galectin-3, and inhibited by luteotrophic human chorionic gonadotrophin (hCG) and prostaglandin E (PGE). α 2,6-sialic acids were accumulated to 3β-HSD-negative luteal cells, suggesting that an inhibition of galectin-1-sugar-binding by α 2,6-sialylation is involved in luteal regression. We propose that there is a "galectin switch" associating with the CL function.

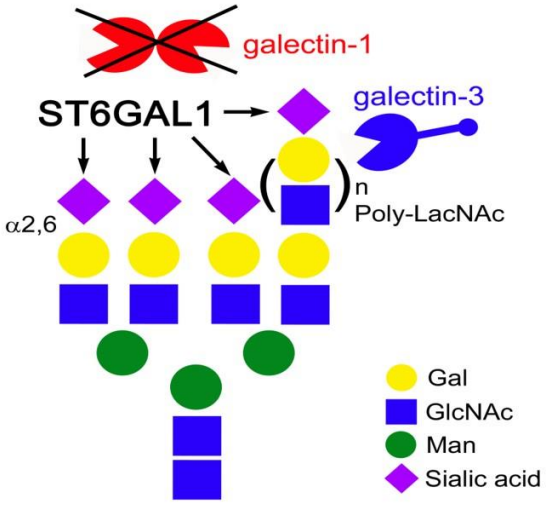

Fig. 1 Subtype specific sugar-binding affinity of galectin-1 and galectin-3. Both galectins bind to LacNAc motif. ST6GAL1 catalyzes α2,6-sialylation on terminal galactose (Gal) to block the binding of galectin-1. GlcNAc; *N*-asetylglucosamine, Man; mannose.

PGE is a major luteotrophic molecule and secreted from luteal cells by hCG/LH stimulation. Galectin-1 supports hCG/LH/PGE-activated cAMP/PKA signaling to maintain luteal function, and hCG/LH/PGE enhance the galectin-1 expression in luteal cells. However, the potential ability to produce PGE and reactivity against hCG/LH in luteal cells are attenuated with the age of the CL. Revealing how this phenomenon occurs is important for understanding the mechanism of spontaneous luteolysis in women because uterus-derived factors such as PGF are not involved in luteolysis in women.

We next focus on the glycosphingolipids in luteal cells and investigated how glycosphingolipids regulate hCG signaling in human luteal cells. D-PDMP, an inhibitor of glycosphingolipid synthesis, decreased the basal and hCG-stimulated production of progesterone and PGE, suggesting that glycosphingolipids are important for luteal function in

women. Ganglioside GM1 is sialic acid-containing glycosphingolipids and a major component of lipid raft, a membrane microdomain that is involved in signal transduction. Galectin-1 binds to ganglioside GM1 and probably supports hCG signaling by accumulating its receptor (LHCGR) on ganglioside GM1-rich lipid raft. Administration of ganglioside GM1 did not affect the luteotrophic action of hCG, whereas ganglioside GM3, the most simple ganglioside, attenuated the hCG-stimulated cAMP/PKA activation. Because the production of ganglioside GM3 is enhanced by luteal cell-derived steroids, it is likely that ganglioside GM3 is accumulated in luteal cell membrane with age of the CL, resulting in attenuation of cAMP/PKA signaling (Fig. 2). These results suggest that quantity and quality of glycosphingolipids are key factors to regulate the function of human luteal cells.

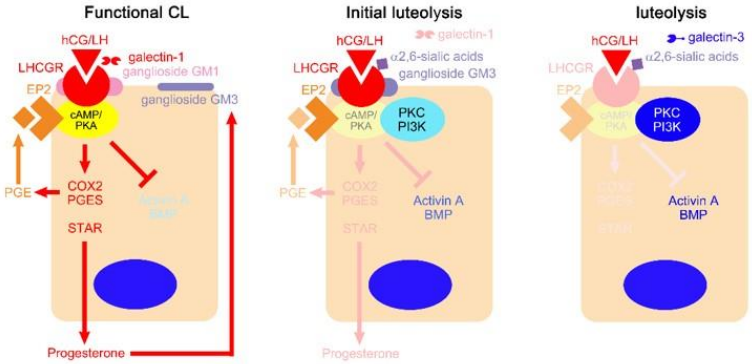

Fig. 2 Possible contribution of glycosphingolipids to spontaneous luteolysis in women. Accumulation of ganglioside GM3 attenuates cAMP/PKA signaling, resulting in an increase of a2,6-sialylation and luteolytic factors (Activin A and BMP). Finally, luteal cells totally lack the ability to produce progesterone and PGE as well as glycosphingolipids. Loss of glycosphingolipids results in change in signal transduction pathway towards PKC and PI3K. BMP; bone morphogenetic protein, COX2; cyclooxygenase 2, PGES; prostaglandin E synthase, STAR; steroidogenic acute regulatory protein.

VASPIN – new adipokine in the ovarian physiology: expression and direct effect on signaling pathways, steroidogenesis, proliferation and apoptosis. In vitro studies on the porcine model.

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Introduction: The reproductive system in female is regulated precisely by an intricate interplay of hormones produced by the hypothalamus, pituitary and the ovaries. The interplay of hormones results in ovarian cyclicity in females, which in consequence leads to fertilization, delivery by the maintenance of pregnancy of offspring. Moreover, it is now clear that female fertility strong depends on the energy metabolism status in women and also domestic animals, including pig. For example, obesity and some metabolic disorders impairs women fertility through an effect upon the control of ovarian functions, ovulation, oocyte development, embryo and endometrial development, implantation and pregnancy loss [1]. Relevant energy status of sows determines the normal reproductive functions, e.g. sows with low weight require a longer time to first estrus and their offspring are less numerous and characterized by low birth weight [2, 3]. The metabolic function in the body is mediated, in part, by its ability to secrete numerous metabolic peptides like oxytocin, ghrelin, kisspeptin or adipose tissue hormones- adipokines: leptin, resistin, adiponectin, or visfatin, which modulate food intake, energy homeostasis, lipid and glucose metabolism, insulin resistance and also reproduction [4,5]. However, vaspin, new metabolic hormone, has never been studied in the ovary.

Aim of the study: Using porcine ovarian follicles, as a animal model, we studies: i). vaspin mRNA and protein expression, immunolocalization in the ovarian follicles; plasma and follicular fluid concentration of vaspin during oestrous cycle in two prolific breeds of pigs: fat Meishan (MS) and lean Large White (LW); ii). effect of several factors like gonadotropin, IGF1, insulin and steroids: P4- progesterone, T- testosterone, E2- estradiol) on ovarian vaspin expression; and understanding molecular mechanism involved by activation of different kinases: mitogen-activated protein kinases (MAPK/ERK1/2), Akt/phosphatidylinositol 3-kinase (Akt/PI3), Janus kinase (Stat/JAK2), 5'AMP-activated protein kinase (AMPKα) and nuclear factor kappa-light-chain-enhancer (NF-kB); iii). assessment of the putative direct action of vaspin on various kinases phosphorylation, steroidogenesis, proliferation and apoptosis..

Results: We found higher vaspin mRNA and protein expression in the ovarian follicles and adipose tissue at 10–12 days of the oestrous cycle in MS compared to LW. Moreover, vaspin expression, as well as its concentration in plasma and follicular fluid, decreased in ovarian follicles of LW during days of the oestrous cycle, while the opposite results were noted in MS. Immunohistochemistry showed vaspin in granulosa, theca, cumulus cells and oocytes as well as in adipocytes. Vaspin level in the ovary increased by gonadotropin, insulin, IGF-1 and steroids stimulation through kinases JAK/Stat, ERK1/2, PI3K and AMPK, as well as factor NF-κB. In the next part of our study, we observed that vaspin), in a time-dependent manner, increased phosphorylation of various ERK1/2, Akt, Stat3, AMPK α and PKA, while it decreased expression of NFKB2. We observed that vaspin, in a dose-dependent manner, increased basal steroid hormones secretion (progesterone and estradiol), mRNA and protein expression of steroid enzymes and the mRNA of gonadotropins (FSHR, LHCGR) and steroids (PGR, ESR2)

receptors. The stimulatory effect of vaspin on basal steroidogenesis was reversed when ovarian cells were cultured in the presence of a PKA pharmacological inhibitor (KT5720) and when GRP78 receptor was knocked down (siRNA). Finally, our in vitro studies documented that vaspin have stimulatory effect on cell proliferation and inhibitory on cellular apoptosis – related protein expression.

Conclusion: These findings all show vaspin expression and function in the ovarian cells, and provide the physiological framework to bring new insights of vaspin in the regulation of female reproduction.

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[1] Mikhael et al. 2019 Hypothalamic-Pituitary-Ovarian Axis Disorders Impacting Female Fertility. Biomedicines 4;7(1).

[2] Quesnel et al. 2007 Influence of litter size on metabolic status and reproductive axis in primiparous sows. J Anim Sci 85; 118–128.

[3] Gaughan et al. 1997 Effect of body composition at selection on reproductive development in large white gilts. J Anim Sci 75; 1764-72.

[4] Sepandar et al. 2019 The association between resting metabolic rate and metabolic syndrome may be mediated by adipokines in overweight and obese women. Diabetes Metab Syndr. 13(1):530-534.

[5] Rak A et al. 2017 Adiponectin and resistin: potential metabolic signals affecting hypothalamo-pituitary gonadal axis in females and males of different species. Reproduction 153(6):R215-R226.

Multiple roles of hypoxia in bovine corpus luteum function

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Introduction

Development of the corpus luteum (CL) occurs after ovulation, and is accompanied by active angiogenesis. When conception does not occur, the CL regresses with the decrease of progesterone (P4) synthesis, accompanied by the apoptosis of luteal cells. During the ovarian cycle, blood flow to the ovary changes [1], causing changes in the transport of nutrients, hormones and gases including O_2 to the ovary. Ovarian blood flow in cows has been reported to decrease during luteal regression, and to be kept at low levels during luteal formation after ovulation [1] (Fig. 1). Thus, during luteal regression and formation, the ovary is characterized by a low oxygen condition (hypoxia) caused by the decreased blood supply. Cellular responses to hypoxic conditions are strongly influenced by hypoxia-inducible factors (HIFs) [2]. HIFs are hypoxia-specific transcription factors that have roles in inducing several physiological processes including angiogenesis and glycolysis. Our research has been focusing on the roles of hypoxic conditions in the regulation of ovarian function, with emphasis on luteal life and death.

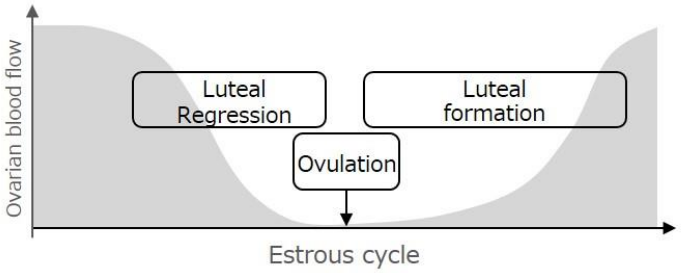

Fig. 1. Changes in ovarian blood flow to the ovary during the estrous cycle in cows.

Hypoxia in functional luteolysis

Luteal regression is characterized by a decrease in P4 production (functional luteolysis), followed by a decrease in luteal size (structural luteolysis), which is largely achieved by apoptosis. Since the decrease of blood flow during luteal regression occurs in parallel with the decrease of serum P4 level in cows [1], the decrease of blood flow has been suggested to be related to functional luteolysis. However, it remains unclear how the decrease of blood flow induces luteolysis. We hypothesized that oxygen deficiency is related to luteolysis. In experiments using cultured bovine mid luteal cells, P4 production decreased under hypoxia (3% O_2). The expression and activity of a cytochrome P450 side-chain cleavage enzyme (P450scc, CYP11A1), which converts pregnenolone into P4, were also decreased by hypoxia. These findings suggest that hypoxia has a role in functional luteolysis in cows.

Hypoxia in structural luteolysis

Apoptosis is an essential part of structural luteolysis [3]. We investigated whether hypoxia is related to structural luteolysis in cows by examining its effect on the viability of bovine cultured mid luteal cells and the integrity of their nuclei. We found that hypoxia induced luteal cell death and DNA fragmentation. We concluded that apoptosis was induced in cultured mid luteal cells under hypoxic conditions. The expressions of caspase-3, an effector caspase

in the apoptosis cascade, and Bcl-2 nineteen-kilodalton interacting protein 3 (BNIP3), which facilitates apoptosis and mitophagy under hypoxic conditions, were induced by hypoxia. The above findings suggest that the oxygen deficiency in the CL, which is caused by a decreased blood supply to the ovary, is one of the factors that accelerate structural luteolysis.

Hypoxia in luteal formation

Vascular endothelial growth factor (VEGF) is known to induce angiogenesis during luteal formation in cows, and its transcription is strongly induced by HIF1 [4]. The early luteal tissue just after ovulation is thought to be under hypoxic conditions, because of the destruction of the vasculature by ovulation. Therefore, we tested the hypothesis that luteal angiogenesis after ovulation is induced by hypoxia. In bovine CL, HIF1A protein expression was higher at the early and developing luteal stages than at the other luteal stages. Hypoxic conditions induced the protein expressions of HIF1A and VEGF in cultured bovine developing luteal cells. HIF1 is known to induce the expressions of genes for mitophagy (BNIP3) and glycolysis (glucose transporter 1; GLUT1). Our results showed that the expressions of BNIP3 and GLUT1 were high at the early luteal stage, and were up-regulated under hypoxia in early luteal cells. These findings suggest that hypoxic conditions caused by decreased blood supply and degraded vasculature immediately after ovulation are needed to form the new CL, and that activation of HIF1 and its downstream signals has a major role in this process.

Conclusion

The overall findings suggest that hypoxia plays multiple roles in both the formation and regression of the CL. Hypoxia induces angiogenesis during formation of the CL, and decreases P4 synthesis and promotes apoptosis during its regression. (summarized in Fig. 2). Further studies on how the length and degree of hypoxia control the fate of cells in each luteal stage and what other factors, such as hormones, regulate HIF1 signals will contribute to a better understanding of the roles of hypoxia in ovarian physiology.

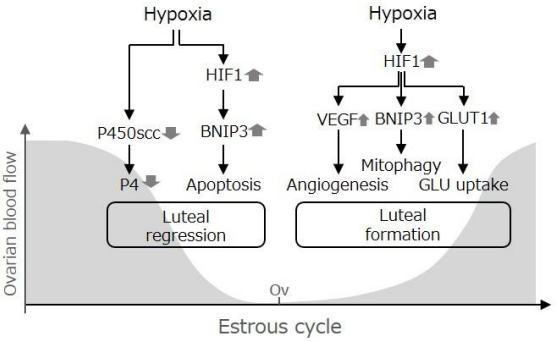

Fig. 2. Multiple roles of hypoxia in the formation and regression of the CL. HIF1: hypoxia-inducible factor 1, P4: progesterone, P450scc: cytochrome P450 side-chain cleavage enzyme, BNIP3: Bcl-2 nineteen-kilodalton interacting protein 3, VEGF: vascular endothelial growth factor, GLUT1: glucose transporter 1, GLU: glucose, Ov: ovulation.

References:

[1] Wise et al. *J Anim Sci* 1982; 55: 627-637.
[2] Wiesener & Maxwell. *Ann Med* 2003; 35: 183-190.
[3] Juengel et al. *Endocrinology* 1993; 132: 249-254.
[4] Forsythe et al. *Mol Cell Biol* 1996; 16: 4604-4613.

The action of transcription factor Gata4 on gonadal promoter regulation is modulated via PKA and ERK1/2 pathway in steroidogenic cells

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Specific objectives: Gonadal gene expression and function are tightly regulated by pituitary trophic hormones. These hormones predominantly signal through the cAMP/PKA pathway leading to increased gene expression. In fact, our previous studies have identified GATA4 as a novel downstream effector of cAMP/PKA signaling on a variety of gonadal promoters including StAR. Several studies, however, have suggested that the MAP kinase (MAPK)-ERK phosphorylation cascade is also involved in regulating gonadal gene expression. Our goal of this study was to determine the role of ERK1/2-mediated phosphorylation of GATA4 in several gonadal gene regulation.

Methodologies and Results: Our present data show that serum-induced StAR transcription in MA-10 Leydig cells is significantly reduced by blocking the ERK1/2 pathway (one of the kinases activated by MAPK). Chromatin immunoprecipitation demonstrate that GATA4 binding to the StAR promoter is positively regulated by this ERK1/2 pathway. Overexpression of a constitutively active form of MEK1 (MEK1 CA: an activator of ERK1/2 pathway), much like PKA, enhanced the transcriptional activity of GATA4 on different gonadal promoters, revealing that both pathways are important for the GATA4-dependent transcription of gonadal genes. Interestingly, the activation of ERK1/2 pathway also enhanced GATA4 action on target gonadal promoters by potentiating the transcriptional synergism between GATA4 and its known transcriptional partners NR5A1 and NR5A2.

Summary: All together, we propose a model whereby GATA4 acts as an effector of two different signaling pathways (cAMP/PKA and ERK1/2) in gonadal cells.

The effect of PGF2α on synthesis and release of progesterone through peroxisome proliferator-activated receptors in the bovine corpus luteum

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors. Three isoforms have been described: alpha (PPARα), delta (PPARδ), and gamma (PPARγ). The activity of PPARs can be modified by arachidonic acid (AA) metabolites e.g. prostaglandin F2-alpha (PGF2α). They are involved in the regulation of reproductive processes i.e. gametogenesis, ovulation, corpus luteum (CL) regression, and the embryo implantation. The effect of PPARs on progesterone (P4) synthesis depends on cell type, stage of cell differentiation, stage of the ovarian cycle, and/or animal species. Therefore, we hypothesized that synthesis and release of P4 through PPARs may change in the bovine CL under the influence of PGF2α. The aim of the study was to determine the mRNA expression of StAR, CYP 11A, 3βHSD mRNA in the bovine CL explants and P4 concentration in the culture medium under the influence of PGF2α in vitro. Bovine CL explants were obtained post mortem from ovaries of cows (n=9) at late luteal phase of the estrous cycle (15-17 days of the cycle). Corpora lutea sections were cultured according to the adopted scheme with PPAR antagonists combination (PPARα antagonist GW6471, PPARδ antagonist GSK3787 and PPARγ antagonist GW9662; 10-5M) and/or a PGF2α receptor (FP) antagonist (AL8810; 10-5M) to block receptor transmission for 6 h, and then stimulated with PGF2α (10-6M) for a further 24 h without medium exchange. Control group consisted explants not stimulated during the incubation. The mRNA expression was evaluated by real-time PCR. Concentration of P4 in the culture medium was determined using RIA. The results were statistically analyzed by Kruskal-Wallis non-parametric ANOVA followed by a Dunn's post hoc test. Increase of mRNA expression for StAR in the bovine CL explants was observed after 24 h PGF2α stimulation in experimental groups: APAL1/2 (group of CL explants stimulated with FP receptor antagonists, and PPARα and PPARδ antagonists) ($P<0.01$), APAL2/3 (group stimulated with the FP receptor antagonist, and PPARδ and PPARγ antagonists) ($P<0.05$), and APAL1/2/3 (group stimulated with PPARα and PPARδ and PPARγ antagonists) ($P<0.01$), compared to the control group. In turn, the expression of 3βHSD at the gene level was downregulated in the APAL1/2/3 experimental group ($P<0.05$) compared to the control group. There were no differences in CYP 11A mRNA expression in all studied groups compared to the control group ($P>0.05$). In addition, P4 concentration in the culture medium was lower ($P<0.01$) in the PAL experimental group (group of CL explants stimulated with FP receptor antagonist) after 24 h PGF2α stimulation compared to the control group.

The overall results indicate that PGF2α has effects on the StAR and 3βHSD mRNA expression profile in the bovine CL explants and on culture concentration of P4 suggesting that PPARs may regulate genes required for development, maintenance and regression of bovine CL.

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Progesterone receptor isoforms in bovine corpus luteum

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Progesterone (P4) is a steroid hormone that is produced by the corpus luteum (CL), placenta and ovarian follicle. The physiological effects of this hormone on target cells are achieved by binding to a specific nuclear progesterone receptor (PGR) belonging to the transcription factor-dependent receptor family. The PGR has two main isoforms, isoform A (PGRA) and isoform B (PGRB). The whole PGRA sequence is common to the PGRB sequence. In addition, PGRB has an additional sequence that distinguishes it from PGRA.

Our research, with using degenerated primers, revealed than in cow PGR also occur in two isoforms. A 429-nucleotide DNA sequence of the PGRB isoform was obtained. This sequence showed 79% similarity to the pig and horse sequence, 75% to the human sequence, 71% to the rat sequence and 68% to the mouse sequence [1].

Further research showed variable levels of mRNA and protein levels of PGRA and PGRB isoforms in CL during the estrous cycle. The highest level of PGRA and PGRB mRNA was found at the beginning of the estrous cycle, and thereafter it was gradually decreased to the end of the cycle. The high mRNA and protein levels of both isoforms at the beginning of the estrous cycle suggested their involvement in P4 regulation in newly formed CL. A comparison of the mRNA and protein expression profiles of the PGRA and PGRB isoforms revealed a 500-2000-fold lower mRNA concentration of PGRB during the cow cycle. In contrast, the difference in protein levels ranged from 2-8-fold in favor of PGRA. Isoform PGRA works as an inhibitor of PGRB. Therefore, the dominant mRNA and protein expression of PGRA in relation to PGRB may indicate that this isoform is a regulatory element of P4 activity, protecting CL against the possible effects of the overproduction of this hormone [1].

The mRNA and protein level of PGRA and PGRB isoforms were differently regulated by luteotropic and luteolytic factors in bovine endometrium. This may suggest that these factors through different impact on the mRNA and protein levels of PGRA and PGRB isoforms may regulate the effect of P4 in endometrial cells [2].

We found also that the most popular PGR antagonists as onapristone (ZK299) and mifepristone (RU486) affected the mRNA and protein expression of the PGRA and PGRB isoforms. This action was depended on the concentration of the given inhibitor. Obtained results may signify that the final physiological effect induced by the antagonist depends on the isoform of PGR that is associated with the compound [3].

The last step of PGR receptor activation is the attachment of additional transcriptional regulators called coagulators. This group consists coactivators, activating the transcription of target genes, and corepressors, inhibiting the transcription of such genes. We found that mRNA and protein level for P300/CBP-associated factor (PCAF) coactivator and the nuclear receptor corepressor 1 (NCOR1) in cow CL were the highest in the middle of the ovarian cycle in comparison to the levels at the beginning and the end of the cycle. In contrast in endometrium, the mRNA and protein levels NCOR1 and PCAF were higher at the beginning of the estrous cycle and decreased slightly to the end of the estrous cycle. The results showed

also that the mRNA and protein levels of PCAF and NCOR1 were corelated with the P4 level in both investigated tissues. This may suggest that P4 is involved in the regulation of their expression. While similar levels of coregulator and corepressor expression in CL and endometrium may denote that they compete for a binding site located in the PGR receptor [4].

Our latest research concerned the participation of methylation of PGR isoform promoters in the regulation of the PGR action. We showed that the methylation percentage of the PGRA promoter was approximately 2.5-3 times higher in CL, while in the endometrium it approximately 0.7-2.5 times higher than the methylation of the PGRB promoter during the estrous cycle. Conversely, the total methylation level of both isoforms in CL was approximately 2-fold higher than in the endometrium. These data could indicate that the increased methylation of the PGRA isoform promoter may be a regulatory mechanism of the inhibitory activity of PGRA against PGRB and thus influences the regulation of P4 activity in CL and endometrium [5].

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References:

1. Rekawiecki R, Kowalik MK, Kotwica J. (2015) Cloning and expression of progesterone receptor isoforms A and B in bovine corpus luteum. Rep. Fertil. Develop. 27:1029-37.
2. Rekawiecki R, Kowalik MK, Kotwica J. (2016) Luteotropic and luteolytic factors regulate mRNA and protein expression of progesterone receptor isoforms A and B in the bovine endometrium. Rep. Fertil. Develop. 28: 907-913.
3. Rekawiecki R, Kowalik MK, Kotwica J. (2015) Onapristone (ZK299) and mifepristone (RU486) regulate the messenger RNA and protein expression levels of the progesterone receptor isoforms A and B in the bovine endometrium. Theriogenology 84: 348-57.
4. Rekawiecki R, Kowalik M.K., Kotwica J. (2017) The expression of progesterone receptor coregulators mRNA and protein in corpus luteum and endometrium of cows during the estrous cycle. Anim. Reprod. Sci. 183:102-109.
5. Rekawiecki R, Kisielewska K, Kowalik MK, Kotwica J. (2018) Methylation of progesterone receptor isoform A and B promoters in the reproductive system of cows. Reprod. Fertil. Dev. 30:1634-1642.

Generating mechanisms for spontaneous rhythmic contraction of bovine oviducts

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Spontaneous phasic contraction of mammalian oviducts occurs at peri-ovulation and is essential for the transport of gametes and embryos. Our previous studies revealed the regulatory mechanisms of contraction modulator production by bovine oviductal epithelial and stromal cells. However, knowledge of the system that generates spontaneous contraction of oviducts remains unclear. In the intestinal and urinary tracts, it has been demonstrated that spontaneous membrane depolarization, induced by inward or outward flow of several ions, increases intracellular Ca^{2+} levels in smooth muscle cells (SMCs) and results in spontaneous phasic contraction. In addition, ion currents also regulate pacemaker cells, which are endogenous generators of myocyte contraction. Based on these findings, our present study aimed to clarify the fundamental mechanisms underlying spontaneous contractions in bovine oviducts.

First, to clarify which ion channels are involved in the spontaneous rhythmic contraction of bovine oviducts, we investigated the effects of ion channel and gap junction blockers on contraction of oviduct strips using the Magnus system. The targets were as follows: (1) voltage-dependent Ca^{2+} channels (VDCC) and the receptors responsible for Ca^{2+} release from the endoplasmic reticulum—inositol trisphosphate receptor (IP3R) and ryanodine receptor (RyR); (2) Na^{+} and Cl^{-} channels that act as depolarization initiators; (3) voltage-gated potassium channels (VGKC) and Ca^{2+}-activated potassium channels (BK and SK channels) that act as re-/hyper-polarization regulators; and (4) gap junctions that mediate propagation of depolarization to neighboring cells. For these targets, we found that: (1) the VDCC blocker decreased the amplitude of contractions, resulting in loss of contraction. Co-inhibition of both IP3R and RyR decreased the number and amplitude of contractions. These results suggest that Ca^{2+} influx via VDCC, or release from the endoplasmic reticulum, is necessary for spontaneous contractions; (2) the Na^{+} channel blocker did not affect contractions, whereas Cl^{-} blockers decreased the frequency of contractions. This suggests that Cl^{-} channels are involved in the initiation of depolarization; (3) a VGKC blocker decreased the number and amplitude of contractions. BK channel blocking decreased the number of contractions, although BK and SK channel blockers increased the amplitude of contractions. These results suggest that VGKC and Ca^{2+}-activated K^{+} channels are involved in regulation of re-/hyper-polarization. (4) A gap junction blocker suppressed contractions, suggesting that depolarization propagates via gap junctions.

Next, intracellular Ca^{2+} imaging was performed in cultured bovine SMCs to evaluate the precontractile cellular activity. Intracellular Ca^{2+} was visualized by Fluo4. Movies using Ca^{2+} imaging were recorded and analyzed. When the area covered by smooth muscle actin-positive cells exceeded 90% (90% confluence), quick spontaneous Ca^{2+} oscillation was observed, but only a few slow Ca^{2+} oscillations were observed at 70% confluence. This indicates that spontaneous activity of SMCs can be observed in culture, and that cell-to-cell contact is required for the initiation of Ca^{2+} oscillations. We also found two types of Ca^{2+} signaling patterns: Ca^{2+} waves and Ca^{2+} flashes. A Ca^{2+} wave is defined as an increase in intracellular Ca^{2+} that propagates across a whole cell, while a Ca^{2+} flash is a temporary increase in global intracellular Ca^{2+}. The number of cells showing Ca^{2+} waves and the frequency of periodic Ca^{2+}

waves was higher in cells at high density than those at low density. In contrast, neither the number of the cells showing Ca^{2+} flashes nor the frequency of periodic Ca^{2+} flashes showed significant differences between high and low cell density cultures. Although the roles of Ca^{2+} waves and flashes in SMCs are still unclear, this study indicated that the precontractile activity of cultured bovine oviductal SMCs can be evaluated using Ca^{2+} imaging methods. The regulatory mechanisms and roles of each Ca^{2+} dynamic pattern in oviductal SMCs will be investigated in future studies. This study is supported by JSPS KAKENHI (JP17H05041).

The effect of lysophosphatidic acid (LPA) on ovarian follicle function, oocyte fertilization and early embryo development in cows

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Assisted reproductive technologies (ART), including in vitro embryo production (IVP) have been successfully applied to animal reproduction with the aim of breeding strategies for improved production of valuable, healthy offspring. Despite the progress in IVP techniques over the years, further improvements of in vitro embryo culture systems are required for the enhancement of oocyte and embryo developmental competence, therefore continued effort to define the optimal media for IVP is still needed. The presented data focus on the current knowledge of lysophosphatidic acid (LPA) as a potential supplement of oocyte maturation, fertilization and embryo culture media as well as current views on the potential involvement of LPA signaling pathway during early embryo development.

Our research proved that LPA may exert regulatory influence not only on the bovine uterus, but also ovary and preimplantation embryo. In the ovary, granulosa and theca cells of the follicle as well as steroidogenic cells of the active corpus luteum are the source and target for LPA. In the bovine ovarian follicle LPA regulated follicle development via the stimulation of estradiol (E2) production. LPA also modulated TNFα dependent apoptosis of the granulosa cells in the atretic and transitional follicles. Moreover, this lysophospholipid participated in the induced by caspases, apoptosis of granulosa cells, in the atretic follicles via the activation of LPAR2 and LPAR3. On the other hand, LPA through LPAR1, also influenced granulosa cells viability, stimulating MCL1 expression and inducing anti-apoptotic processes in healthy follicles. We documented that in the healthy group of ovarian follicles, LPA acting via LPAR1 stimulated the expression of ERβ, interacted with E2 and thus influenced on granulosa cells differentiation and proliferation.

At the estrous cycle, after ovulation, during CL formation and progesterone (P4) secretion, LPA exerted auto- and para- crine role in the bovine ovary. At the time of P4 secretion, the well known luteolytic factors such as nitric oxide (NO) or TNFα with IFNγ could not induce luteolysis in the presence of LPA. This protective, on steroidogenic cells, LPA role might occur in two ways. During functional luteolysis, LPA abrogated the inhibitory influence of NO or TNFα with IFNγ on P4 synthesis in the CL. On the other hand, LPA inhibited, dependent on cytokines and NO structural luteolysis of the CL via the influence on the proapoptotic factors in steroidogenic cells (activity of caspaze 3, Bax/Bcl2 artio, Fas/FasL complex and TNFα/TNFR1).

During the estrous cycle in the cow, LPA originating from the CL played auto/para-crine role in its development and P4 synthesis. On the other hand, during early pregnancy, LPA, being synthesized mainly in the uterus, exerted two kinds of influence on the CL. Indirectly stimulating the synthesis and secretion of endometrial PGE2 or through the stimulation of IFNτ dependent gene expression in the CL. During the luteal steroidogenesis, we documented that LPA

stimulated P4 secretion via the influence on the terminal enzyme in its synthesis - 3βHSD. Moreover, we demonstrated that LPA augmented the stimulatory effect of interferone tau (IFNτ) on the IFNτ- dependent gene expression (OAS1 and ISG15).

We also found that oocyte and also blastocyst can be the place of LPA synthesis and target of its action in the cow. During the process of in vitro oocyte maturation, LPA increased the number of metaphase II oocytes, which resulted in the enhanced oocyte maturation rate. Moreover, we documented that the supplementation of the maturation medium with LPA decreased the number of apoptotic nuclei in the cumulus cells thus inhibited apoptosis in the oocyte-cumulus complex. We also found that the supplementation of the maturation medium with LPA increased glucose uptake via the influence on mRNA for glucose transporter (GLUT1) expression and also increased lactate production via the stimulation of mRNA for phosphofructokinase (PFKP) in the oocyte-cumulus complexes. The obtained data account for LPA –dependent redirection of glucose metabolism towards glycolysis. Moreover, LPA stimulated the expression of amfiregulin (AREG) and epiregulin (EREG) during cumulus expansion at oocyte maturation. At last, LPA increased the quality of in vitro matured oocytes via the influence on the expression of oocyte quality markers- follistatin (FST), growth and differentiation factor 9 (GDF9) in the oocyte and catepsins (CTS) B, K and Z in the cumulus cells. On the other hand the supplementation of the culture medium with LPA stimulated the expression of OCT4, SOX2, IGF2R, BAX and BCL2 in the preimplantation blastocysts.

Summarising, the obtained data enhance the existing knowledge of the bovine reproductive system function as well as the processes during early embryo development, which in the future might become the theoretical background for the optimization of assisted reproduction techniques in cows as well as help to implement new diagnostics methods of early embryo mortality.

Multi-step process of ciliogenesis in bovine oviductal epithelium during the estrous cycle

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The oviductal epithelium is composed of ciliated and non-ciliated cells. Ciliated cells produce a stream of oviductal fluid that flows toward the uterus, and non-ciliated cells produce an oviductal fluid that is rich in amino acids and various molecules to provide an optimal microenvironment for sperm capacitation, fertilization, and embryonic development. In cattle, ciliated cells in the oviductal epithelium are abundant in the follicular phase, whereas the number of non-ciliated cells gradually increases along with the formation of the corpus luteum. However, the mechanism underlying this cyclic change in the proportions of the two cell types is not understood. Our previous study indicated that ciliated cells are derived from non-ciliated cells. Therefore, the objective of the present study was to clarify the different cell types in the bovine oviductal epithelium and the process of ciliogenesis from non-ciliated to ciliated cells.

Bovine ampullary oviductal tissues and epithelial cells were collected for immunohistochemistry and mRNA expression analysis (n = 8), respectively, from a local slaughterhouse at four different estrous stages (stage I: days 1–4 after ovulation, stage II: days 5–10, stage III: days 11–17, and stage IV: days 18–20). Immunohistochemical analysis was used to study the number of cells that were positive for cilia marker (acetylated-α-tubulin), proliferation marker (Ki67), non-ciliated cell marker (PAX8), and ciliogenesis markers (FOXJ1 and MYB) in the epithelial cells during the estrous cycle. The total number of positive and negative epithelial cells along a 1-mm basement membrane was counted. Quantitative RT-PCR was used to examine the mRNA expression of FOXJ1 and MYB in the isolated oviductal epithelial cells.

The oviductal epithelial cells expressed either FOXJ1 or PAX8. All the acetylated-α-tubulin+ cells were positive for FOXJ1, but there were few acetylated-α-tubulin−/FOXJ1+ cells. Both FOXJ1+ and PAX8+ cells expressed MYB, but Ki67+ cells did not express it. Some MYB+ cells expressed acetylated-α-tubulin. The number of Ki67+ and MYB+ cells was the highest at stage IV ($P < 0.05$), whereas the number of FOXJ1+ and acetylated-α-tubulin+ cells was the highest in the following stage I ($P < 0.05$), indicating an association between ciliogenesis and estrous cycle. The mRNA expression of MYB was the lowest at stage IV ($P < 0.05$), whereas that of FOXJ1 was the highest at stage IV ($P < 0.05$). Thus, based on immunological classification, the oviductal epithelium contains at least seven cell types at different stages of ciliogenesis. Moreover, ciliogenesis is initiated at stage IV and terminated in the following stage I to provide an optimal environment for gamete transport, fertilization, and embryonic development.

Function of the porcine oviduct under different physiological and hormonal conditions

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Oviductal epithelium is actively involved not only in transport, gametes maturation and fertilization but it's also the source of many factors occurring in oviductal fluid eg. prostaglandins, cytokines, various growth factors and others, which creates proper environment for early embryo development. New methods used in improving animal breeding, like in vitro fertilization, embryo transfer or cloning, require modification of the estrous cycle and gaining a high degree of control over the timing of ovulation as well as inducting superovulation. Oestrous synchronization and superovulation are common tools used for obtaining oocytes and embryos. However, it has been recognized that pharmacological manipulations of the oestrus cycle, apart from the expected effects, may also lead to some unwanted disturbances in longer term regulation of female reproductive tract. The most popular protocol for oestrous cycle induction involves applying a combination of equine chorionic gonadotropin (eCG) for follicular growth stimulation and human chorionic gonadotropin (hCG) to induce ovulation. Presented study was performed to determine the influence of insemination as well as treatment with hCG and eCG on expression of main factors determining oviductal function: VEGF- and prostaglandins- synthesis pathways in the porcine oviducts on Day 3 after insemination. Our study revealed that hCG /eCG treatment change prostaglandins and VEGF synthesis in dose dependent manner. Synchronization of ovulation caused decrease of expression of PTGS2 and VEGFA protein as well as decrease of PGE_2 content in the porcine oviductal isthmus, but amount of $PGF_{2\alpha}$ in oviductal tissue of gilts treated with hCG /eCG was significantly elevated. Furthermore , the PGE_2 to $PGF_{2\alpha}$ ratio in isthmus decreased significantly after hormonal treatment. Superovulation increase PGFS and CBR1 expression. Insemination itself increase expression of *PTGS2*, *mPGES* mRNA and mPGES protein and PGE2 content, but decrease VEGFA protein expression in isthmus. Differential expression of the factors analyzed in gilts treated with exogenous gonadotrophins suggests that hCG/ eCG stimulation affects prostaglandins and VEGF synthesis pathway.

Because mechanisms responsible for this regulation remain unclear, the *in vitro* study was performed to determine the effect of hCG and FSH on cultured porcine oviductal epithelial cells (POEC). POEC, obtained in peri-ovulatory period, were incubated with hCG (1ng/ml or 50 ng/ml), FSH (10 ng/ml) or hCG/FSH (1ng/ml or 50ng/ml) for 24 or 48 hours. Gene expression for prostaglandin and VEGF synthesis pathways were determined by qPCR and PGE2, PGFM and 6-keto PGF1α content in the incubation media using EIA kit. Statistical analyses were performed using two-way ANOVA, followed by Bonferroni's post hoc test.

POEC cultured with FSH alone showed decreased expression of PGs synthesis. Incubation of POEC with FSH alone decreased prostacyclin synthase (PGIS) levels, but without any influence on prostacyclin synthesis and IP receptor expression. We observed decreased CBR1 mRNA expression, which coincided with PGFM concentrations in the incubation media. This strongly confirms the major contribution of the CBR1 enzyme in the synthesis of $PGF_2\alpha$ by oviductal tissue via PGE_2 conversion and the direct influence of FSH on $PGF_2\alpha$ synthesis. This study, in addition to the PGs data, resulted in altered expression of the VEGF system in POEC treated with FSH and hCG. The Flt-1 and KDR mRNA levels were lowered after incubation with 10 ng of FSH.

In conclusion, this study showed that POEC stimulation with hCG and FSH dramatically change PGs and VEGF system synthesis pathway. Any disturbances in its expression may change the local environment within the oviduct and in consequence affect gamete transport and fertilization and/or embryo early development.

The novel techniques of in vitro maturation of porcine oocytes using 3D culture systems.

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Good quality oocytes are exceedingly essential for reproductive ability of the female, especially for successful in vitro fertilization (IVF), which is one major assisted reproductive technology for female infertility. Therefore, to clarify the unknown signaling pathways involved to impart developing competence of the oocytes during in vitro maturation (IVM) and subsequent embryonic development to the blastocyst stage, we established effective methods of the porcine cumulus-oocyte complex (COCs) long-term culture in a 3D-system.

Porcine ovaries were excised from prepubertal gilts at a local slaughterhouse. Only healthy, medium-sized follicles (4-6 mm in diameter) were selected for COCs isolation under a stereoscopic microscope. COCs of grade I, possessing homogeneous ooplasm and uniform, compact CCs were considered for further 3D in vitro maturation procedure. In the 2D model of culture, after isolation and washing, COCs were transferred into the medium drop (TCM199, 50% follicular fluid) under mineral oil. In the 3D culture model COCs with a drop of medium were encapsulated with hydrophobic polytetrafluoroethylene powder particles, to form microbioreactors, defined as Liquid Marbles (LM). In the other 3D culture system tested, COCs were encapsulated in 5 µl alginate beads (AD) and then placed in 96-well plates. COCs matured for 96 hours. Every 24 hours ½ medium was changed. After termination of culture in each of the culture systems use, analysis (light microscope) of the degree of cumulus cell expansion and nuclear maturation frequency were performed. All experiments were carried out in quadruplicate in five separate cultures (n = 5 independent experiments).

Oocytes maturating in the 2D system (16.6%) showed moderate cumulus cells expansion, while oocytes maturating in both 3D culture systems (LM: 43.3 ± 3.1%; AD: 41.3 ± 2.8%) showed advanced expansion of cumulus cells. The degree of maturation of cultured oocytes measured on the basis of the presence of the 1st polar body did not differ significantly between LM (4.3 ± 1.1%) and AD (5.7 ± 1.4%), which, considering the lack of exogenous hormones (eCG and hCG) is a very good result. Interestingly, in 2D cultures no oocyte was observed with maturated nucleus (no polar body). These results clearly indicate that further experiments on porcine oocytes should be carried out using one of the tested 3D systems.

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The biology of telomeres and TERRA in bovine oocytes- two aspects of reproductive ageing

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OBJECTIVE: Maternal aging-associated reduction of oocyte viability and age-related subfertility is a common feature in many mammalian species, and still poorly described. Late exit from the developmental line during oogenesis presumably contributes to telomere shortening. When telomeres are transcribed they generate non-coding RNAs known as Telomeric Repeat-Containing RNA (TERRA), a very recent discovery. To our knowledge, there are no reports about the expression of TERRA during early bovine embryo development, which is very similar to the human species. Therefore, we studied TERRA expression dynamics in oocytes, and throughout early embryonic cleavage stages up to blastocyst formation in a bovine model.
MATERIALS AND METHODS: Bovine GV oocytes were aspirated from collected ovaries at a local abattoir, matured, fertilized, and cultured in vitro until day 7 in a standard laboratory protocol. Samples were collected starting from the GV, MII, zygote until blastocyst stage and fixed on glass slides. TERRA foci were stained by a RNA-FISH protocol, and RNase treated samples were considered as negative controls, and pictures were captured with the help of confocal microscopy. Relative TERRA spots were determined by the summatory fluorescence intensity per nucleus.
RESULTS: TERRA is localized in the GV stage inside of the germinal vesicle, in the MII stage in the ooplasm, and from the zygote to blastocyst stage the foci are nuclear. Mean number of TERRA foci from GV to the 4-cell embryos were counted as 2.43. After this stage, namely in the 8-cell and later cleavage stages, there was a significant 3-fold increase($P<0.01$) in mean TERRA foci with 7.452 counted spots per nucleus.
CONCLUSIONS: Our data show for the first time that TERRA expression is activated at the 8-cell cleavage stage in bovine embryos, a time of significant telomere reprogramming and embryonic genome activation, what could be a possible pathway for a better understanding of reproductive ageing.

The History And Current View Of Polish Konik Horse

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Polish Konik horses are the only native breed of horses originating directly from wild tarpans (Equus caballus gmelini Ant.), which could be found in the areas of former Poland, Lithuania and Prussia until the end of the 18th century, and in some areas even up to the beginning of the 19th century. At that time, tarpans living in the wild were caught and then often used to mate with local horses. In 1936, professor Tadeusz Vetulani, researcher and propagator of primitive horses, began an experiment to prove that Polish Konik horses originated from the forest variety of tarpans (Equus caballus gmelini Ant. form of silvatica Vet). For that purpose, a group of horses, most closely related in their exterior appearance to tarpans, was purchased from the local inhabitants and placed in a forest reserve in Białowieża. After professor Vetulani's death in 1952, the experiment on the restoration of forest tarpans was stopped.

It was resumed in 1955 in Popielno (53°45'16.4"N, 21°37'42.1"E), where Polish Konik horses breeding has been continued up till now in two maintenance systems: the reserve and the stable ones. Polish Konik horses are an excellent model for analysing the processes and behaviour patterns of their wild ancestors, as well as in the wild horse population. Stud books for this breed have been published since 1962 by the Polish Horse Breeders' Association (PZHK). Since 1984 it has become a closed studbook and any infusion of a different breed is unacceptable. Through analysis of the numbers of Polish Koniks one will find a constant upward tendency in the population. Currently, about 1447 mares and 158 stallions are active in the breeding (2018). In accordance with the assumptions adopted in the FAO program for the preservation of genetic resources of farm animals based on the convention on biological diversity, this breed is still considered as facing extinction because the number of breeding mares in the European Union does not exceed 5000.

A sustainable and rationally conducted animal husbandry assumes maintaining biodiversity, and thus a diversified genetic pool, and the conservation of valuable phenotypic features. Breeding selection conducted to obtain and improve different breeds of horses has largely contributed to the genetic depletion of many breeds, which has led to a decline in horse health, lack of resistance to adverse environmental conditions, vulnerability to injuries and

negative factors in the environment. The widely-conducted natural selection in the case of Polish Konik horses, i.e. with a very limited human intervention, resulted in the creation of a horse breed resistant to environmental conditions. The above-average resistance and endurance of the Polish Konik horses is especially visible in animals living in a forest reserve. Of course, under reserve breeding conditions, the cultivation is generally limited to catching foals, so as to maintain a relatively constant number of horses in a particular area. On the other hand, rigorous selection should be conducted in stable breeding. However, one of the assumptions of the breeding program for Polish Konik horses is the increase in population. In recent years it has been increasing steadily, but considering the fact that the work on reproducing the breed started with very limited breeding material, the changes in that breed should be constantly monitored. The analysis of the affiliation of stallions and mares to separate genealogical lines indicates their uneven representation [Jaworski, 1997], which influences the insufficient diversity of genetic variability and even threatens its loss. Pedigree analyses show that in the Polish Konik horses population, in which 35 female families and 6 male lines were identified, only some present satisfactory breeding activity. Among the male lines, the stallion lines of the lowest representation include the Liliput and Glejt I lines. Among 35 female families, only 16 are active and 6 of them present clear increases (Liliputka I, Karolka, Zaza, Urszulka, Tarpanka I, Traszka), while the remaining ones show either slight development or stagnation (Tygryska, Popielica, Wola, Białka, Ponętna, Misia II, Dzina I, Tunguska, Bona, Geneza). The breeding is aimed at strengthening a variety of genetic characteristics, striving for a healthy offspring, and improving animal functional traits. If the selection were indeed carried out in accordance with the above objectives, it would exclude from reproduction some individuals which represent rare genealogical lines, but are less likely to reproduce and are less likely to get offspring. The unique nature of the breed and the limited amount of breeding material necessitate a strict reproductive control in Polish Konik horses. The mating importance in this breed is related to the possibility of using a much larger number of stallions to reproduce, which at the same time slows the growth of inbreeding [Górecka and Jezierski, 1995] than in the case of artificial insemination with the material from a small group of males. It should be noted that the Polish Konik horse population is limited and the stud books for this breed are closed, which means that no horse from outside the breed can be introduced to breeding. Assuming that there is also a reserve breeding of Polish Konik horses, where the herds are usually created in a spontaneous manner, a steady increase in inbreeding connected with this breed may be expected, leading to deterioration in the health of the breed, manifested, for example, in reproductive problems. Nevertheless, spontaneous ethological mechanisms, including among others expulsion of maturing daughters from the herds belonging to the stallions or excluding the mares from mating by their fathers, cause the growth of inbreeding to be lower than expected. Moreover, by analysing inbreeding coefficients, Jaworski and Jezierski [1999] concluded that it amounts to 0.237 between the stallions and mares within the herds and 0.33 between the stallions and mares from outside the herds. Thus, this mechanism effectively protects the population from the increase in homozygosity.

The utility value of Polish Konik horses both in stable and outdoor keeping is assessed on the basis of field tests. Mares and stallions have to pass an initial performance test before

they may be entered in the Studbook. Then, at the age of 3-4 years stallions are to undergo an obligatory basic performance test under saddle or in harness and mares have 4 years to pass the test starting from the day of their registration. These tests serve to improve gaits and the traits important in recreational applications. Movement of Koniks has improved much in recent years both in terms of the length of stride and engagement of the hindquarters. In terms of conformation Polish Konik horses are more similar to draft horse breeds, while their other advantages such as their gentle nature, friendly attitude to a human and willingness to work, contribute to the fact that they are mostly used for riding both by children and adults. Thanks to a small size, the appropriate temperament and ease of making contact with humans they are popular in hipotherapy. A unique form of the usage of Polish Konik horses is their role in protection of the landscape, such as the prevention of plant succession on open grasslands. Biting off and trampling stems of young plants, and gnawing the bark from young trees slows down growth of undesired plants and reduces their succession. By acting as so-called “living lawn mowers” Koniks help to control the vegetation and carry out landscape management.

The forest reserve in Popielno is located on a peninsula, surrounded by four lakes. Since 1962, the total area of the forest available to freely roaming herds of the Polish Konik horses has been 1620 ha. Interference by humans in the life of the herds is restricted to a minimum. In a majority of cases the herds had formed naturally, through fights for mares or when a new stallion took control over the herd after the removal of the old one. The composition of the herds is stabile and usually has not changed for years, with exception of the young mares, which are driven away from the home herds by their fathers. This spontaneous ethological mechanism, which leads to migration of the young mares between herds, safeguards against a drastic increase in inbreeding. An excellent adaptation of the Polish Primitive Horses to the difficult environmental conditions is proved by their high fertility. The incidence of pregnancies reaches 91% on average over the whole 40 years period studied. The lifespan of mares in the forest reserve is about 30 years and they remain fertile until the age of 24-27 years. The cases of retained fetal membranes or dystocia are sporadic in this breed. The cases of twin pregnancies naturally terminated were also sporadic; however once, in 1989, a twin pregnancy resulted in fully developed twins. The length of pregnancy may differs in the Polish Konik mares kept in stable. The shortest observed pregnancy was 320 days and the longest was 358 days with an average 331,06±8,2 days. An average pregnancy length in case of dropping a colt was 332,46±7,17, and in case of dropping a filly was 330,5±6,2 days. The breeding carrier of the Polish Konik mares is usually very intensive and expanded for years. The satisfying reproductive rates are the evidence of a high reproductive potential of Polish Konik horses. Breeding the Polish Konik horses in Popielno has saved extinction of a unique population originating from the ancient tarpan. At present, the population of the Polish konik is increasing, however, it must be noted that restoration of the breed began with very limited breeding material and therefore special precautions should be taken to conserve the genetic pool and all changes occurring within the population must be monitored carefully.

Status and prospect of Horse breeding and reproductive treatment in Japan

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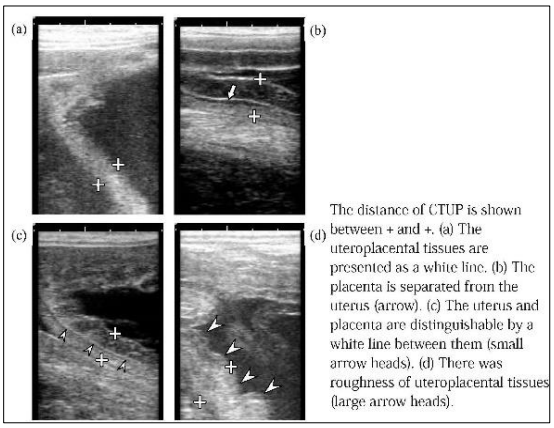
Horse industry in Japan

Although there are only 80,000 horses in Japan, 55% of them are Thoroughbreds (TB) for the racing industry and others include heavy draft horses, riding horses, and native ponies. Thoroughbred breeding in Japan stands fifth in the world. Approximately 7000 TB foals are born every year in Japan. And about 95% of their broodmares are bred and raised in Hokkaido, a north island. None of the other regions of the world where Thoroughbreds are bred and raised have severe cold climate in the winter as it has in Hokkaido (see photo). For several years, Japan Racing Association (JRA) has also been conducting multifaceted research related to racehorses, such as that related to nutrition, exercise physiology, ethology, genetic science, and clinical sciences and pathobiology. One area of advanced research that has recently attracted considerable attention is equine reproduction for efficient breeding.

Obihiro is famous for holding heavy draft horse racing (see photo). Approximately 60% of these horses are bred in the eastern part of Hokkaido. Thus, our university should focus on the problem in Heavy draft horses in addition to other breeds.

Diagnosis of placental disorder in Heavy draft mares

There are a lot of pregnancy loss caused by infectious or non-infectious cases in Heavy draft horses. In this study, combined thickness of uterus and placenta (CTUP) and uteroplacental imaging were investigated in heavy draft horses. In 35 pregnant heavy draft horses, CTUP was measured and ultrasonographic images were obtained in Month 7–12 of pregnancy. Mares were divided into three groups: those pathologically diagnosed as having placentitis (placentitis group, n = 3); those who had abortion, premature birth, or fetal malformation (abnormal group, n = 7); and the remaining 25 animals who had no abnormal findings (normal group). In the normal group, CTUP increased as pregnancy progress and was higher than those reported previously in Thoroughbreds. Increased CTUP was considered to reflect placentitis and abnormal pregnancies. Ultrasonographic images showing placental separation were obtained in 67% of the placentitis group (2/3), 29% of the abnormal group (2/7), and 20% of the normal group (5/25). Ultrasonographic images showing uteroplacental roughness or distinguishability, and pathological placental edema were observed even in the normal group (figure). These findings suggest that the



The distance of CTUP is shown between + and +. (a) The uteroplacental tissues are presented as a white line. (b) The placenta is separated from the uterus (arrow). (c) The uterus and placenta are distinguishable by a white line between them (small arrow heads). (d) There was roughness of uteroplacental tissues (large arrow heads).

detection of increased CTUP and placental separation may become a diagnostic aid for detecting abnormal pregnancy in heavy draft horses [1].

Embryos transfer from a Hokkaido native pony after artificial insemination using frozen semen

Embryo transfer (ET) technology allows the donor mare to potentially produce multiple foals in a year. In Japan, there has been no report of foal born through ET after artificial insemination using frozen semen. In this season, special riding crossbred horses (Connemara pony x Hokkaido native pony) were produced through ET technology. A non-surgical transcervical procedure was used to collect embryo from the uterus of the donor mare at day 7 post-ovulation and transferred fresh into the uterus of recipient mare. Four embryos were collected from a single donor mare and were transferred to recipients in spring 2018 [2]. Three out of 4 recipient mares (75 %) established successful pregnancy and delivered a healthy foal each in 2019 (see photo). These are the first foals produced through embryo transfer in Japan after artificial insemination using frozen semen. We are expecting that this new crossbred would be an ideal riding breed for disable people.

The relationship between size of sebaceous glands in back skin and plasma testosterone concentration in male brown bear

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In brown bears (Ursus arctos), bipedal back-rubbing against tree is considered as a marking behavior. Because the number of this behavior increases during the breeding season in males, it is speculated that male bears may convey reproductive information using chemical signals. We previously reported an oily sweet smelled secretion and enlarged sebaceous glands (SGs) in male back skins during the breeding season. Because the SGs become enlarged during the breeding season, we speculated that plasma testosterone (T), which increases during the breeding season, may regulate the size of SGs. Thus, in the present study, we examined the relationship between plasma T concentrations and sizes of SGs in back skins. In the experiment 1, we monitored plasma T concentrations and sizes of SGs in 8 intact and 3 castrated males during the non-breeding, transitional and breeding seasons. The skin samples and bloods were collected from anesthetized captive bears. In intact males, sizes of SGs increased during the transitional and breeding seasons, and decreased during the non-breeding season, accordingly with the changes of plasma T concentrations. A significant positive correlation was found between plasma T concentrations and sizes of SGs in intact males during the transitional and breeding seasons. In contrast, SGs of castrated bears with basal T levels shriveled during the all seasons. Furthermore, immunohistochemical analysis revealed that SGs in back skins were positive for androgen receptors. In the experiment 2, to clarify the effect of T on the SGs directly, silicon tubes filled with testosterone were implanted subcutaneously in an intact male during the non-breeding season. T treatment resulted in the high T concentration and marked enlargement of SGs. These results suggest that T regulates the size of SGs in back skins of male brown bears.

Authors