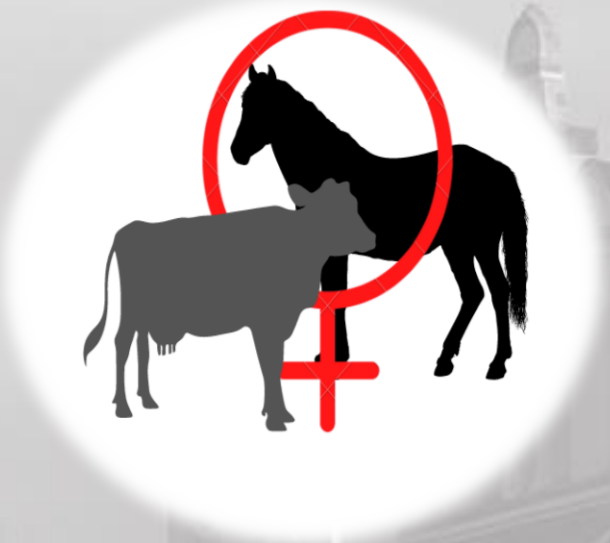
5th International Conference on Uterine Disorders in Farm Animals:

Endometritis as a cause of infertility in domestic animals

22nd – 25th June 2022, Cracow, Poland



Welcome to the Conference

Dear Colleagues,

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

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, 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.

The first edition was held in Olsztyn in 2013 under special EU program Regpot – project Refresh that was implemented to improve research standards of the Institute and integration with the European Research Area and regional development.
The second one, in Gdansk, in 2015 was carried out 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 organized with the funds from our next project KNOW: Leading National Research Center in Veterinary Sciences: "Healthy Animal – Safe Food".
The fourth conference, in Warsaw was a **joint Polish-Japanese Seminar entitled "Cutting edge of Reproductive Physiology - Key processes for birth of a new life"** and was held together with our partners from Japan.

This year, we meet in Cracow for the 5th edition and it gives me a great pleasure to welcome all the seventy participants from ten countries to this conference. Unusually, we meet after 3 and not 2 years, as due to COVID-19 pandemic we had to postpone the conference. Also new this time is the hybrid format of the conference, so we welcome not only all the participants gathered here, but also all of you connected with us remotely. This year's conference is also a satellite meeting of the ICAR 2020+2 taking place in Bologna, Italy starting on June 26th. Over the years we have gathered a group of regular participants in our conference and if we may say good friends with common passion for reproductive biology of domestic animals. We therefore really look forward to meeting you after such a long break and we are sure that we will have many exciting discussions.

We want to thank all of our invited speakers who have accepted our invitation to come to Cracow. 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 we want to thank:
Anna Szóstek-Mioduchowska, Karolina Łukasik, Beenu Moza Jalali, Magdalena Weidner - Glunde from the Department of Reproductive Immunology and Pathology, IAR&FR PAS and Monika Bugno - Poniewierska from the Department of Animal Genetics, Breeding and Ethology, University of Agriculture in Krakow.

The Conference is organized with support of IRZBŻ PAN, MEiN "Doskonała Nauka" and PAN "DUN".

The organizers of the conference strongly condemn Russia's military aggression against Ukraine. At this critical time, we express our solidarity with the Ukrainian people.

We wish all of you many fruitful discussions, meetings with old friends, establishing new partnerships and having a pleasant stay in Warsaw

Dariusz Jan Skarżyński, Tomasz Janowski

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

- MEiN “Doskonała Nauka” grant
- Polish Academy of Sciences - “DUN” grant
- Institute of Animal Reproduction and Food Research of Polish Academy of Sciences

GENERAL INFORMATIONS

The Conference Venue will be the Kossak Hotel (Plac Kossaka 1, Kraków), lectures will be held in the conference room at 6th floor.

FOOD SERVICE

Meals

Lunches will be provided with in Kossak Hotel Restaurant "Percheron" at lobby floor according to the schedule of the meeting.

Coffee Breaks

Coffee and cookies will be available in foyer close to the presentation room.

SOCIAL EVENTS

Welcome

Wednesday, 22nd June, 7:30 p.m. (free for registrants) at Kossak Hotel Restaurant "Percheron", later guided Cracow sightseeing tour

Gala Dinner

Thursday, 23rd June, 8:00- 11:00 p.m. (free for registrants). The Restaurant is located in walking distance from the Venue Hotel.

Restauracja Szara Gęś w Kuchni
Rynek Główny 17
Kraków

Arabian Horses State Stud

Saturday, 25th June 8:30 a.m – 3 p.m. Scientific excursion to Michalow Arabian Horses State Stud (free for registrants). Buss will stop in front Kossak Hotel.

5th International Conference on Uterine Disorders in Farm Animals:

Endometritis as a cause of infertility in domestic animals

Wednesday, June 22nd

14:00 – 15:00 Registration

14:45 – 15:00 Opening Ceremony – **Prof. J. JANOWSKI & Prof. D. SKARZYŃSKI**

Preconference Session: ***Genetic and biotechnological aspects of equine reproductive health – in memory of Prof. William Twink Allen***

Chairman: Prof. Amanda DE MESTRE
Chairman: Prof. Graca FEREIRRA-DIAS

15:00 – 15:30 **Prof. Marian TISCHNER** – in memory of Prof. William Twink Allen

15:30 – 16:00 Invited lecture: *It's not always the endometrium: genetic variants of the conceptus associated with pregnancy loss throughout gestation*
Prof. Amanda DE MESTRE

16:00 – 16:30 Invited lecture: *Mare Endometrosis: From histopathology to epigenetics*
Prof. Graca FERREIRA-DIAS

16:30 – 16:50 Coffee break

16:50 – 17:10 *Research trends in biotechnology of horse reproduction over the last two decades inspirated by Krakow-Cambridge cooperation - summary of own research*
J. KOCHAN, M. TISCHNER, A. OKÓLSKI, A. NOWAK, W. MŁODAWSKA, M. TISCHNER JR, J. GABRYŚ , M. BUGNO-PONIEWIERSKA

17:10 – 17:30 *Semiautomatic detection of local vasodilation based on two vascular measures in inflamed equine endometrium*
Ł. ZDROJKOWSKI, T. JASIŃSKI, A. NIWIŃSKA, B. PAWLIŃSKI, M. DOMINO

17:30 – 17:50 *Immunohistochemical expression of Myeloperoxidase in the equine endometrium (online presentation)*
S. PARRILLA-HERNÁNDEZ, F. Reigner, E. Feyereisen, C. Munaut, T. Franck and S. Deleuze

19:30 **''Welcome reception" with Cracow sightseeing tour**
At Kossak Hotel Restaurant

Thursday, June 23rd

08:00 – 09:00 Registration

09:00 – 09:45 **Plenary lecture – *Protecting the bovine endometrium against damage caused by pathogenic bacteria* (online lecture)**
Prof. Martin SHELDON

09:45 – 10:15 Coffee break

10:15-13:10 Session I: Pathophysiology of Uterine Functions

Chairman: Prof. Tal RAZ
Chairman: Prof. Claudia KLEIN

10:15 – 10:45 Invited lecture: *Postpartum ovarian and uterine functions associated with metritis and endometritis in dairy cows*
Prof. Tal RAZ

10:45 – 11:15 Invited lecture: *Excessive TAGLN and CCN2 expression in equine endometrial fibrotic glands*
Prof. Claudia KLEIN

11:15 – 11:30 Coffee break

11:30 – 11:50 *How EVs affect reproduction*
A. ANDRONOWSKA

11:50 – 12:10 *Transcriptomic analysis of mare endometrium reveals molecular changes in immune response and metabolism at different stages of endometrosis*
A. SZÓSTEK-MIODUCHOWSKA, A. WÓJTOWICZ, A. SADOWSKA, B.M. JALALI, M. SŁYSZEWSKA, K. ŁUKASIK, A. GURGUL, T. SZMATOŁA, M. BUGNO-PONIEWIERSKA, G. FERREIRA-DIAS, D.J. SKARZYNSKI

12:10 – 12:30 *The role of interleukin 13 in the development of endometrosis in the mare*
A. WÓJTOWICZ, A. SADOWSKA, FERREIRA-DIAS, A. SZÓSTEK-MIODUCHOWSKA

12:30 – 12:50 *Substrate stiffness modifies gene expression of equine endometrial fibroblasts in vitro*
E. ZU KLAMPEN, D. HERRMANN, C. KLEIN

12:50 – 13:10 *DNA methyltransferases in equine endometrial fibroblasts*
J. ALPOIM-MOREIRA, A. WÓJTOWICZ, A. SADOWSKA, A. SZOSTEK-MIODUCHOWSKA, M.R. REBORDÃO, D.J. SKARZYNSKI, G. FERREIRA-DIAS

13:10 – 14:10 Lunch

14:25 – 15:00 Around Animal Reproduction - Poster session - Flash Talk

14:25 – 14:30 *The effect of interleukin 6 on mRNA expression of fibrotic markers in equine endometrial fibroblasts*
E. ŻEBROWSKA, G. FERREIRA-DIAS, A. SZÓSTEK-MIODUCHOWSKA

14:30 – 14:35 *Genetic polymorphism of NOTCH4 gene is associated with anestrum in cows*
K. Gh. M. MAHMOUD, S. IBRAHIM, A. SOSA, H. R. H. DARWISH, E. A. ALMADALY, D. E. ILIE, M. H. HASANAIN, M. F. NAWITO

14:35 – 14:40 *EGF regulates expression of extracellular matrix proteins in porcine endometrium through STAT3 activation*
P. LIKSZO, K. ŁUKASIK, B.M. JALALI

14:40 – 14:45 *Changes in transcriptome profile in equine corpus luteum during early pregnancy,*
K. LUKASIK, M.B. JALALI, A. BACLAWSKA, A. GURGUL, M. BUGNO-PONIEWIERSKA, D. J. SKARZYNSKI

14:45 – 14:50 *Expression pattern of apoptotic genes in corpus luteum during thermal stress in Egyptian buffaloes,*
S.M. GALAL, S. IBRAHIM, K. MAHMOUD, O. ADEL, A. A. SHOKRY, El-Belely M S, Ismail S T

14:50 – 14:55 *Gene expression and interaction between aryl hydrocarbon receptor, interleukin 17 and transforming growth factor β1 in equine endometrial fibrosis,*
A. SADOWSKA, A. WÓJTOWICZ, E. ŻEBROWSKA, M. SŁYSZEWSKA, G. FERREIRA-DIAS, A. SZÓSTEK-MIODUCHOWSKA

14:55 – 15:00 *Possible factors which determine testosterone levels in Japanese black bears*
J. TOMIYASU, M. KAYANO, K. HAZANO, M. MATSUI, Y. NEMOTO, T. NAGANUMA, S. KOIKE, K. YAMAZAKI

15:00 – 18:20 Session II: Effects of uterine disorders in reproductive health of farm animals

Chairman: Prof. Christine AURICH
Chairman: Prof. Wojciech BARAŃSKI

15:00 – 15:30 Invited lecture: *Association of the breeding induced endometrial response with subsequent fertility in mares*
Prof. Christine AURICH

15:30 – 16:00 Invited lecture: *Reproductive tract diseases – endometritis and what else?*
Prof. Wojciech BARAŃSKI

16:00 – 16:20 *Effect of heat stress on the occurrence of endometritis in dairy cows*
M. TEKIN, C. GUSE, M. IWERSEN, M. DRILLICH, K. WAGENER

16:20 – 16:40 *Hastening first postpartum ovulation in early lactation may reduce the incidence of endometritis in dairy cows*,
S. A. Druker, R. SICSIC, M. KEDMI, A. KAPLAN, T. RAZ

16:40 – 17:00 Coffee break

17:00 – 17:20 *Nucleolar size of large luteal cells increases in mares with endometritis*
M. J. ESTRADÉ, F. PEREYRA MONTANTS, S. CASTRO, N. DZIUGYS, C. LARRANAGA, B. VARELA, R.C. MATTOS, G. PEDRANA

17:20 – 17:40 *Circulating and endometrial polymorphonuclear leukocyte function dynamics in postpartum dairy cows with subclinical or clinical endometritis*
L. LIETAER, **O. BOGADO PASCOTTINI**, S. HEIRBAUT, K. DEMEYERE, L. VANDAELE, E. MEYER, V. FIEVEZ, J. LEROY, G. OPSOMER

17:40 – 18:00 *The vascular and perivascular morphometric features and their correlations in equine endometrium affected by endometrosis*
T. JASIŃSKI, Ł. ZDROJKOWSKI, K. SIEWRUK, B. PAWLIŃSKI, M. DOMINO

18:00 – 18:20 *Inflammation effect on the endometrial percentage of reproductive hormone receptors and the height of the superficial and glandular epithelium in anestrus type II dairy cows*
M. TRELA, Ł. ZDROJKOWSKI, D. DOMAŃSKA, O.WITKOWSKA-PIŁASZEWICZ, K. SIEWRUK, B. PAWLIŃSKI, M. DOMINO

20:00 – 23:00 **Gala dinner – *Restauracja Szara Gęś w Kuchni***

Friday, June 24th

09:00 – 09:45 **Plenary lecture – *How do viruses cross the porcine endometrium to reach embryos/fetuses and cause reproductive failure*?**
Prof. Hans Nauwynck

09:45 – 10:15 Coffee break

10:15 – 13:00 Session III: The role of bacteria and viruses for the pathogenesis of uterine disorders

Chairman: Prof. Rodolfo DE LA SOTA
Chairman: Prof. Marc DRILLICH

10:15 – 10:45 Invited lecture: *Carryover effect of uterine diseases on subsequent pregnancy losses in lactation*
Prof. Rodolfo DE LA SOTA

10:45 – 11:15 Invited lecture: *Research in bovine endometritis – are we asking the best questions?*
Prof. Marc DRILLICH

11:15 – 11:30 Coffee break

11:30 – 11:50 *The most common pathogens isolated from the uterus in mares before the breeding season in 2018-2020 in the Mazovia region of Poland*
D. DOMAŃSKA, O. WITKOWSKA-PIŁASZEWICZ, M. TRELA, B. PODESZEWSKI, M. DOMINO

11:50 – 12:10 *Growth characteristics of Trueperella pyogenes in an uterine in vitro model*
M. IBRAHIM, K. WAGENER, M. DRILLICH, M. EHLING-SCHULZ, C. GABLER

12:10 – 12:30 *Relationship between cultivable aerobic microbiota in the uterus and oviduct of postpartum dairy cows*
K. WAGENER, L. NEUBRAND, H. AWWAD, M. TEKIN, H. POTHMANN, V. HAVLICEK, U. BESENFELDER, M. EHLING-SCHULZ, M. DRILLICH

12:30 – 12:50 *The effect of uterine lavage on the isolation of bacteria in mares with subclinical endometritis*
M. SIKORA, J. BUCZKOWSKA, R. KOZDROWSKI, J. KRÓL, M. MARCINEK

12:50 – 13:10 *Isolation and host range determination of bacteriophages specific to selected equine uterine pathogen (online presentation)*
M. KÖHNE, S. KITTLER, M. PLÖTZ, H. SIEME

13:10 – 14:10 Lunch

14:25 – 15:00 Around Animal Reproduction 2 - Poster session - Flash Talk

14.25 – 14.30 *Application of the microflow technique for the selection of stallion semen*
M. BUGNO-PONIEWIERSKA, J. KOCHAN

14:30 – 14:35 *Endometrial miRNA expression profile during pre-implantation period of pregnancy in the mare*
A. SADOWSKA, T. MOLCAN, A. WÓJTOWICZ, K. ŁUKASIK, K. PAWLINA-TYSZKO, A. GURGUL, T. SZMATOŁA, M. BUGNO-PONIEWIERSKA, G.FERREIRA-DIAS, D. J. SKARZYNSKI, A. SZÓSTEK-MIODUCHOWSKA

14:35 – 14:40 *IL-6 induced STAT3 activation increases VEGF expression and capillary formation in porcine endometrial endothelial cel*
B. M. JALALI, K. LUKASIK

14:40 – 14:45 *ADAMTS metalloproteases and their regulation by transforming growth factor-β1 in mare endometrium*,
M. SŁYSZEWSKA, E. ŻEBROWSKA, A. WÓJTOWICZ, A. SADOWSKA, G. FERREIRA-DIAS, A. SZÓSTEK-MIODUCHOWSKA

14:45 – 14:50 *New insight into pregnancy maintenance in she-camel: Molecular interaction among ovulatory site, uterine body and uterine horns*
O. ADEL, S. IBRAHIM, K. MAHMOUD, S.M GALAL, M. FATHI, A.A.M. SEIDA

14:50 – 14:55 *Mammary gland carcinoma in a dog caused the hypertrophic osteopathy*,
O. WITKOWSKA-PIŁASZEWICZ, D. DOMAŃSKA, M. TRELA, B. PAWLIŃSKI

14:55 – 15:00 *Genotyping of NOTCH2 gene in fertile and anestrum cows*,
K. Gh. M. MAHMOUD, A. S.A. SOSA, H. R. H. DARWISH, S. IBRAHIM, M. H. HASANAIN, A. M. SAKR, A. Sh. E. SHAMS, D. E. ILIE Y.F. AHMED

15:00 – 18:00 Session IV: Methods of diagnosis and therapy of uterine disorders in farm animals

Chairman: Prof. Igor CANISSO
Chairman: Prof. Geert OPSOMER

15:00 – 15:30 Invited lecture: *Persistent Breeding-Induced Endometritis in Mares — A Multifaceted Challenge: From Clinical Aspects to Immunopathogenesis and Pathobiology*
Prof. Igor CANISSO

15:30 – 16:00 *Invited lecture: An overview of diagnosis and treatment protocols for uterine diseases in cattle*
Prof. Geert OPSOMER

16:00 – 16:20 Coffee break

16:20 – 16:40 *Comparison of cytological, microbiological and histopathological findings of genital canal in healthy cows and cows with vestibulo-vaginal sphincter dysfunction*
E.S. ÖZDEMIR-SALCI, Ö.YAVAŞ, Ö.YILMAZ, G.SÖNMEZ, S.KAHYA-DEMIRBILEK, K.SEYREK-İNTAŞ

16:40 – 17:00 *A new method of cytological sampling from the endometrium of dairy cows*
D. TOBOLSKI, Z. POLAK, M. KUPA

17:00 – 17:20 *The role of microRNAs in endometritis and their potential as a diagnostic biomarker*" *(online presentation)*
S. IBRAHIM, M. O. TAQI, K. Gh. M. MAHMOUD, M. F. NAWITO

17:20 – 17:40 *Endometritis in breeding mares from Spain: Microbial prevalence and antimicrobial susceptibility (online presentation)*
A. DORREGO, M. PÉREZ-SANCHO, M. UGARTE RUIZ, P. GAGO, E. CAMINO, A. BUENDÍA, L. DE JUAN, F. CRUZ-LÓPEZ

17:40 – 18:00 *Cows with subclinical endometritis show lower IL1RA / IL1B ratio in uterine secretions detectable in vivo and at the abattoir (online presentation)*
A. KNEIDL, S. KIRSCH, F. WEBER, A. HELFRICH, S. SCHABMEYER, J. SCHNEIDER, W. PETZL, H. ZERBE, **M. M. MEYERHOLZ**

18:00 **Closing Ceremony, Awarding of best presentation and poster**

Saturday, June 25th

08:30 **Scientific excursion to Michalow Arabian Horses State Stud**

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Ministerstwo
Edukacji i Nauki

INVITED LECTURE

It's not always the endometrium: genetic variants of the conceptus associated with pregnancy loss

A. M. DE MESTRE[1], C. SHILTON[1], J. ROACH[1], A. KAHLER[1], R. MOUNCEY[2], D. C. WATHES[2], B. DAVIS[3], T. RAUDSEPP[3]

[1]*Department of Comparative Biomedical Sciences*
[2]*Department of Pathobiology and Population Sciences, The Royal Veterinary College, London, United Kingdom*
[3]*College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA*
Corresponding author email: adamestre@rvc.ac.uk

Embryonic and fetal loss remain one of the greatest challenges in reproductive health with 5 - 10% of established day 15 pregnancies and subsequently 5% of day 70 pregnancies failing to produce a viable foal. The underlying reason for these losses is variable but ultimately most cases will be attributed to a primary pathology of the mare (such as endometrial pathology, endocrine function, immunopathology and oocyte characteristics) or the embryo/feto-placental unit (inherited from the stallion or mare via the germline or acquired during development). In both cases, defects could be intrinsic to the tissue or alternatively the response of that tissue to extrinsic factors such as pathogens, nutrients, and environmental contaminants. Whilst previous research has focused on both intrinsic and extrinsic factors that impact the environment in which the embryo develops, surprisingly little is known about defects intrinsic to the embryo. Both large and small genetic variants intrinsic to the conceptual tissues are commonly associated with miscarriage in women but to recently little was known about their role in fetal viability in the mare.

In order to address this gap, we set out to develop a bank of fetal and placental tissues from three phenotypes: Early Pregnancy Loss ((EPL); 15 to < 70 days gestation), abortion (70 to 300 days gestation) and stillbirth (300 days to birth). DNA isolated from both allantochorion and/or fetus was hybridized to Axiom Equine 670K or GGP 70K SNP Genotyping Arrays allowing us to explore a number of candidate variants including aneuploidy, Copy Number Variation (CNVs) and more recently Single Nucleotide Polymorphisms (SNPs).

Combining our published (Shilton et al, 2020) and unpublished data, we have identified autosomal aneuploidy as the most common lethal variant occurring in 20.6% (95%CI 12.0-31.6%) of EPLs (15/73). A single case of partial trisomy 6 was identified in abortion (1/104) and a partial trisomy/monosomy in a stillbirth (1/24). Our analysis of CNVs revealed a significant genome enrichment of CNVs in the allantochorion but not fetuses of EPLs when compared with age matched viable pregnancies suggesting premature acquisition of CNV in the placenta may be sufficient to cause a genetic imbalance and disrupt early development. Additionally, specific CNVs containing key developmental genes were uniquely found in the fetus and/or placentae of two or more EPLs but were absent in all viable individuals supporting an additional role for specific microdeletions. Collectively this data suggests large genomic variants are significant contributors to lethality in the first two months of gestation but can also lead to lethality in mid to late pregnancy. Future work is focusing on SNPs inherited through the germline that may contribute to these phenotypes.

INVITED LECTURE

Mare Endometrosis: From histopathology to epigenetics

G. FERREIRA-DIAS[1,2], M. R. REBORDÃO[1,2,3], J. ALPOIM-MOREIRA[1,2], A. AMARAL[1,2,4], A. Z. SZÓSTEK-MIODUCHOWSKA[5], D. J. SKARZYNSKI[5,6]

[1]*CIISA-Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;*
[2]*Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS);*
[3]*Polytechnic of Coimbra, Coimbra Agriculture School, Coimbra, Portugal*
[4]*Faculty of Veterinary Medicine, Universidade Lusófona, Lisboa, Portugal*
[5]*Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland,*
[6]*Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Poland*

Histopathological alterations in the mare endometrium, characterized by irreversible chronic degenerative changes, mainly the deposition of collagen fibres, were first described by Kenney in the late 70´s. This disease, later named endometrosis, has been a major concern for the horse industry, being responsible for large economic losses due to infertility. The only available standardized approach to diagnose endometrosis is the histopathological grading of endometrial biopsy according to Kenney and Doig. This system considers several criteria, as inflammation and fibrosis, among others. However, interpretation of histopathological lesions in the endometrial biopsy may not be consensual. Currently, studies are being conducted to establish blood-markers of endometrosis, as non-invasive diagnosis methods. Thus, our last study showed that in mares with endometrosis, blood collagen type 3 might be a useful biomarker, as a complementary less invasive diagnostic approach of fibrosis and as a fertility predictor. But, when the precise diagnosis of endometrosis is reached, no efficient treatment for this disease is currently available.

Deep knowledge of the pathogenesis of endometrosis is crucial to reach its cure. From earlier times, it is known that soon after sperm gets into the uterine lumen, a post breeding endometritis (PBE), which is a physiological event in the mare, driven by pro-inflammatory cytokines/chemokines, develops and attracts immune cells. Despite decades of research, it is controversial what drives some mares´ endometrium to develop endometrosis. Mares´ aging, but not so much parity, predisposes to deficient immune system function or tissue remodeling capacity that impairs extracellular matrix (ECM) homeostasis and facilitates fibrogenesis. Neutrophils are the major inflammatory cells of the innate immune system to first arrive at the mare uterus after breeding to phagocytize damaged cells, spermatozoa or microorganisms and fight endometritis. In addition to the classical antimicrobial host defense mechanisms of neutrophils**,** they release DNA strands entangled by proteins from their cytoplasm and nucleus to the surroundings, forming Neutrophil Extracellular Traps (NETs). This phagocytosis-independent system of pathogen destruction also occurs in the mare endometrium, when challenged by bacteria causing endometritis. Besides the antimicrobial role of the proteins present in NETs (histones, myeloperoxidase, cathepsin G, and elastase), they might provoke a deleterious effect on the endometrium of cyclic mares by stimulating collagen formation and fibrosis establishment, whose severity depends on endometrium histopathological grade and estrous cycle stage. Promising data from the *in vitro* use of specific inhibitors of enzymes present in NETs might be an important contribute to develop endometrosis therapeutical means.

Endometrosis could develop when PBE does not resolve timely and turns into a prolonged PBE, leading to persistent neutrophilia with NETs formation and collagen deposition, showing the connection between inflammation and fibrosis. As such, persistence of endometritis, flips the inflammatory uterine milieu to a fibrotic environment, resulting in endometrosis establishment. In the mare endometrium, resident fibroblasts differentiate into myofibroblasts responsible for collagen production and fibrogenesis, by action of pro-fibrotic cytokines, growth factors, and other proteins secreted by inflammatory cells and damaged cells. The pro-fibrotic cytokines affect metalloproteinases (MMPs) and their tissue inhibitors (TIMP). As such, endometrosis severity may be modulated by TGF-β1, which induces *in vitro* myofibroblast differentiation, up-regulates the transcription of ECM components and alpha smooth muscle actin (*αSMA*), mediated by MMPs and TIMPs. In addition, in mare endometrium, the expression of several interleukins, cytokines and growth factors, such as connective tissue growth factor (CTGF), pro-inflammatory monocyte chemoattractant protein-1 (MCP-1), and nuclear factor kappaB (NF-κB) has been associated with the presence of inflammatory cells (neutrophils, eosinophils, lymphocytes), and with the establishment of endometrosis.

Prostaglandins have also been involved in fibrogenesis modulation. As in other tissues, also in the mare endometrium, while $PGF_{2\alpha}$ stimulates fibrogenesis, PGE_2 inhibits fibrosis, up-regulates MMP-2 and MMP-9 transcription, and down-regulates MMP-13. Thus, NETs enzymes might induce endometrial collagen deposition in mare endometrium through $PGF_{2\alpha}$ fibrogenic action, while the anti-fibrotic role of PGE_2 is suppressed. A vicious cycle is created when equine spermatozoa in contact with the mare endometrium trigger $PGF_{2\alpha}$ release, subsequent neutrophil chemotaxis into the uterus and NETs formation that further stimulate $PGF_{2\alpha}$ production.

The progression of fibrosis has lately been ascribed to epigenetic modifications that affect gene expression profiles, but not the DNA sequence. Since epigenetic alterations can be reversed, in contrast to genetic changes, this was evaluated in mare endometrium, as a promising therapeutic tool for endometrosis. Methylation of DNA is one of the most studied epigenetic events in mammals. In the most severe stage of endometrosis (category III), DNA methylation enzymes (DNMTs) and collagen are altered. Thus, fibrogenesis in mare endometrium might be related to downregulation of anti-fibrotic genes and an increase in DNMTs expression. It is known that the aging process in mares increases the severity of endometrosis. This finding might also be explained by epigenetic changes, since *DNMT3B* transcripts raise, and the correlation between *DNMTs* and types of collagens changes in the equine endometrium. Nevertheless, DNA methylation is a broad process. As such, assessment of specific methylation sites of DNA methylation (CpG islands) in mare endometrium is crucial to get a precise information of which gene is methylated. We have shown that epigenetics modulation play an important role in endometrosis pathogenesis, by inhibiting anti-fibrotic genes (*MMP2* and *MMP9*), rather than activating fibrotic genes. The disturbed uterine milieu in the presence of a persistent PBE, and epigenetic changes also associated with aging in mare endometrium might be involved in endometrosis development. Therefore, epigenetic manipulation and specific inhibitors might be promising targets for therapeutic use in mare endometrosis. However, it still lacks a complete understanding of endometrosis pathogenesis, as the basis for therapy, either aiming at NETs components or choosing the epigenetics approach.

Funded by the bilateral Polish-Portugal research project under the agreement of MS&HE and FCT (NAWA project PPN/BIL/2018/1/00250?u/0001) and LA/P/2020-AL4AnimalS.

Research trends in biotechnology of horse reproduction over the last two decades inspirated by Krakow-Cambridge cooperation - summary of own research

J. KOCHAN, M. TISCHNER, A. OKÓLSKI, A. NOWAK, W. MŁODAWSKA, M. TISCHNER JR, J. GABRYŚ, M. BUGNO-PONIEWIERSKA

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The present abstract is a brief overview of research on developing assisted reproduction technology (ART) in horses over the last two decades period at the Universty of Agriculture in Krakow, in Department of Animal Reproduction. Much of our research were insprated by cooperation with Prof. Twink Alen and could be described as 'embryo-focused'. At the beginning of the 21st century, the main focus of our interest was embryo transfer. Then our most important goal to this day has been obtaining of equine embryos in vitro using different methods (ICSI, cloning, parthenogenetic activatin). In the meantime, a scientific trend in biotechnology of horse reproduction became cryopreservation of embryos and then oocytes. Five years ago, our team has acquired outstanding geneticists and we have started a new chapter in our research. We are currently investigating the genetic basis of the formation of the embryonic capsule in horses. We are improving the embryo biopsy procedure for embryo genetic diagnostics. Finally, we are trying to improve the efficiency of in vitro maturation of mare oocytes adding extracellular vesicles. Our whole team is fortunate to have been a part of the early development of horse ART and continue progress in reproductive biotechnolgy in horses.

Semiautomatic detection of local vasodilation based on two vascular measures in inflamed equine endometrium

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Endometritis is crucial problem in equine reproduction. Chronic endometritis may harder to diagnose than acute, however it decreases fertility in mares. The study aimed to compare selected vascular measures in equine endometrium concerning the intensity and type of equine endometritis. Endometrial samples (n=32) were collected, fixed, cut into slices and stained by hematoxylin-eosin (HE) and immunohistochemistry (IHC) with primary, secondary antibodies and NuclearGreen or Hoechst. HE-stained slides were scanned with a semiautomatic brightfield system (TissueFaxsPlus), and the vessel area (VA, nm²) and vessel perimeter (VP, nm) were quantified. IHC-stained slides were examined under confocal microscope LeicaTCSSP8 (qualitative) and TissueFaxsPLUS (quantitative). Immune cells differentiating was based on expression of CD45/CD66 (granulocytes, GRA), CD45/CD14 (macrophages, MAC), CD45/CD3 (lymphocytes, LYM) end expressed as percentage of all detected cells. Samples were assigned to four groups (n=8, each) representing: healthy endometrium (C, control), and mild (+), moderate (++), and severe (+++) endometritis. Kruskal-Wallis test was used to compare measures (mean±SD) between groups. Studied groups differed in LYM (C:0.27±0.09%; +:2.23±1.24%; ++:5.66±6.04%; +++:14.25±11.50%; $p<0.0001$) but not GRA (C:0.17±0.22%; +:0.75±0.49%; ++:0.55±1.03%; +++:0.69±0.42%; $p=0.055$) and MAC (C:0.89±0.45%; +:0.80±0.20%; ++:0.88±0.64%; +++:0.91±0.19%; $p=0.764$). Thus the chronic endometritis was recognized. Vessel measures differed between groups in area (C:1469±1015 nm²; +:2114±1111 nm²; ++:2367±1920 nm²; +++:2104±2097 nm²; $p=0.014$) but not perimeter (C:238±310 nm; +:161±121 nm; ++:158±109 nm; +++:348±2505nm; $p=0.185$), probably due to SD > mean in VP values. Thus the inflammation-dependent local vasodilation was recognized, based on rather VA than VP. The scanning cytometry-based semiautomatic vessel area detection may be considered more effective than the detection of vessel perimeter in the case of vascularization determination in equine inflamed endometrium.

Immunohistochemical expression of Myeloperoxidase in the equine endometrium

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Myeloperoxidase (MPO) is a marker of neutrophil activation. However, in the uterine lumen of mares with negative cytological results, MPO was constantly detected at variable concentrations. The aim of this study was to investigate whether MPO is present in the equine endometrium in physiological conditions to better understand the origin and the role of this enzyme in uterine immunity. Endometrial biopsies (n=11) obtained from mares in estrus with histological detection of three or less neutrophils per field (400x) were studied. Immunohistochemical analysis using MPO-specific horse antibody was performed. MPO was detected in endometrial epithelial cells as well as in secretory products within glandular lumen in all mares. Middle and basal glands presented maximal MPO immunostaining with a predominant intracytoplasmatic apical reinforcement. In the adluminal epithelium, only some areas were immunopositive in which cells with different staining intensities were mixed with immunonegative cells. In some mares presenting neutrophils in sub and transepithelial areas, a uniform diffuse intracytoplasmic immunostaining was observed. The immunostaining of the luminal epithelium may be a consequence of MPO internalization, while the cytoplasmic apical labeling suggests synthesis and secretion of MPO by endometrial glands. Our results show a constitutive presence of MPO in the mare's endometrium during estrus suggesting a contribution of the enzyme to the mucosal immune system. Further studies are necessary to confirm MPO synthesis and/or internalization by equine endometrial cells and its detailed roles.

Protecting the bovine endometrium against damage caused by pathogenic bacteria

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Bacterial infections of the uterus cause metritis or endometritis in up to 40% of postpartum dairy cattle. Metritis and endometritis are important because they result in delayed conception or infertility. The annual cost of treatment, lost milk production and replacing infertile animals is about $2 billion across the EU and US. However, resilient cows remain healthy and fertile, even when exposed to the same pathogenic bacteria.

Resilience to bacterial infections depends on immunity and tolerance. Immunity and inflammatory responses help clear pathogens from the uterus. Tolerance is the ability to cope with tissue and cell damage caused by the pathogens or by excessive inflammation. Immunity and inflammatory responses to pathogens are well characterized in the bovine endometrium. However, less is known about tolerance to the damage caused by bacteria in the endometrium.

Many species of pathogenic bacteria damage cells by secreting toxins that form pores in their plasma membrane. Membrane pores enable the delivery of other bacterial virulence factors into cells, result in the leakage of cytosolic molecules that bacteria can use as nutrients, and facilitate pathogen invasion. Endometrial pathogenic bacteria that secrete pore-forming toxins include *Trueperella pyogenes*, *Staphylococcus aureus* and *Escherichia coli*.

Trueperella pyogenes is the pathogen most associated with the severity of endometritis and infertility in dairy cattle. *Trueperella pyogenes* secretes pyolysin. Pyolysin is a cholesterol-dependent cytolysin that forms pores in cholesterol-rich areas of the plasma membrane. Endometrial stromal cells are particularly sensitive to pyolysin, which is important because the protective epithelium is lost after parturition. Therefore, there is interest in protecting endometrial cells against pore-forming toxins.

Cytolysin accessible cholesterol in plasma membranes is essential for binding of cholesterol-dependent cytolysins. We first considered whether reducing cellular cholesterol could protect against pyolysin. Cellular cholesterol was reduced using three approaches: (a) cyclodextrins to bind cholesterol; (b) activators of cellular cholesterol efflux; and (c) inhibitors for enzymes in the cholesterol biosynthesis pathway. All three approaches protected cells against pyolysin-induced cell damage. The treatments reduced pyolysin-induced leakage of potassium ions and lactate dehydrogenase protein from cells, reduced cytoskeletal changes, and prevented cytolysis.

The next consideration was whether physiological regulators of accessible cholesterol in the plasma membrane could alter cell-intrinsic protection against pore-forming toxins. Accessible cholesterol in plasma membranes is regulated by oxysterols, which are oxidised forms of cholesterol. Using mass spectrometry, we found the oxysterols 27 – hydroxycholesterol and 25 – hydroxycholesterol in peripheral plasma, uterine fluid, and ovarian follicular fluid. Furthermore, endometrial epithelial cells released additional 25-hydroxycholesterol in response to a pyolysin challenge. Treatment with 27-hydroxycholesterol or 25-hydroxycholesterol protected endometrial epithelial and stromal cells against pore formation and the damage caused by pyolysin. Treatment with 27-hydroxycholesterol also protected endometrial cells against *Staphylococcus aureus* α-hemolysin. The oxysterols limited pyolysin-induced leakage of potassium and lactate dehydrogenase from cells, reduced cytoskeletal changes, and prevented cytolysis. Oxysterol cytoprotection against pyolysin was partially dependent on acyl-coenzyme A:cholesterol acyltransferase (ACAT) reducing cytolysin-accessible cholesterol in the plasma membrane. In addition, oxysterol cytoprotection was partially dependent on activating liver X receptors, which stimulate cellular cholesterol efflux. Collectively, these findings imply that oxysterols may help defend the endometrium against pathogenic bacteria.

In conclusion, tolerance of damage caused by bacterial infections is important for resilience to pathogens in the endometrium. The mechanisms of tolerance are underexplored but are important for developing new strategies to prevent and treat disease. Our findings imply that reducing accessible cholesterol in the plasma membrane is a cell-intrinsic mechanism of tolerance. These finding could lead to pharmaceuticals to enhance endometrial tolerance to pathogens. *Funding: BBSRC (BB/K006592/1) and NIH (R01HD084316).*

INVITED LECTURE

Postpartum ovarian and uterine functions associated with metritis and endometritis in dairy cows

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Postpartum uterine inflammatory diseases are among the most prevalent diseases in bovine dairy herds worldwide, resulting in major economic losses, mainly through decreased reproductive performance and milk production. Metritis is an inflammatory condition of the uterus, which in Israel is diagnosed at a routine clinical examination of all dairy cows at 5-12DIM (days-in-milk). Clinical findings include fetid mucopurulent vaginal discharge and palpation of large, gas, or fluid-filled uterus. The existence of additional systemic clinical signs (e.g., fever, depression) leads to a diagnosis of septic metritis. Cytological Endometritis (CEM) is diagnosed from 21DIM onward by evaluating the percentage of polymorphonuclear cells (PMN) in endometrial cytology obtained typically by cytobrush. Previous studies suggest possible links between postpartum ovarian function and uterine health.

In the last few years, we have conducted several studies to explore the pathophysiology and risk factors of metritis and endometritis. In primiparous cows, metritis was associated with delayed postpartum resumption of ovarian activity, while in multiparous cows, metritis was associated with their metabolic status but not with ovarian activity. Interestingly, induction of ovarian activity by hormonal treatment early in lactation was associated with a reduced incidence of CEM later in lactation.

At 5-12 DIM, histological analysis of uterine biopsy samples indicated differences in the epithelium integrity, bacterial invasion, and PMN infiltration between metritis and healthy cows. However, luminal and mucosal bacterial load, as measured by 16S qPCR, did not differ between healthy and metritis cows. Uterine cytological analysis at 5-10DIM revealed that in most cows, mucosal PMN% was either very low or very high regardless of disease status. However, PMN% was found to be related to bacterial load. Metagenomic analysis of 16S rRNA gene sequencing revealed that in metritis cows, there was a typical bacterial community with higher relative abundances of the phyla Bacteroidetes and Fusobacteria. This community was composed mainly of the genera Bacteroides, Porphyromonas, Fusobacterium, and Tissierellaceae spp. In contrast, the bacterial community composition of healthy cows was more diverse. However, some healthy cows had a 'metritic-like' bacterial community, with no clinical signs of metritis, suggesting differences in uterine response. Analysis of gene expression by mRNA-seq methods indicated 152 differentially-expressed genes (DEGs) found when comparing metritis to healthy cows; most were immune-related DEGs.

In a longitudinal study, we found a significant decrease in uterine bacterial load over time (5-12DIM → 30-40DIM → 60-70 DIM). However, metritis in early lactation was a significant but not obligatory risk factor for the development of CEM later in lactation. In addition, we found that optimally, primiparous and multiparous cows should be diagnosed for CEM by different %PMN thresholds and sampling timings (primiparous: 30-40DIM, a threshold of ≥7%PMN; multiparous: 60-70DIM, a threshold of ≥4%PMN); such approach provides a better prediction of reproductive prognosis. 16S rRNA gene sequencing analysis could not identify a specific bacterial community or differences in uterine bacterial load between CEM and healthy cows at 30-40DIM and 60-70DIM. However, expression by mRNA-seq between CEM cows and healthy cows yielded many differentially-expressed genes, most of them inflammatory gens, that can be attributed to the presence of luminal PMN.

Further understanding of the risk factors and the pathophysiology of uterine inflammatory diseases may enable the development of new preventive and therapeutic approaches, potentially improving cows' health and welfare and dairy herds' economy.

INVITED LECTURE

Excessive TAGLN and CCN2 expression in equine endometrial fibrotic glands,

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Endometrial fibrosis is a common problem in mares, resulting in subfertility. While the extent of degenerative changes is associated with increased age of the affected mare, the pathogenesis of this disease is unknown. A hallmark of endometrosis is markedly increased endometrial stromal and peri-glandular fibrosis resulting in the formation of "nested glands". The myofibroblast is the central player of fibrosis and serves as the primary collagen-producing cell within fibrotic tissue when activated. To date, the underlying mechanism of the transition of fibroblast to myofibroblast in equine endometrial fibrosis is unknown. The aim of the current study was to localize expression of transgelin (*TGLN*), an early marker of smooth muscle differentiation, cellular communication network factor 2 (*CCN2*), previously known as connective tissue growth factor, platelet derived growth factor beta (*PDGFB*) and Milk fat globule-EGF factor 8 protein (MFGE8) in equine fibrotic endometrium using in situ hybridization. CCN2, PDGFB and MFGE8 are proteins implicated in the pathogenesis of fibrotic conditions. Pronounced staining was evident for TGLN for the majority, but not all fibrotic glands, similar results were found for CCN2. A subset of fibrotic glands was also positive for MFGE8.

How EVs affect reproduction.

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Extracellular vesicles (EVs) are nanosized membrane-enclosed compartments that mediate cell-to-cell communication, both in normal physiology and in pathological conditions and are secreted by nearly all cel types. EVs, which are present in seminal, follicular, oviductal, and endometrial fluids, as well as in embryo secretions, carry molecular constituents that impact gamete maturation, fertilization, early embryo development, and embryo-maternal communication. The distribution, concentration, and molecular cargo of EVs are regulated by steroid hormones and the health status of the tissue of origin, and thus are influenced by menstrual phase, stage of conception, and the presence of infertility-associated diseases. Our observation showed that bovine follicular fluid and FF-derived EVs can induce changes in the gene expression of the bovine oviductal cells which, although observed in vitro, may be reflective of in vivo responses. Moreover, using *in vitro* embryo-maternal cross-talk model we present the evidence of non-contact transfer of embryonic RNA transcripts to endometrium. We also found that the concentration and size of EVs released by preimplantation bovine embryos are influenced by embryo quality and may indicate their prospective development potential. Therefore if EVs can be used as a biomarkers, could contribute to a better understanding of fertility disorders.

Transcriptomic analysis of mare endometrium reveals molecular changes in immune response and metabolism at different stages of endometrosis

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Endometrosis is a degenerative chronic condition of the equine uterus, defined as a fibrotic process that develops around the endometrial glands and/or in the stroma. Equine endometrosis leads to changes in the uterine microenvironment and early pregnancy loss. In our study, we aimed to carry out a global analysis of mRNA using high throughput mRNA sequencing of mare endometrium at different stages of endometrosis. Additionally, we investigated the action of the potential regulators of the expression of altered genes in endometrial cells *in vitro*. In Exp. 1., uteri were collected *post-mortem* from cyclic mares at the follicular phases of estrous cycle (n=36) at a local abattoir. The endometrial tissues were divided into four groups (n=9 for each) according to Kenney and Doig´s categories: I, II A, II B, III of endometrium classification. Isolated RNA was used for library construction using the TruSeq RNA Sample Prep v2 kit. In Exp. 2., fibroblasts isolated from healthy category I (n=5) and endometrosis category IIB (n=5) endometrium at the follicular phase of the estrous cycle were treated with TGF-β1 (10 ng/ml) or IL-17 (10 ng/ml) for 48h. Then, the transcription of DEG and fibrotic markers were determined using qPCR. In the comparison of the transcriptomes of endometrial tissue in categories IIA *vs* I, 61 genes were up-regulated and 205 genes down-regulated. In category IIB, in contrast to category I, 677 genes were up-regulated and 547 genes down-regulated. In category III *vs* category I, 15 genes were up-regulated and 24 genes down-regulated. In category IIA endometrial tissue, the predicted activation of DEGs was annotated to processes, such as inflammatory response, organismal injury and abnormalities, lipid metabolism, small molecule biochemistry, hematological system development and function, and tissue morphology. In category IIB endometrial tissue, the predicted activation of DEGs was annotated to processes, such as organismal injury, and abnormalities, cell-to-cell signaling and interaction, inflammatory response. Transforming growth factor-β1 and IL-17 affect DEG expression on mare endometrium depend on the presence and stage of endometrosis.
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The role of interleukin 13 in the development of endometrosis in the mare

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Endometrosis is a chronic condition with developing fibrosis of mare endometrium. As a consequence of endometrosis, embryo implantation failure results in economic losses in the horse-breeding industry. The pathogenesis of endometrosis remains not completely understood. Interleukin (IL)-13, which is secreted by activated T helper 2 lymphocytes, regulates both physiological and pathological processes. Studies focused on fibrosis in different species and tissues indicated a meaningful role of IL-13 in the development of organ fibrosis. However, the role of IL-13 in the pathogenesis of endometrosis remains unknown. Thus, we aimed at the evaluation of the role of IL-13 on processes associated with the development of endometrosis. For this purpose, we localized IL-13 and its receptor (IL-13R) in mare endometrium (n=3 at each stage of endometrosis) using IF-P. We further cultured mare endometrial fibroblasts (n=5, category I endometrium) in 2D (48h and 96h) and 3D (48h and 144h) *in vitro* culture systems without or with IL-13 (10 ng/mL). The effect of IL-13 on fibroblast proliferation was assessed using an MTT-based assay. The action of IL-13 on gene expression of fibrosis markers, such as *collagen (Col)1A1, Col3A1, α-smooth muscle actin (SMA), metalloproteinase 2 (Mmp2), Mmp9, tissue inhibitor of metalloproteinase 1 (Timp1)* and *Timp2* was determined using qPCR. The presence of IL-13 and IL-13R was determined in epithelial, stromal, and glandular cells of mare endometrium. Interleukin 13 increased ($p<0.01$) mare endometrial fibroblast proliferation. Treatment with IL-13 affected ($p<0.05$) *α-SMA, Col1a1, Col3a1, Mmp2, Mmp9, Timp1* and *Timp2* gene expression. Moreover, IL-13 treatment decreased the endometrial ratio of *Mmp2* to *Timp1* and *Mmp9* to *Timp1* gene expression. Obtained results strongly suggest the role of IL-13 in the processes associated with the initiation and development of endometrosis. The profibrotic effect of IL-13 may depend on the regulation of the expression of extracellular matrix proteins and enzymes involved in endometrosis development.

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Substrate stiffness modifies gene expression of equine endometrial fibroblasts in vitro

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Equine endometrial fibrosis is a common cause of equine infertility in ageing mares.
In the development of equine endometrial diseases, stromal fibroblasts play a central role, through phenotypic transition to myofibroblasts which proliferate and secrete excess amounts of extracellular matrix. Transforming growth factor beta (TGFB) has been identified as pivotal cytokine driving the aforementioned phenotypic transition. Fibroblasts can be studied in cell culture systems, but it has been shown that they react very sensitive to the stiffness of their surrounding environment, leading to increased expression of myofibroblast markers. This sensitivity can subsequently lead to falsified interpretations when working with fibroblasts in cell culture to study the pathogenetic mechanisms of fibrosis. Our aim was therefore to investigate the gene expression of smooth muscle markers, signaling proteins, and extracellular matrix proteins in equine endometrial fibroblasts, depending on the stiffness of the substrate on which they have been cultured. Additionally, we investigated the effect of transforming growth factor beta on gene expression depending on substrate stiffness.
We observed a dose-dependent effect of substrate stiffness on gene expression by stromal fibroblasts. Furthermore, we observed a substrate stiffness dependent ability of TGFB to alter gene expression.
In conclusion, we demonstrated that gene expression of various signaling and extracellular matrix proteins of equine stromal fibroblasts is influenced by the stiffness of the substrate and exposure towards TGFB. The impact of influences varies depending on the gene examined. Some genes show a higher, other genes show a lower dependency. Scientists should keep this in mind, when performing cell culture experiments with fibroblasts.

DNA methyltransferases in equine endometrial fibroblasts

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Endometrosis is a chronic degenerative fibrotic disorder with collagen deposition in mare endometrium, mainly collagen type I (COL1) and type III (COL3). Transforming growth factor β1 (TGFβ1), a main pro-fibrotic signal for myofibroblast differentiation, promotes COL1 gene expression. Neutrophil extracellular traps are released to fight pathogens. Their enzymes, as elastase and cathepsin G, can also act as pro-fibrotic factors in mare endometrium, inducing COL1 deposition. Prostaglandins (PG) E_2 and $F_{2\alpha}$ may have anti and pro-fibrotic effects, respectively. Epigenetic changes can modulate fibroproliferative diseases, as we suggested for equine endometrosis. DNA methylation, a stable epigenetic marker, can be assessed through DNA methyltransferases (DNMTs) action. This study aimed to evaluate mare endometrial fibroblasts epigenetic modulation, after *in vitro* treatment with TGFβ1, elastase, cathepsin, PGE_2 or $PGF_{2\alpha}$ for 72h. *DNMT1*, *DNMT3A*, *DNMT3B*, *COL1A1* and *COL3A1* transcription was evaluated by qPCR. Upregulation of *DNMT1* and *DNMT3A* mRNA occurred after elastase and cathepsin treatment, and *DNMT3A* mRNA, after PGE_2. *DNMT3B* mRNA decreased with elastase and increased with cathepsin. *COL1A1* and *COL3A1* increased after TGFβ1 treatment and decreased after PGE_2. This suggests that fibroblast hypermethylation, due to increased *DNMTs* transcripts with the antifibrotic PGE_2, may be responsible for *COL1A1* and *COL3A1* lower transcription, and therefore, a possible epigenetic regulation. Nevertheless, for the other studied factors, the absence of association between *DNMT*s and *COL1A1* and *COL3A1* transcription suggests no epigenetic involvement.

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The effect of interleukin 6 on mRNA expression of fibrotic markers in equine endometrial fibroblasts

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Interleukin (IL)-6 is a pleiotropic cytokine, produced mainly by monocytes and macrophages, and plays a role in the fibrosis of various tissues and organs. Macrophages secrete IL-6, which affects the inflammatory reaction. Results of recent studies indicate that IL-6 increases the expression of extracellular matrix components (ECM) in various tissues. In previous studies, changes in the expression of IL-6 in mare's endometrosis were observed. Endometrosis is defined as endometrial fibrosis in the mare endometrium, characterized by excessive collagen (COL) deposition and disturbed expression of metalloproteinases (MMPs) responsible for ECM degradation and their tissue inhibitors (TIMPs). IL-6 increases the proliferation of equine endometrial fibroblasts. However, there is no study about the effects of IL-6 on the expression of fibrotic markers in endometrial fibroblasts. The goal of this study was to determine the effect of IL-6 on mRNA expression of ECM components, MMP, and TIMP in equine endometrial fibroblasts. Fibroblasts isolated from healthy mares endometrium (n=4) were treated with IL- 6 (10 ng/mL) for 48 and 72 hours to determine mRNA expression of *FN1, Col1a1, Col3a1, Mmp-2, -3,-9, Timp-1* and-*2*. The treatment with IL-6 up-regulated the mRNA expression of *FN1* ($P < 0.01$), *Col3a1* ($P < 0.01$), and *Mmp2* ($P < 0.001$) after 48 hours, and *Mmp9* ($P < 0.01$) after 72 hours. Moreover, IL-6 treatment had no effect on mRNA expression of *Timp1-2* ($P > 0.05$). The results suggest that IL-6 could be involved in the development of endometrosis via regulating the mRNA expression of ECM components, MMP, and TIMP in endometrial fibroblasts.
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Genetic polymorphism of *NOTCH4 g*ene is associated with anestrum in cows

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The occurrence of ovarian inactivity is one of the main causes of fertility deficiency in cows. The genetic handling of this phenomenon could be a forward step for improving animal fertility. Notch pathway is an active player in many ovarian events including, ovarian angiogenesis, follicular growth, oocytes maturation, and production of steroid hormones. The aim of this work was to identify the genetic polymorphism of *NOTCH4* gene in cows and providing information about the desirable alleles and special SNPs involved in anestrum. The animals were diagnosed with inactive ovary by delayed estrous cycle from 6-12 months after parturition as well as ultrasonography. The blood samples were collected from 183 animals (69 with inactive ovaries and 114 normal with active ovaries) for DNA extraction. The primers for *NOTCH4* gene were prepared using Primer3 program version 4.0. The polymerase chain reaction and SSCP technique were carried out. The results showed thatcows with ovarian inactivity recorded an incidence of 22%. There was a genetic polymorphism in *NOTCH4* gene with different patterns associated with anestrum in cattle. The sequencing data determined the SNPs related to anestrum. In conclusion, *NOTCH4* gene could be a promising marker associated with fertility in Egyptian cattle for the first time.

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EGF regulates expression of extracellular matrix proteins in porcine endometrium through STAT3 activation

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Background: One of the prerequisites for the development of endometrial receptivity for embryo implantation is remodeling of extracellular matrix (ECM), cell migration and adhesion taking place during peri-implantation period in pigs. During this period, both endometrium and conceptuses secrete a epidermal growth factor (EGF) that can lead to activation of transcription factors such as STAT3. As STAT3 has an established role in this process mentioned above, we evaluated whether EGF induces STAT3 activation in porcine endometrium and if this activation is associated with change in the expression of genes associated with ECM.
Methods and Results: We used a predefined porcine ECM and adhesion molecule quantitative PCR arrays consisting of 84 ECM and cell adhesion-related genes and evaluated their expression in porcine endometrial explants, collected on day 12-13 of the estrous cycle, treated with EGF or with inhibitor of STAT3 activation, Stattic in combination with EGF. Binding of STAT3 to promoters of select ECM genes was evaluated by chromatin immunoprecipitation assay.
We observed an increase in the expression of *COL5A3, ICAM1, ITGA5, ITGA7, MMP12, MMP3, LAMA1, SELP, SELL,* and *VCAM* in the endometrial explants treated with EGF. On the other hand, genes that showed a decreased expression included *COL2A1, MMP8,* and *TNC*. The expression of these genes was further evaluated by qPCR and compared with their expression in explants that were first treated with Stattic to inhibit STAT3 activation and then incubated with EGF for 24 hrs. The effect of EGF on the expression of *MMP3, MMP8, MMP12, ICAM1, SELL* and *ITGA5* was reversed in explants where STAT3 was inhibited by Stattic. Moreover, EGF induced a 2.7-fold and 7.3-fold increase in STAT3 binding on *MMP3* and *MMP12* promoters, respectively.
Conclusions: Activation of STAT3 signaling pathway can be responsible for extracellular matrix remodeling during period of embryo-implantation in pigs.

Changes in transcriptome profile in equine corpus luteum during early pregnancy

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Corpus luteum (CL) insufficiency is a multifactorial, endocrine disorder that is a substantial reason of early embryo mortality and implantation failure. To describe novel agents regulating equine CL function during early pregnancy we carried out global mRNA analysis using high throughput mRNA sequencing.
Corpora lutea were obtained post-mortem at Day 8-12 after ovulation (n = 9) and from pregnant mares (n = 3/group) on Days 16-18 or 26-28 after insemination (DP). RNA isolated from CLs was used for library construction using TruSeq RNA Sample Prep v2 kit. The libraries were eventually sequenced in single 50-bp run (1×50bp) on the HiScanSQ system using TruSeq SBSv3 Sequencing kit. The differentially expressed genes (DEGs) were annotated in KOBAS 3.0 web server and analyzed in terms of their biological functions using Ingenuity pathway analysis (IPA).
A total of 1116 and 102 DEGs were identified in CLs obtained from 16DP and 28DP as compared to mid-luteal CL, respectively. 824/1116 and 77/102 genes were successfully annotated. Functional analysis of DEGs revealed significant enrichment of molecular and cellular processes such as cell growth, lipid metabolism and molecular transport in pregnant CL and decrease in processes related to immune response. PTGER4, ACKR2, IL1RN (Z-score > 2.0) and IFNb, IL1b, PRL (Z-score > - 2.0) were identified as upstream regulators of some of DEGs. Comparison of CL from 16DP versus 28DP revealed 540 DEGs of which 473 were annotated and linked to functions such as cellular movement, cellular growth and proliferation and inflammatory response. ADIPOQ, PRKAA1/2, CXCL12 (Z-score > 2.0) and TGF, VEGF (Z-score > - 2.0) were identified as upstream regulators of many DEGs.
These data reveal that changes in luteal transcriptome during early pregnancy is related to maintenance of luteal function and cell survival mechanisms.

Expression pattern of apoptotic genes in corpus luteum during thermal stress in Egyptian buffaloes

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Apoptosis is a crucial process for maintenance a functional corpus luteum (CL). The current study aimed to investigate the role of some apoptotic genes (TNFα, BAX, CASP3, AGTR2, FASLG, NOS2 and HSP70) at different stages of CL during thermal stress. The present work was conducted by collecting 70 paired ovarian samples from local abattoir from buffaloes. The CLs were classified morphologically into; early, mid, and late stages. The obtained samples were cut and snapped frozen at -80ºC for RNA isolation and qRT-PCR. The level of TNFα, BAX, AGTR2 and NOS2 genes was lowered ($P<0.001$) at different stages of CL during hot opposed to cold seasons. Additionally, relative abundance of CASP3 and FASLG was decreased at mid and late stages of CL during hot season. The HSP70 mRNA was increased ($P<0.001$) in response to hot season. It could be concluded that decline of AGTR2 was associated with decrease of NOS2 gene expression, which consequently affect TNFα, BAX, CASP3 mRNAs. Moreover, apoptosis might be affected by direct role of AGTR2 on CASP3 during thermal stress. The findings emerged from our study suggested the impact of thermal stress on some genes controlling apoptosis of CL in Egyptian buffaloes.

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Gene expression and interaction between aryl hydrocarbon receptor, interleukin 17 and transforming growth factor β1 in equine endometrial fibrosis

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Mare endometrosis is defined as periglandular and/or stromal endometrial fibrosis. This condition destroys tissue architecture and impairs endometrial function, which results in early pregnancy dysfunction and embryo loss. An increasing body of evidence indicates the involvement of aryl hydrocarbon receptor (AhR) in the progression of tissue fibrosis. Numerous studies have reported an association between AhR and transforming growth factor (TGF)-β1, the master regulator of fibrotic processes, as well as T helper (Th) cell differentiation, specifically, Th17 cell subset. To the best of our knowledge, there is no study about the involvement of the AhR and Th17 cells and their mediator – IL-17, in the development and progression of endometrosis in the mare. Thus, the main aim of the current study was to determine the endometrial gene expression of AhR and IL-17 during equine endometrial fibrosis (category I – negative control, IIA, IIB and III Kenney and Doig´s endometrium categories; n=3 for each category) by means of qPCR. Moreover, we examined the impact of TGF-β1 and IL-17 on AhR gene expression in fibroblasts isolated from mare endometrium in categories I, IIA, and IIB of Kenney and Doig (n=3 for each category) using qPCR. The AhR gene expression was the highest in category I endometrium *(P<0.05)*, and did not differ between category IIA, IIB and III. Gene expression of IL-17, in turn, was similar, regardless of the endometrium category. The treatment with TGF-β1 up-regulated *(P<0.005)* the gene expression of AhR in category IIA endometrium, while IL-17 down-regulated AhR gene expression in category IIB of endometrosis *(P<0.01)*, when compared to category I endometrium. Our findings suggest the involvement of AhR in the development of endometrosis in the mare, as well as AhR gene expression modulation by TGF-β1 and IL-17.

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Possible factors which determine testosterone levels in Japanese black bears

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Testosterone, mainly secreted from the testis, plays an important role as a regulator of male reproductive function, for example, spermatogenesis, development of the accessory reproductive organs, and reproductive behaviors. In spite of its importance, testosterone levels of male bears demonstrate individual variation even during the breeding season. To uncover the causative underlying factors of the individual difference in testosterone levels, the present study examined associations of potential factors (season, time of sampling, body condition index, head circumference, and age) with the testosterone level in 80 blood samples collected from wild Japanese black bears (*Ursus thibetanus*) captured in barrel traps in the Ashio–Nikko Mountains and Okutama Mountains in central Honshu Island, Japan. Body condition index and head circumference were used as indicators of nutritional condition and body size, respectively. The plasma testosterone level was higher during the breeding season (May–July) than during the non-breeding season (August–November). The body condition index was significantly and positively associated with the plasma testosterone level. None of the other factors were significantly associated with the plasma testosterone level. Thus, the body condition index may be essential for maintaining high plasma testosterone levels. Because energy-rich food lacks during the breeding season right after the hibernation, the nutritional status of bears declines. Therefore, only male bears with good nutritional status might be able to maintain high plasma testosterone levels. Our study implies the importance of field research to clarify the reproductive physiology of wild bears with variable nutritional status.

INVITED LECTURE

Association of the breeding induced endometrial response with subsequent fertility in mares

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Endometritis in the mare begins as a normal physiological inflammatory response to breeding that involves both a mechanical and immunological response pathway activated to rid the uterus of semen and bacteria. This endometrial response is characterized by a rapid increase in the pro-inflammatory cytokines IL1ß, IL-8 and IFN-Ɣ followed by an influx of inflammatory cells, mainly polymorph nuclear neutrophils. With successful resolution of this inflammation, the mare's uterus will provide a hospitable environment for the development of the semi-allogenic conceptus. In mares with impaired physical uterine clearance mechanisms (i.e. susceptible mares), however, the inflammation may persist and develop into a bacterial infection. This condition also known as persistent breeding induced endometritis (PBIE) is considered the main underlying cause for reduced pregnancy rates in affected mares.

To prevent the development of PBIE, injection of ecbolic drugs, such as oxytocin and prostaglandin (PG) F2α and their analogues, has become a routine treatment after breeding of mares with the aim to improve uterine mechanical clearance. Whereas treatment with oxytocin or its analogue carbetocin is often continued until after ovulation if the endometrial inflammation has not ceased, continuation of treatment with $PGF_{2\alpha}$ and its analogues is not recommended because of their known detrimental effects on luteal function. More recently, there was also the suggestion of detrimental effects of carbetocin on luteal function. To the best of our knowledge there is no information on the relationship of endometrial $PGF_{2\alpha}$ release in mares with PMIE and corpus luteum development during the early postovulatory period. There is also no proven evidence if a postovulatory treatment with ecboclic drugs of mares may impair corpus luteum function and thus further contribute to low conception rates in susceptible mares. In the presentation, possible interactions between the inflammatory endometrial response to insemination of estrous mares, periovulatory ecbolic treatments and their effects on luteal function and early conceptus development will be presented and discussed in the light of recent investigations.

INVITED LECTURE

Reproductive tract diseases – endometritis and what else?

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Effective reproductive management leading to conception and maintenance of the pregnancy by the cows is crucial for dairy farmers. Breeding success can be influenced by metabolic disorders, infectious diseases affecting reproductive tract, mechanical injuries after previous parturitions, feeding and managemental strategies. The most known disorders of the reproductive tract of dairy cows are metritis and endometritis studied over decades but there are also less common problems that can worsen reproductive outcomes. Among them cervicitis (with and without simultaneous endometritis), pyometra and salpingitis can be listed. Although their prevalence is lower they should also be considered as possible causes of infertility. Cervicitis can be diagnosed on the basis of rectal, ultrasound and cytological examination and can be correlated to different types of endometritis. Before 35 days postpartum (dpp) can be diagnosed in up to 70% of cows, between 42 and 50 dpp in 60.8% and in 19% of subfertile dairy cows. As the method of choice local antimicrobial treatment is recommended, but is not allowed in all countries. Pyometra is a disease when fluid or pus accumulate in the lumen of the uterus with the presence of corpus luteum. Progesterone produced by corpus luteum closes uterine cervix and growth of the germs inside is promoted. *Trichomonas foetus* and bacteria like *Trueperella pyogenes, Fusobacterium necrophorum, Prevotella melaninogenica* and anaerobic Gram-negative are the most often found. Culture-independent methods were also used to know bacteria present in the exudate and the most prevalent belonged to *Fusobacteriaceae, Mycoplasmaceae, Bacteroidaceae, Pasteurellaceae* families. According to the previous studies *T. pyogenes* seemed to be the most pathogenic and producing toxin – pyolizin that binds cholesterol destroys cell wall leading to the death of the cell. But present studies showed *F. necrophorum* the most abundant in uterine samples obtained from cows with pyometra. A few treatment protocols including prostaglandin F2alfa and different antibiotics are recommended and impaired fertility is observed even after successful cure. Clinical salpingitis is not a very important clinical problem in bovine reproduction but the present studies brings new and interesting data concerning bacterial flora and inflammatory processes present in fallopian tube. There are no good, reliable diagnostic tools that can be used to diagnose that disorder in alive animals. Clinical signs are very rare and limited only to enlarged, palpable structure between uterine horn and the ovary. Studies performed on organs from abattoirs showed wide range of bacteria present both in fallopian tube and uterine lumen. In most cases microflora responsible for endometritis was harvested but also atypical like *Ureaplasma diversum, Chlamydia abortus* and *trachomatis* or *Neisseria gonorrhoeae.* Analysis of inflammatory processes in reproductive tracts showed concurrent endometritis and salpingitis in most cases and similar situation was found in repeat breeding buffalo-cows. As is it almost impossible to diagnose salpingitis no recommended treatment is present. Metritis and endometritis are the most predominant diseases of tubular reproductive organs in dairy cows, but other less often present disorders should also be considered while gynecological examination is performed as they influence possibility to conceive and maintain pregnancy.

Effect of heat stress on the occurrence of endometritis in dairy cows

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The objective of the study was to examine the effect of heat stress (HS) under central European conditions on the occurrence of clinical and subclinical endometritis (CE and SE) and reproductive performance. A total of 1825 dairy cows were included at the day of calving and examined for signs of puerperal metritis (PM) and subclinical ketosis (SK) on day 5 postpartum (pp). On day 28 pp, vaginal discharge was assessed for the diagnosis of CE (mucopurulent or purulent discharge). Cytobrush samples were collected from animals with clear discharge and the percentage of polymorphonuclear cells (PMN) was determined for the diagnosis of SE (≥5% PMN). The body condition score (BCS) was assessed on the day of calving and on day 28 pp. Ambient temperature and relative humidity were recorded using calibrated data loggers (Tinytag-Gemini Datalogers Ltd., Chichester, the UK) located inside the barn. The temperature humidity index (THI) was calculated and THI of 68 was set as threshold for HS. According to the THI, animals were divided into three groups: 'no-HS' (without HS, n=491), 'low-HS' (below median, n=665) and 'moderate-HS' (above median, n=669). No negative effect of HS on the occurrence of CE, SE, and PM was found ($P \geq 0.05$). The occurrence of CE was associated with SK, PM, parity and BCS changes from calving to diagnosis ($P < 0.05$). Survival analysis showed that CE affected calving to conception interval and time to first artificial insemination ($P < 0.05$); however, SE and HS did not affect any of these two. Future studies should investigate underlying mechanisms linking CE to follicular development and embryonic loss.

Hastening first postpartum ovulation in early lactation may reduce the incidence of endometritis in dairy cows

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Endometritis is prevalent in bovine dairy herds, resulting in major economic losses. Previous studies suggest that cows spontaneously ovulate early in lactation have reduced endometritis incidence. Our objective was to evaluate the efficacy of two hormonal treatment regimens for hastening ovulation in early lactation (treatment initiation at 24-27DIM) and explore the associated incidence of cytological endometritis (CEM).
Based on transrectal ultrasonographic examination (presence of corpus luteum, CL) combined with milk progesterone cow-side test at 24-27DIM, postpartum dairy cows (n= 450) were divided into four groups: ***Positive control*****:** cows spontaneously ovulated by 24-27DIM, no treatment. Cows that did not spontaneously ovulate were divided randomly into the following (n= 101 cows/group): ***Select–synch***: GnRH analog and PGF2 7d later; ***Select–synch-CIDR*****:** GnRH analog and PGF2 7d later, with 7d CIDR; ***Negative control***: no hormonal treatment (two saline injections 7d apart). Ovaries' status was evaluated five times by transrectal ultrasonography (24-27DIM; 31-34DIM; 38-41DIM; 45-49DIM; 66-69DIM). CEM diagnosis was performed based on endometrial cytobrush at 38-41DIM and 66-69DIM.
A total of 114 cows (27%) spontaneously ovulated by 24-27DIM. Both hormonal protocols efficiently induced ovulation and increased the number of cows ovulating during the 70DIM voluntary waiting period (Select-synch 92.1%, Select-synch-CIDR 89.1%; Negative control 71.3%; P=0.0466). Furthermore, the Select-synch protocol was associated with a decreased risk for CEM compared to the negative control, particularly in cows without postpartum ketosis (18.6% vs. 35.4%; OR=0.42, 95%CI 0.18-0.90; *P*=0.0295). We concluded that hormonal treatments could induce ovarian activity in early lactation and subsequently improve uterine health.

Nucleolar size of large luteal cells increases in mares with endometritis

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Endometritis can decrease progesterone (P4) production by the corpus luteum (CL), among other deleterious effects on fertility. P4 production may depend on the number and size of luteal cells. We hypothesize that endometritis during ovulation could affect luteal cytoarchitecture and decrease serum P4. The aim of this experiment was to count and measure large luteal cells (LLC) and small luteal cells (SLC) in the CL of mares with endometritis versus control mares. Cyclic mares (n=13) were randomly assigned to Endometritis Group (EG, n=6), receiving an intrauterine infusion of 1 x 10_9 Streptococcus equi zooepidemicus one day before ovulation, and Control Group without endometritis (CG, n=7). On day 5 post ovulation blood for P4 measurement was extracted, and the ovary containing the CL was surgically removed. CL were paraformaldehyde fixed and parafin embedded, cut in 5 μm sections and stained with Hematoxylin Eosin. Twenty images per mare were selected at 400 x. LLC and SLC were counted. Nuclei of LLC and SLC, and nucleoli of LLC, were measured in 100 cells per mare. Serum P4 was lower in EG compared to CG (9,6 ± 1,7 ng/mL versus 11,2 ± 1,3 ng/mL, p=0.014). LLC showed round euchromatic nucleus with one prominent round nucleolus. Nucleolar area of LLC was larger in EG compared to CG (10,2 ± 0,7 μm_2 versus 8, 9 ± 0,5 μm_2, p=0.027). No differences were found between groups in the number of LLC and SLC nor in nuclear measurements. Nucleolar size, structure and proteome are related to nucleolar functional state, and altered by cellular stress. The present data demonstrates that endometritis increases nucleolar area in LLC, which could be related to the presence of stress proteins in LLC caused by endometrial inflammation. Further studies are needed to clarify the significance and mechanisms underlying these findings.

Circulating and endometrial polymorphonuclear leukocyte function dynamics in postpartum dairy cows with subclinical or clinical endometritis

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We aimed to compare the circulating polymorphonuclear leukocyte (cPMN) and endometrial PMN (ePMN) viability and function dynamics in postpartum dairy cows with subclinical (SCE) or clinical endometritis (CE). To do so, blood samples from 38 Holstein cows were collected at -7, 9, 21, and 36 d relative to calving, and endometrial cytology samples from 32 Holstein cows were harvested at 9, 21, and 36 d postpartum. The uterine health status was assessed at 36 d postpartum, and cows were classified as healthy (absence of mucopurulent discharge and ≤ 5% ePMN), SCE (absence of mucopurulent discharge but > 5% ePMN), or CE (mucopurulent discharge or worse and > 5% ePMN). Viability and function parameters phagocytosis (PC), oxidative burst, and intracellular proteolytic degradation were evaluated via flow cytometry. Cows with CE had a lesser proportion of cPMN viability (84.5 ± 2.1%) and greater apoptosis (14.4 ± 2.0%) than healthy (92.4 ± 1.3 and 6.7 ± 1.3%, respectively) or SCE (95.3 ± 2.4 and 3.8 ± 2.3%, respectively) at 9 d postpartum. Interestingly, cPMN intracellular proteolytic degradation was lower (6.2 ± 0.1 MFI) in SCE than healthy (6.7 ± 0.08 MFI) or CE (6.8 ± 0.1 MFI) at d 9 postpartum. The proportion of necrotic ePMN was higher for healthy (49.6 ± 5.1%) than SCE (27.4 ± 7.3%) and CE (27.7 ± 7.3%) cows at 36 d postpartum. Also, at 36 d postpartum, the proportion of ePMN for PC was higher in CE (47.0 ± 8.6%) compared to healthy (18.4 ± 7.6%) cows, but it was not different than SCE (25.9 ± 8.7%). Results of the present study modestly suggest that cPMN viability and function at 9 d postpartum contribute to the pathogenesis of uterine disease. Remarkably, ePMN found in cows with SCE at 36 d postpartum are mostly viably but dysfunctional.

The vascular and perivascular morphometric features and their correlations in equine endometrium affected by endometrosis

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Vascular changes in endometrosis are thought to decrease perfusion in uterine tissues. The study aimed to compare the vascular and perivascular measures and transcription of profibrotic enzymes concerning the degrees of equine endometrosis. Endometrial samples were collected from corpus uteri from n=36 mares, and stored in formalin. The samples were histologically processed, cut, and stained with hematoxylin-eosin (HE) and Masson's Trichrome (MT). The degree of equine endometrosis was determined on HE slides and assigned into groups as follows: I (n=8), IIa (n=8), IIb (n=8), and III (n=8). HE-stained and MT-stained slides were then scanned with a semiautomatic brightfield system (TissueFaxsPlus), and the vessel area (VA, nm²) and vessel perimeter (VP, nm), and perivascular fibrosis (PF; μm²) were quantified. Kruskal-Wallis test was used to compare measures (mean±SD) between I, IIa, IIb, and III groups, as well as Spearman correlation coefficient (ρ) was used to indicate features relations. Studied morphometric features were higher (VA $p<0.0001$; VP $p=0.004$; PF $p<0.0001$) in III (VA:2037±3250 nm^2; VP:200±163 nm; PF:4313±7615 μm^2) than in I (VA:892±576 nm^2; VP:153±93 nm; PF:1100±1830 μm^2) and IIa (VA:1186±1739 nm^2; VP:154±100 nm; PF:1195±2213 μm^2) groups. PF was also higher in III than in IIb (1834±3276 μm^2), whereas no differences were found between IIb and III for VA (IIb:1572±2064 nm^2) and VP (IIb:163±120 nm). Strong positive correlations were calculated for VA and VP in groups I ($\rho=0.73$; $p<0.0001$), IIa ($\rho=0.71$; $p=0.002$), IIb ($\rho=0.82$; $p=0.048$), and III ($\rho=0.71$; $p=0.030$). However, no significant correlations were noted between PF and remaining studied morphometric features. The perivascular fibrosis, typical for equine endometrosis, seems to not affect the vessel size in endometrium.

Inflammation effect on the endometrial percentage of reproductive hormone receptors and the height of the superficial and glandular epithelium in anestrus type II dairy cows

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The prolongation of the time from calving to next fertilization is one of the important case affecting reproductive efficiency of dairy cows. Concerning anestrus type II cows, this study aimed to preliminary compare measures of hormone receptors and epithelium height in inflamed and healthy endometria. Among 33 cows with anestrus type II, biopsy samples were taken on before insertion of progesterone vaginal inserts. Samples were histologically processed, cut, and hematoxylin-eosin (HE) immunofluorescent (IF) stained. On HE-stained slides, inflammatory cell infiltration (ICI) was manually evaluated and histometric analysis was performed (CellSens Standard Image Analysis software). ICI, predominantly lymphocytes, was present in 6 samples assigned as endometritis (E), whereas, of the remaining samples, 6 slides were assigned to the control group (C). In histometric analysis, the height of the superficial epithelium (HSE), the diameter of inactive uterine glands (DIG), and the diameter of the secretory-active uterine glands (DSG) were determined. On IF-labeled slides, estrogen (ERα, ERβ) and progesterone (PR) receptors were visualized using specific primary mouse monoclonal antibodies. Data series of C and E groups were compared using unpaired t-test with Welch's correction and presented as mean±SD. No differences were found for ERα (C:5.95±2.37%; E:4.54±3.34%; p=0.465), ERβ (C:5.77±1.96%; E:4.45±2.03%; p=0.328), and PR (C:3.74±0.92%; E:3.15±1.33%; p=0.441) endometrial percentage of immunopositive cells. However, histometric measurements were always higher (HSE:p=0.025; DIG:p=0.002; DSG:p=0.021) in E (HSE:17.11±0.58μm; DIG:75.15±2.35μm; DSG:90.13±17.81μm) than in C (HSE:15.98±0.70μm; DIG:66.86±3.17μm; DSG:61.92±5.73μm) group. In anestrus type II, besides the effect of the reproductive hormone, other inflammatory-related factors are suspected to alter the height of the superficial and glandular epithelium in the bovine endometrium.

How do viruses cross the porcine endometrium to reach embryos/fetuses and cause reproductive failure?

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Viruses have developed during co-evolution with their host several mechanisms to invade and to reach embryos and fetuses with the purpose to become spread in the environment and to be transmitted to other naïve animals. This is in part their way of persisting in a population. It can be done by replicating in embryos/fetuses and the maternal/fetal interface (placenta) to high levels causing interruptions of gestation (return to estrus, abortion and early farrowing) or by replication only in the fetuses and not in the endometrium resulting in a normal gestation and mummification of the infected young non-immunocompetent fetuses. Both situations result in a massive viral shedding. Examples are parvovirus (PPV), porcine circovirus 2 (PCV2), enteroviruses, porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV) and classical/African swine fever virus (C/ASFV). An alternative way is to infect fetuses in a restricted/silent way (PRRSV/PCV2) or persistent way (CSFV) leading to piglets that may shed the virus and infect littermates.
In the past, researchers believed that the spread through the endometrium was caused by a direct viral spread. As even antibodies cannot cross this barrier, viruses make no chance in reaching the embryos/fetuses by this way. A fast cell-to-cell spread and microchimerism are powerfull processes that allow the virus to escape from the local innate (mainly mediated by cytotoxic NK cells) and sometimes even adaptive immunity (antibodies and cytotoxic T-lymhocytes). Some examples will illustrate this. PRV infects blood monocytes and is a master in escaping from immunity by internalization of the membrane-expressed viral glycoproteins upon binding of antibodies. They are highly sticky and upon binding to the endothelial cells of the endometrial blood vessels, they transfer the virus from cell to cell without using the hostile extracellular environment. This happens in a fast and efficient way. Replication in the endometrium and fetal tissues results in a fast intrauterine spread and death of the fetuses. In contrast, PRRSV is present in the blood in a cell-free state and is infecting the intravascular sialoadhesin/CD163 positive macrophages, which are mainly present at the end of the gestation. These macrophages are largely attracted starting from 70 days of gestation. As these macrophages invade the endometrium and even may cross the maternal/fetal placenta (microchimerism), they are transferring the virus to endometrial and fetal sialoadhesin/ CD163 positive macrophages. Apoptosis of these infected cells and bystander cells lead to malfunction of the placenta and the death of the fetus. Upon PCV2-infection in seronegative sows (rare situation), infected leukocytes (T-lymphoblasts and monocytes) are carrying the virus to the pregnant uterus and via microchimerism the virus ends up in the fetuses. The infected T-lymphoblasts are actively infected and release infectious virus in the fetuses, leading to an explosive replication in the proliferating fetal cells. Replication in fetuses without replicating in the endometrium results in mummication and normal gestation. Upon the induction of a specific immunity, the infected T-lymphoblasts disappear. However, infected monocytes are still present. They do not produce an offspring; the viral genome is released upon uptake of the virus and stays in the cell. It is not known if such an aborted infection may be reactivated.
In conclusion, viruses developed over time different efficient strategies to cross the endometrial tissues and reach embryos/fetuses in order to spread upon reproductive failure.

INVITED LECTURE

Carryover effect of uterine diseases on subsequent pregnancy losses in lactation

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The objective of this study was to assess of pregnancy losses (PL) up to 210 days of gestation due to uterine diseases in grazing dairy cows. A retrospective study including a total of 24,232 records of first, second and third plus pregnancies (PREG) within the same lactation from cows of 1, 2 or 3 plus lactations (LACT) calving from January 1st, 2010, to December 31st, 2018, was used. All cows had retained fetal membranes (RFM) were diagnosed by trained farm personnel. Puerperal metritis (PM) was diagnosed every week, and clinical endometritis (CE) and pyometra (PYO) diagnosed every other week by the veterinarian. Pregnancy diagnosis (PD) was performed buy veterinarian every two-weeks between 30-44 days post-AI. Pregnancies were recorded as single or double during each diagnosis (TWIN). Pregnancy loss (PL) after confirmation of pregnancy (n=6,600) were classified as 1) cows that had a dead embryo at PD with ultrasonography 30-44 days after, 2) cows that were diagnosed not pregnant at the PD reconfirmation between 60 and 90 days post-AI, 3) cows that returned to estrus and were diagnosed not pregnant at the next examination after detected in heat, and 4) cows that were diagnosed pregnant and returned to estrus 30 days after PD and were inseminated. The risk of PL was analyzed by logistic regression using two models. The first model included the effects of lactation number (LACN), season of the year that became pregnant (SEAP), number of services became pregnant (NSP), and health status (HEALTH; healthy [no diseases], uterine [RFM, PM, CE, PYO], no uterine [anestrous, mastitis, lameness], and both [uterine + non uterine]). The second model included the effects of LACN, SEAP, NSP, RFM, PM, CE, PYO, and TWIN. Statistical significance was set at P<0.05.
The occurrence of PL was 27.5%. Healthy cows had lower PL than cows with uterine, non-uterine and both diseases (24.8 vs. 29.0, 26.1 and 28.1%; P<0.001). The odd of losing pregnancy was higher in cows with uterine diseases compared to healthy cows (odd ratio [OR] 1.3, 95% confidence interval [95%CI] 1.18-1.42; P<0.001). Cows with 2+ lactations, cows that became pregnant in summer, and cows that had more services to become pregnant had higher PL (P<0.001). The occurrence of uterine diseases and PL and the OR of PL are shown in Table 1.

Table 1. Occurrence of uterine diseases and pregnancy losses in pregnant cows and the odds of losing pregnancy.

		RFM	PM	CE	PYO	TWIN
Occurrence (%)		4.0	9.3	17.9	3.9	2.6
PL (%)						
	0	27.3	27.2	26.4	26.9	27.3
	1	32.7	30.4	32.4	42.0	33.1
OR (95%CI)						
	0	1	1	1	1	1
	1	1.14	1.11	1.30	1.83	1.17
		(0.99-1.31)	(1.01-1.23)	(1.21-1.40)	(1.60-2.09)	(0.98-1.38)
	P	0.06	0.03	<0.01	<0.01	0.07

Reference: no disease (RFM, PM, CE, PYO), single pregnancy (TWIN).

INVITED LECTURE

Research in bovine endometritis – are we asking the best questions?

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Several research groups worldwide have investigated bovine endometritis since decades. A simple and rough literature search using PubMed (https://pubmed.gov) and the search terms "(endometritis OR uterine infection) AND (cow OR cattle)" resulted in 1435 publications in the last 60 years. Interestingly, the number of publications in the last 20 years (2002-2021; n=1078) has increased significantly compared to the previous 40 years (1962-2001: n=492). This can be partly explained by a general increase in publication activity or more extensive electronic filing of publications in databases. Furthermore, we did not check the references found for their eligibility, since the intention of the literature search was not to carry out a literature review; it was only intended to illustrate the research activities in this area. There is no doubt that our knowledge of uterine diseases in cattle has increased enormously. This applies to everything from the underlying mechanisms of infection, inflammation and immune response, to the wide diversity of the uterine microbiota, and to strategies for the prevention and treatment of endometritis. We as a scientific community have identified risk factors at both individual and herd levels and gained insight into genetic predispositions for endometritis. Finally, we are aware of the later negative effects on reproduction and the economic impact on the profitability of farms. As a research and scientific community, we have asked many GOOD and important questions, tested countless hypotheses and entered previously unimagined spheres. We know a lot - but has this changed the situation on the farms? The prevalence of endometritis is still high at up to 40% and more and the affected cows still have a decreased chance to get pregnant in time. Some treatment approaches are less common than they used to be, e.g., the intrauterine application of disinfectants, and others, e.g., antimicrobial or hormone-based strategies, have been refined or modified. New approaches, e.g. vaccination against endometritis or intrauterine infusion of dextrose have been proposed. However, the variety of treatment options also suggests that no strategy can be considered successful enough to cover the majority of cases. So the authors of this presentation ask themselves: are we asking the BEST questions? What kind of research is needed and what methods are appropriate and available today that could significantly improve uterine health?

Endometritis is a complex disease with multiple risk factors and a variety of bacteria involved. For the authors, one of the central questions for the development of new prevention and treatment strategies is how to keep the microbiome in a physiological state and, thus, avoid pathologies. In the past, the most common and pathogenic bacteria have been studied in detail and intriguing mechanisms have been described. Current research aims to understand the interactions within the microbial community, and future research will likely analyze the interactions and dynamics of the entire microbiome from parturition to conception. Considering the enormous amount of different bacteria in the uterus and others entering from the environment as well as the huge amount of generated data, this will not be possible without e.g. the support of specialists, not only in microbiology but also in e.g. bioinformatics. In addition, the composition of the microbiome is influenced by internal factors such as the immune response, which in turn is related to energy balance or other nutritional factors, and by external factors such as hygiene. These and other factors at herd level, e.g. housing density or barn climate, should be taken into account if an overall picture of the dynamics of the microbial composition is to be drawn and the turning point from physiological to pathological conditions is to be found. Data scientists and modeling capacities are required for these complex analyzes and the development of prediction models. Based on all of the abovementioned analyses, different approaches to modulating the microbiome can then be tested and brought into practice. This is just one of several considerations in which direction future research might go. Other approaches, which are already being conducted, are based, for example, on a better understanding of the uterine defense mechanisms with the long-term goal of being able to influence it positively.

The authors of this presentation do not have the BEST questions and certainly, we do not have the best answers, but we hope to stimulate a fruitful discussion on future research strategies.

The most common pathogens isolated from the uterus in mares before the breeding season in 2018-2020 in the Mazovia region of Poland

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Subclinical endometritis is one of the major case of mares' infertility. Therefore, identification of pathogens is one of the most important challenges for practitioners. This study aimed to summarize the bacterial cases of mares infertility isolated in Mazovian mares. The study enrolled 52 mares, presented to the detailed examination of reproductive tract in the years 2018-2020. The standard examination protocol included a detailed interview, basic clinical examination, and detailed gynecological examination with ultrasound and bacteriological examination. Uterine swabs were collected, and the aerobic and anaerobic bacteria growth rate was noted as mild (+), moderate (++), or severe (+++). In 4% of samples, no bacterial growth was found. In 44% contamination with monoculture was noted, whereas in 52% more than one type of battery was grown from the swab. Aerobic and anaerobic bacteria were found in 96% and 19% of samples, respectively. The mild and severe contaminations were noted both in 63% of samples, whereas, the moderate only in 46% of samples Within aerobic bacteria, *Streptococcus* spp. was the most common isolated bacteria, contaminating 71% of samples. Subsequently, *Escherichia coli* was isolated from 56% of samples, *Acinetobacter* spp. from 17%, *and Staphylococcus* spp. form 10% samples. The remaining four aerobic species were found in less than 10% of samples. Within anaerobic bacteria, *Arcanobacterium hippocoleae* was the most common isolated bacteria, contaminating 8% of samples. The remaining four anaerobic species were found in 2% of samples, each. *Streptococcus* spp. and *Escherichia coli* were the most common pathogens isolated from the uterine swab in the Mazovia region of Poland.

Growth characteristics of *Trueperella pyogenes* in an uterine *in vitro* model

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Among different bacterial populations colonizing the bovine uterus, some, as, e.g., *T. pyogenes* (TP) are associated with clinical endometritis, whereas others, such as *Lactobacillus buchneri* (LBB) can be regarded as protective species and may have the potential to be used as probiotic. The interaction between TP, LBB and the uterine microenvironment, however, is not well understood. Therefore, the objective of this study was to examine the growth rate of TP in the presence of bacteria-free filtrate (BFF) of LBB, bovine estrual mucus (BEM), estradiol (E2), and progesterone (P4). TP was isolated from a postpartum dairy cow's uterus with clinical endometritis, whereas LBB was isolated from a healthy cow. TP and LBB were grown in BHI and MRS broth, respectively. LBB-BFF was added to TP culture at a rate of 10% and 20%. Sterile BEM was added at a rate of 10%. E2 and P4 concentrations were 10 pg/ml and 5 ng/ml, respectively. The OD_{600} of TP cultures were measured at 0, 12 and 24h, and difference in OD was calculated. In the presence of BEM, the growth rate of LBB was greater ($P<0.05$) after 24h. In contrast, the growth rate of TP was not affected by BEM. In the presence of LBB-BFF at a rate of 10% and 20%, the growth rate of TP was significantly lower after 12 and 24h, respectively. E2 and P4 did not affect the growth rate of TP. In conclusion, BEM might provide an advantage for protective bacteria over pathogenic bacteria around estrus. Moreover, LBB might release factors that decrease the proliferation of pathogenic TP.

Relationship between cultivable aerobic microbiota in the uterus and oviduct of postpartum dairy cows

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The objective of the study was to describe the relationship between uterine and oviductal infection in cattle *in vivo*. Cows were examined by vaginoscopy 28±3 days postpartum (dpp) and classified as healthy (HE; n=12) or with clinical endometritis (CE; n=7). Samples were taken 28±3 and 56±3 dpp from the uterus with a cytobrush (Ut) as well from both oviducts (Ov) with a mini-cytobrush, guided by transvaginal endoscopy. Samples were cultivated aerobically on agar plates, colonies were counted (bacterial growth density, BGD) and species were identified by MALDI-TOF MS. The diversity of bacterial species was greater in HE than in CE (Ut-HE: n=53 vs Ut-CE: n=19; Ov-HE: n=106 vs Ov-CE: n=52). Some bacteria, such as *Streptococcus pluranimalium*, *Escherichia coli* and *Trueperella pyogenes* were present only in the uterus. *Staphylococcus haemolyticus* and *Staphylococcus chromogenes* were found only in the oviduct. The most prevalent species in the uterus was *Streptococcus pluranimalium* (Ut-HE 14.6%; Ut-CE 31.3%). The microbiota in HE oviducts was dominated by *Corynebacterium camporealensis* (Ov-HE 12.9%; Ov-CE 2.8%). *Staphylococcus haemolyticus* was the most abundant species in CE cows (Ov-CE 33.3%; Ov-HE 3.0%). The prevalence of bacterial findings and BGD in the uterus and oviduct did not differ significantly between CE and HE cows. There was a positive correlation between BGD in the uterus 28±3 dpp and the oviducts 56±3 dpp ($r=0.5$, $P<0.05$). The results showed a partially shared microbial community in the bovine uterus and oviduct, presumably originating from ascending infections.

The effect of uterine lavage on the isolation of bacteria in mares with subclinical endometritis

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Bacterial endometritis remains a major cause of subfertility or infertility in mares. The most common cause of this disease is *Streptococcus equi subspecies zooepidemicus*, but in some cases these bacteria cannot be isolated by standard methods.
Aim: To investigate if uterine lavage can stimulate dormant forms of bacteria and facilitate their isolation from the uterus of mares suspected of subclinical endometritis.
Materials and methods: 10 mares, 5 and 15 years of age, were qualified for the study. Only mares unsuccessfully breed with a fertile stallion for at least three times were included. Physical and a gynecological examination were conducted. All mares had uterine swabs and biopsies taken twice. The first swab and biopsy were collected before uterine lavage, and the second 48h after infusion of 1l Ringer's lactate fluid into the uterus.
Results: *Streptococcus equi subspecies zooepidemicus* and *Escherichia coli* were mostly isolated. Before and after flushing the uterus, bacteria were isolated in 1/10 and 3/10 mares respectively when the material was collected with a swab, and in 3/10 and 9/10 mares correspondingly when material was obtained by endometrial biopsy. After inoculation with Ringer lactate, bacteria were isolated from a significantly higher number of mares than before the flushing, when the culture was done from a biopsy (p=0.04).
Conclusion: Subclinical dormant persistent infection in the endometrium of the mare can be activated possibly by uterine infusion of Ringer lactate, facilitating the isolation of bacteria.

Isolation and host range determination of bacteriophages specific to selected equine uterine pathogens

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Chronic bacterial endometritis is among the major problems in equine reproduction. *Streptococcus equi ssp. zooepidemicus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are among the most important pathogens for the condition. Bacteriophages are the viruses of bacteria, having a species- and strain-specific lytic activity. Due to their bactericidal capacities bacteriophages can be used as an alternative treatment approach for bacterial infections. The study aimed to isolate bacteriophages with lytic activity against *Streptococcus equi ssp. zooepidemicus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* and to determine their host range. Bacteriophage samples were obtained from manure, uterine lavage fluid and drain water of horse husbandries (n=14). For isolation of bacteriophages, bacterial isolates of the equine genital tract (*Streptococcus equi ssp. zooepidemicus* (n=1), *Klebsiella pneumoniae* (n=1), and *Pseudomonas aeruginosa* (n=1)) were inoculated with the bacteriophage samples. For purification, bacteriophage plaques were repeatedly cultivated, harvested and filtered. For determining the host-range, the obtained bacteriophages were inoculated with bacterial isolates provided by two diagnostic laboratories (*Streptococcus equi ssp. zooepidemicus* (n=37), *Klebsiella pneumoniae* (n=26), and *Pseudomonas aeruginosa* (n=33)). In total, bacteriophages (n=28) were isolated (*Streptococcus equi ssp. zooepidemicus*- (n=13), *Klebsiella pneumoniae*- (n=6), and *Pseudomonas aeruginosa*-specific (n=9)). Bacteriolysis was achieved in 32/37 *Streptococcus*-, 16/26 *Klebsiella*-, and 14/33 *Pseudomonas*-isolates. The bacteriophage showing the broadest host range covered 81% of all tested *Streptococcus*-isolates (30/37). Host ranges of *Klebsiella pneumoniae*- (9/26) and *Pseudomonas aeruginosa*-specific (14/33) covered a lower proportion of tested isolates. In conclusion, isolation of bacteriophages specific to equine uterine pathogens resulted in available bacteriophages (n=28), including promising candidates for further investigations.

Application of the microflow technique for the selection of stallion semen

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Routine semen preparation techniques are not always sufficient for all stallions. Some stallions, despite poor semen parameters, are valuable for breeders who want to get offspring for them. In recent times, advanced sperm selection methods such as the microflow technique have been developed and have been successfully applied to humans. The aim of this study was to try to adapt the microflow technique for the selection of stallion semen, focusing on their predicted benefits for sperm quality.
The experiment was carried out on the semen of 6 stallions, which were combined in equal volumes, obtaining a representative sample, which was left for 24 h at RT in order to reduce the parameters of sperm viability and motility. After 24 hours, the sample was divided into 3 parts, 1 of which was the control sample and the other two test samples were separated using the microflow technique and two different incubation times of 30 and 45 minutes.
For each of the samples, the basic parameters were assessed, i.e. concentration and motility, including the progressive movement of sperm.
In the sample incubated for 30 min, sperm motility increased from 28% to 52.15%, including sperm with progressive movement, from 8.47% in the control sample to 41.91%. After extending the incubation time for 45 minutes, these parameters were even higher: motility 62.43%, sperm with progressive movement 55.33. It should be noted, however, that in the case of both research samples, the concentration of sperm significantly decreased, which was 804.68 M / ml in the control sample, 32.35 M / ml in the sample incubated for 30 minutes, and 40.37 M / ml in the sample incubated 45 minutes.
The basic results of the concentration and mobility analysis showed a significant improvement in the mobility parameters and, above all, the number of progressive sperm cells in separate sperm. Comparing the two incubation times (30 and 45 min), it was found that extending the incubation time to 45 minutes significantly improves the parameters of separate sperm. Further research will concern the characteristics of detailed parameters such as: viability, morphology, DNA fragmentation, apoptosis using the TUNEL technique

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Endometrial microRNA expression profile during preimplantation period of pregnancy in the mare

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Early embryonic development, implantation, and maintenance of pregnancy are dependent on a precisely regulated communication between the conceptus and the uterine environment. An increasing body of evidence highlights a potentially important role of microRNA (miRNA) molecules in the proper embryo–maternal dialogue, especially during the preimplantation period of pregnancy. To the best of our knowledge, there is no study concerning the regulatory role of miRNA during the preimplantation period of pregnancy in the mare. Therefore, the main aim of the current study was to identify changes in the miRNA expression profile between mare endometrium during the preimplantation period of pregnancy (25-28 day of pregnancy; n=3) and endometrium during the mid-luteal phase of the estrous cycle (8-12 of estrous cycle; n=3). Endometrium tissue samples were obtained by biopsy.

By employing next-generation sequencing (NGS), we identified 81 differently expressed miRNAs (DEmiRs; $p_{adjusted}<0.05$, $log2FC\geq1.0/log2FC\leq-1.0$; 48 up- and 33 down-regulated DEmiRs), between examined endometrial tissue. Functional enrichment analysis revealed that target genes predicted for identified DEmiRs were mainly associated with the ligand-gated channel activity, transmitter-gated ion channel activity and neurotransmitter receptor activity, as well as sphingolipid metabolism. Moreover, our results indicate that, during the preimplantation period of pregnancy in the horse, miRNA molecules may play an essential role, especially those involved in cell growth, proliferation, migration, invasion, and apoptosis (eca-miR-21, eca-miR-126-3p, eca-miR-451), endometrial receptivity formation and extracellular matrix remodelling (eca-miR-129b-5p, eca-miR-150, eca-miR-491-5p) as well as angiogenesis and maternal immunotolerance (eca-miR-15b; eca-miR-130a, eca-miR-145).

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IL-6 induced STAT3 activation increases VEGF expression and capillary formation in porcine endometrial endothelial cell

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Background: The establishment of mammalian pregnancy requires an embryo–maternal dialog involves interactions between hormones, cytokines, growth factors, and their receptors. One of the significances of this dialogue is the development of the blood vessel formation, process of angiogenesis, in endometrium for the proper transport of the nutrients and factors required for pregnancy establishment. Vascular endothelial growth factor (VEGF) is the most studied angiogenesis regulator in the endometrium but the mechanism of its induction in porcine endometrium is not well established. In this study our aim was to evaluate role for IL-6 in inducing VEGF expression and angiogenesis through STAT3.
Methods and Results: Endothelial cells were isolated from porcine endometrium on day 13 of the estrous cycle and treated with IL-6 or with inhibitor of STAT3 activation, Stattic in combination with IL-6. Expression of VEGF in endothelial cells (ECs) was evaluated using Western Blot (WB) and immunofluorescence and EC proliferation and extent of angiogenesis was evaluated using MTT cell proliferation and matrigel assay, respectively.
Results: Immunofluorescence and WB studies revealed that treatment of endothelial cells with IL-6 did not have any effect on STAT3 expression but there was an increase in p-STAT3 abundance and translocation of activated (phosphorylated) protein to the nucleus. Inhibition of the STAT3 activation by Stattic completely abrogated the stimulatory effect of IL-6 on EEC. Additionally, IL-6 also increased the abundance of angiogenic factor VEGF in EEC which was reversed in the presence of Stattic. IL-6 also induced a significant increase in the proliferation in ECs, their differentiation and alignement to form capillary like network on Matrigel surface. The capillary formation started as early as three hour after incubation with IL-6. There was a significant increase in the number of branches/area and total segment length of ECs formed in the presence of IL-6. Stattic at 10 uM significantly inhibited cell proliferation and capillary structure formation by ECs.
Conclusion: Cytokine, IL-6 secreted at embryo-maternal interface can have functions other than immune responses in porcine endometrium. It can play an important role in endometrial angiogenesis through STAT3 activation.

ADAMTS metalloproteases and their regulation by transforming growth factor-β1 in mare endometrium

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Endometrosis is a chronic degenerative condition of the equine endometrium. It is characterized by excessive extracellular matrix (ECM) components deposition (fibrosis) around the endometrial glands and in the stroma. It is presumed that fibrosis results from inflammation. Macrophages, important players in inflammation, secrete significant amounts of transforming growth factor (TGF)-β1, known as a key pro-fibrotic factor. The excessive amount of TGF-β1 leads to changes in the expression of ECM components, metalloproteinases, and their inhibitors. ADAMTS metalloproteases are responsible for tissue remodeling and target components of ECM. Ongoing fibrosis suggests changes in their expression and activity. Hence, the study aimed to determine the mRNA transcription of ADAMTS at different stages of endometrosis and to determine whether TGF-β1 treatment affects their mRNA transcription in endometrial fibroblasts. Endometrial tissue in the follicular phase of the estrous cycle was divided into four categories of endometrium. Fibroblasts were isolated from categories I, IIA, and IIB endometria (n=6 each) and treated with TGF-β1 (10 ng/ml) for 48h. In endometrial tissue and treated fibroblasts, mRNA transcription of *ADAMTS1*, *-4*, *-5*, and *-9* was analyzed by qPCR. *ADAMTS* mRNA transcription was altered in different endometrium categories. TGF-β1 up-regulated mRNA transcription of *ADAMTS4* (*P<0.05)* and down-regulated mRNA transcription of *ADAMTS5* (*P<0.01*) in each category, whereas changes in *ADAMTS1* and *ADAMTS9* mRNA transcription were category dependent. Our findings suggest a potential role of ADAMTS in the development of endometrosis.
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New insight into pregnancy maintenance in she-camel: Molecular interaction among ovulatory site, uterine body and uterine horns

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A critical step for pregnancy establishment is the proper regulated dialogue between the endometrium and conceptus to achieve successful implantation and placental formation. So we give insight to the molecular interaction between uterine body and both right & left uterine horns. Our candidate genes is related to implantation & matrix formation (JUN and SLCO2A1), vascularization & placental formation (VEGFA), growth & development (PTEN). Therefore, 40 genital tracts (20 pregnant and 20 non-pregnant) and blood samples were collected from slaughterhouses in Cairo during the breeding season (December, 2020 to March, 2021). In this notion all pregnancies were in the left uterine horn and the corpus luteum was on the right ovaries. Total RNA was isolated from uterine tissues (body and right & left horns), RT-PCR was performed for candidate genes, as well as measuring level of serum progesterone (P4) and histological examination. Data analysis of gene expression was done using two-way ANOVA, followed by Tukey's multiple comparisons test, while the level of P4 was analyzed using the student's t-test. Results showed that JUN, SLCO2A1, VEGFA and PTEN mRNAs were up-regulated (P<0.001) in the uterine body, left and right uterine horns of pregnant groups. In serum, the level of progesterone increased in pregnant she-camels compared to non-pregnant ones. Histological examination revealed that the uterine body of pregnant she-camel, showed numerous congested blood vessels with thickened walls, also the left and right uterine horns of pregnant she-camel showed normal increased glands and less fibrous stroma compared to non-pregnant ones. We supposed that hormonal regulation as well as the ovulatory site (right side), which was contralateral to implantation site (left side) might regulate directly or indirectly the gene expression in both right & left uterine horns and uterine body in order to maintain pregnancy in dromedary she-camel.

Mammary gland carcinoma in a dog caused the hypertrophic osteopathy

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One of the paraneoplastic syndromes may be hypertrophic osteopathy (HO). It is characterized by the symmetrical periosteal proliferation of new bone on the four limbs. The pathogenesis of hypertrophic osteopathy is not completely understood and it is thought to be multifactorial connected with vagal nerve stimulation, circulation shunting, and hormones. OH is commonly connected with metastatic lung disease.
A 14 years old mixed breed dog (35 kg) was referred to the clinic with the problems with movement. Approximately one year earlier the total mastectomy was performed because of severe mammary gland carcinoma. In anamnesis bitch was treated with low doses of steroids two weeks prior to consultation because of lameness and swelling on all four limb.
During physical examination all four limbs were painful to touch and had distal edematous swelling, extending from mid-tibia to mid-metacarpal and tarsal regions. Also and nasal hyperkeratosis was Hematological examination was unremarkable, however in blood biochemistry the alkaline phosphatase (ALP) was elevated (1891, reference range 15–212 [U/l]). The radiological examination revealed an extensive periosteal reaction in all four limbs as well as the lung tumor. Because of severity of the case only the analgesics administration was performed. The bedinwetmab (20 mg Librela SC) was implemented once per month. Approximately five months later, dog was euthanized because of respiratory distress and sever problems in walking. Thus, this case study shows the bedinwetmab efficacy as a pain reliefer in dog with sever OH.

Genotyping of *NOTCH2* gene in fertile and anestrum cows

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Notch signaling plays important roles in the mammalian ovary including, primordial follicle activation, follicle development, regulation of granulosa cell proliferation and corpus luteum (CL) formation. There are no data about NOTCH gene polymorphism in relation to ovarian inactivity in bovine. So, our study aimed to clarify the association between *NOTCH2* polymorphism and ovarian inactivity in cows. The blood samples were collected from 69 cows with inactive ovaries and 114 normal cows with active ovaries.The non-fertile animals had history of anestrum for 6 months to one year after parturition.The cows were examined by ultrasound for determining the status of reproductive system. The ovarian activity was represented by the presence of corpus luteum and follicles larger than 1 mm in size. DNA was extracted from both groups, and then the PCR and single-strand conformation polymorphism techniques (SSCP) were performed for detection of *NOTCH2* gene polymorphism. The results showed a genetic polymorphism with different patterns associated with anestrum in cattle. The sequencing data determined the SNPs related to ovarian inactivity represented by anestrum. In conclusion, *NOTCH2* gene in cattle is polymorphic with different SSCP patterns. These finding suggest that this gene could be used as a genetic marker for ovarian activity in bovinespecies.

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INVITED LECTURE

Persistent breeding-induced endometritis in mares – a multifaceted challenge: from clinical aspects to immunopathogenesis and pathobiology

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Endometritis is ranked as the third most common medical problem seen in equine practice. Persistent breeding-induced endometritis (PBIE) is the most important cause of subfertility in broodmares and embryo donor mares. All mares display a physiological post-breeding inflammatory response, which is resolved within 48 hours; however, 15-20% display a persistent and prolonged inflammatory response, and such type of mare is deemed susceptible to PBIE. Mares susceptible to PBIE have deficient mechanisms of uterine clearance, ineffective anti-inflammatory response, and are prone to develop infectious endometritis. Some mares susceptible to PBIE have anatomic defects such as poor perineal conformation (e.g., elongated and sloped vulva, pendulous uterus, and torn cervix) that can be surgically fixed, and some defects that cannot be fixed (e.g., ventral sacculation). Anatomical defects explain susceptibility to PBIE in part of the mares; however, others; have no anatomical defects; but an exacerbated and persistent post-breeding inflammatory response for unknown reasons. Recent multi-omics studies have shown/confirmed some differences between mares resistant and susceptible to endometritis but have failed to identify the primary cause. Currently, endometritis is diagnosed via transrectal ultrasound (i.e., to identify intrauterine fluid accumulation), endometrial cytology (i.e., to assess inflammatory cells quantity and features), and traditional aerobic culture, and less commonly by endometrial biopsy. While these techniques are currently used in clinical practice, they are prone to false positive and false negative results leading to unnecessary treatment or misdiagnoses. Future studies should be focused on developing rapid and novel diagnostic modalities. Treatment for endometritis includes ecbolics and uterine lavage to promote clearance of the uterus; anti-inflammatories to modulate the exacerbated inflammatory response; non-traditional therapies to be infused or flushed the uterus and antibiotics. The latter is excessively used in clinical practice, and evidence suggests that mares do not necessarily need antibiotic infusion after breeding. The indiscriminate use of antimicrobials favors the development of antimicrobial resistance. Thus, alternative therapies should continue to be investigated; some alternative products, such as platelet-rich plasma, have shown some encouraging results; however, further investigation is warranted. Studies need to be conducted on how the various uterine therapies affect the uterine microbiome and resistome. Endometritis is a challenging disease requiring multidisciplinary knowledge for the most effective diagnosis and therapy.

INVITED LECTURE

Diagnosis and treatment of retained placenta in cattle

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Introduction

Retention of fetal membranes, defined as the fetal placenta not being expelled within 12 to 24h after parturition, lowers productivity and fertility resulting in significant economic losses for the modern dairy industry. Although this health problem is in itself not extremely dangerous nor life threatening for the individual patient, it brings a lot of stress for many modern dairy farmers since it often presents like a start of an avalanche of concomitant transition diseases like metritis, displacement of the abomasum, ketosis, clinical and subclinical endometritis and finally repeat breeding. Therefore, this in se rather harmless but very annoying problem requires special attention and an efficient preventive and therapeutic approach in order to minimize concomitant economic losses. In the present manuscript, we will focus on the diagnosis and treatment of this all too common reproductive disorder.

Definition

Retained placenta (RP) occurs when the fetal membranes after birth are retained for a longer period than physiologically normal in the uterus of the early postpartum cow. Loss of the placenta in the cow occurs during the third stage of parturition and the process of separation has been stated to take less than six hours. The definition of RP varies widely in the literature ranging from retention of the placenta for 6 to 71 hours postpartum (Van Werven et al., 1992). The most commonly used definition is the retention of fetal membranes for 12 to 24 hours or more post partum, although according to some authors, retention for more than 6 hours would probably be a better definition particularly in older cows (Van Werven et al., 1992). The cow and the water buffalo are reported as the only domestic ruminants in which retained placenta is a relatively often reported abnormality accompanied with a variety of secondary problems finally leading to important economic losses (Laven and Peters, 1996). It affects other ruminants like sheep and goats less frequently with little evidence of associated problems in these species.

In some papers, authors differentiate between primary retention of fetal membranes and secondary retention. With primary retention they refer to a real problem of cotyledon dehiscence from the caruncular crypts, while a secondary retention refers to the fact that the detachment has been successfully completed but the placenta has not been expelled out of the genital tract and remains in the uterine cavity although she is completely loose there. Mostly cited reason for this so called secondary retention, is hypocalcemia or any other reason causing inertia of the uterine musculature.

The average annual incidence of RP in cattle varies from 3 to 10-12%, and is known to be highly variable between farms and can reach even up to 30%. Gohary and LeBlanc (2018) estimated the economic cost of a RP case to be 297$, due to production losses, treatment costs, reproductive disorders and increased culling.

Diagnosis of retained placenta in cattle

Based on the definition, the diagnosis of RP in cattle is in principle very easy to make and is indeed most often made by the farmer: when he/she has not found any placenta for 24h or longer after parturition, the cow will be indicated as suffering from RP. In many of these cases, the cow presents herself with a large part of the placenta hanging out of her vulva facilitating the diagnosis.

In practice however especially in circumstances where fresh cows are loosely housed in a maternity pen (in straw for example), cows are free to move and may eat their placenta in an instinctive attempt not to attract potential predators. Eventually, other animals like dogs or cats may ‘steal’ the placenta to eat it. Anecdotical stories are available of fresh cows laying down in tie stalls with extreme lacerations at the vulva caused by a dog that started to eat the (retained) placenta and ended eating a large part of the vulva. So, in cases where cows are free to move and no placenta was found, it is advisable to perform a vaginal examination to double check whether the placenta is retained or not. In most cases, parts of the placenta can be felt in the vagina, cervix or somewhat deeper in the uterus and the diagnosis will be readily made.

Following caesarean section, it can also be harder to diagnose RP. During surgery, large parts of the placenta are cut so that only remnants are left in the uterine cavity. In most cases, the rest of the placenta will be shed the day following the surgery, but in some cases at least some remnants may retain. Especially when only a relatively small part of the placenta has been left in the uterus, this remnant part can be retained quite firmly and deep in the uterine cavity, not being reachable via vaginal exploration. The latter may make the diagnosis very difficult or even impossible. This specific case is however very uncommon in dairy cows, since in dairy cows caesarean sections are very seldom and membranes will be expelled in a whole, not leaving little pieces behind deep in the uterine cavity.
As mentioned higher, RP is in many cases accompanied by one or even multiple other well-known transition diseases. Therefore, veterinarians who are confronted with a cow suspected from or indeed suffering from RP, should not neglect to perform a thorough clinical examination to detect any accompanying disorder. Besides the general clinical examination (respiratory rate, pulse, temperature, hydration status, lymph nodes, mucosae), one should not forget to control for ketosis (for example using cowside ketosticks) and to exclude a potential displacement of the abomasum.

Treatment of retained placenta

Although RP is frequently occurring in modern bovine husbandry, and although the diagnosis is pretty simple, there is a continuing debate about the most efficient treatment strategy for cows attained by this problem.
Although a plethora of studies have been performed to study the results of different treatments, there are multiple reasons why it is very difficult to come forward with generally accepted conclusions and evidence with regard to an efficient RP treatment. One of them is the question: which parameter should be used to evaluate the result of the treatment? Should one focus on the general condition of the cows, like the number of cows with fever; critically evaluate the dry matter intake or milk yield of the cows, or should one focus on the number of cows suffering from metritis, clinical or subclinical endometritis following treatment. After all, in terms of fertility, the most crucial evaluation of the end result of the treatment, should be an in depth evaluation of the key performance indicators for reproductive performance, like the pregnancy rate after first and eventual following inseminations, the number of inseminations per pregnancy and the number of cows culled for reproductive failure among others.
An other reason hampering the scientifically sound interpretation of field studies regarding RP treatment, is the difficulty to include a control group. It is obvious that studies only make sense when control groups that did not receive any or a placebo treatment are included. The latter is a real challenge when performing field studies about RP treatment. Is it for example acceptable from an ethical/animal welfare point of view to not treat an RP cow, or to treat it with a placebo? Furthermore, the farmers who are participating in such field trials might be really challenged to treat the cows belonging to the control group behind the back of the researcher.
Taking these specific challenges into account, we herewith try to give some general conclusions of the studies performed.

Curative treatments

Manual removal

In the very past, at least in Belgium, a lot of large animal practitioners daily treated multiple RP cows by manually removing the remaining membranes. In most cases, veterinarians cleaned and disinfected the perineal area to subsequently enter the genital tract to one after the other manually unpeel each individual placentome. Still, in many countries manual removal is a common procedure in modern cattle practice, mainly for the two logical benefits for the farmer: first it may improve parlour hygiene (eventually necessary for the sale of milk for human consumption in several countries), and secondly, the removal of the source of a disagreeable odour. Those are probably the reasons why in many countries, practitioners still undertake attempts to manually remove the placenta even in cases where this requires rather strong unpeeling activities at the individual placentomes.
Multiple studies have however shown that by trying to unpeel the individual cotyledons from the caruncles, in most cases microtraumata do appear. These microlesions are an easy entry port for bacteria to enter into the peripheral circulation, in many cases presenting an important reason for a higher risk to suffer from a far more severe clinical course of the problem. Therefore, most recent studies discourage to unpeel the placentomes.
There is however 1 exception to this generally accepted rule and that is the treatment of the so called 'secondary retention of the membranes'. As mentioned earlier, this type of placental retention is based on uterine inertia and the subsequent disability of the uterus to expel the placenta. In these cases, the placenta can easily be removed by causing minimal traction and without any unpeeling activity of the placentomes.

So, basically, in case one is confronted with a case of placental retention, we advise to thoroughly clean and disinfect the perineal area of the cow, in order to perform a manual exploration of the vagina and to grasp the placenta. Slight pulling should be applied in an attempt to remove the placenta. In case obvious progression in removing the placenta is easily noticeable, a sustained slight pull should be applied till the whole placenta can be removed out of the genital tract of the cow. In most cases however, immediate resistance is noticeable when pulling the placenta. The latter indicates that the dehiscence of the cotyledons out of the caruncular crypts did not occur (=primary or truly retained placenta). In these case, further attempts to manually remove to the placenta should be immediately stopped to avoid microlesions in the endometrium. The protruding parts of the placenta should be cut off and the back of the cow should be cleaned again.

Antibacterial therapy

Both acute and chronic uterine disease post partum are well known sequelae of RP. Therefore, antibacterial therapy has been commonly used in an attempt to prevent the development of this infectious postpartum uterine disease complex and its subsequent negative effect on fertility.

Antibacterial drugs have most commonly been administered via the uterus. However, when they are applied via this route, they are patently unsuccessful in preventing metritis, even when given repeatedly. Their main effect is a reduction of putrefaction, which decreases the bad odour but may prolong the retention. The effect of this kind of treatment on subsequent reproductive performance is inconclusive. While some trials did show a benefit, many others did not and some even showed a negative effect.

When applying antibacterial drugs into the uterus, one should not forget the very specific environment in which they deposit the drug. The intra-uterine environment early after calving is known to be anaerobic, to contain a lot of debris and lochia which on their turn may contain specific enzymes like penicillinases. Tetracyclines have been named to well maintain their antibacterial activity in this specific environment. Recent data however mention the increasing incidence of resistance against tetracyclines. Furthermore, we are not aware of any well carried out field study that showed that RP cows intra-uterinely treated with tetracyclines outperformed the control cows in terms of fertility parameters.

Besides applying intra-uterine antibacterial therapy, also general antibacterial therapy by injecting the cows parenterally has been studied in multiple field studies done over different continents in the world. In many countries, injecting the cows with penicillin when treating them intra-uterinely with antibiotics, has been applied for long time. The latter was done as a rather preventive therapy to avoid that cows which had not expelled the placenta, should not suffer from febrile metritis and accompanying other diseases.

The choice of antibiotic to be used has been proposed to depend on the concentrations of derivates of the product that are reached in uterine tissues and lochial fluids and on the MIC values of these antibacterial products against the most common bacteria known to cause uterine infections, such as *Escherichia coli* and *Fusobacterium necrophorum* (Okker et al., 2002). In 2006, a large field trial was published by Drillich et al. in which common therapies like manual removal (MR), local antibacterial therapy (AP) and the combination of both (PR) were tested against a reference group (REF). In all groups, cows were injected parenterally with ceftiofur during 3 to 5 days when their body temperature raised above 39,5°C during the first 10 days after calving. In case of continued fever after 5 treatments, cows received a different antibiotic as an escape therapy. Of all animals, 79.8% had a body temperature of ≥39.5°C at least once within 10 d postpartum and were treated with ceftiofur. Occurrence of fever within 10 d postpartum was significantly lower in groups AP and PR compared with REF, but was not different between groups MR and REF. Risk of receiving an escape therapy in case of fever after 5 treatments with ceftiofur, did not differ among groups. Reproductive performance measures did not differ significantly between group REF and any of the comparison groups. Compared with a treatment protocol based only on systemic treatment with antibiotics for cows with a fever, neither intrauterine antibiotics nor manual removal of fetal membranes alone or in combination with local antibacterial therapy reduced proportions of cows needing an escape therapy nor did those treatments improve reproductive measures in the current lactation. Systemic treatment alone based on elevated rectal temperature was effective and reduced the use of antibiotics compared with therapies that included intrauterine antibiotics. In some European countries however, the use of ceftiofur in livestock has been seriously discouraged in an attempt to reduce the incidence of resistance against antibacterial drugs.

Cows that have suffered from RP regardless of an eventual treatment, should be subjected to an in depth clinical examination before breeding, preferably around day 35 after calving. Aim to perform this examination is to identify cows eventually suffering from any sequential uterine disease like (sub)clinical endometritis and pyometra. Since the uterine infection caused by RP presents a significant risk for any of these diseases, close

attention including an appropriate therapy should be applied to these cows in an attempt to safeguard their reproductive performance.

Other curative treatments

There is a plethora of studies done to examine the effect of other than antibacterial curative treatments, like for example the use of ecbolic drugs ($PGF_{2\alpha}$ and its analogues, ergot derivates, oxytocin and β_2-adrenoceptor antagonists). The rationale for their use is that they stimulate uterine contractions and thus physically aid the expulsion of the membranes. The consensus of opinion papers appears however to be that the response to ecbolic drugs is unpredictable and poor.

Besides the use of ecbolic drugs, also the use of local enzyme therapy (collagenase and hyaluronidase) has been tested. Aim of these therapies was to stimulate the detachment of the cotyledons to facilitate sequential manual removal. It was found that hyaluronidase had no effect but that collagenase did facilitate placental separation. So, the conclusion was that this local treatment (infusion into one or both umbilical arteries) is promising although time consuming, cumbersome and probably too expensive.

Much work remains to be done on the use of these drugs, particularly in the light of greater knowledge of placental separation.

Preventive treatment

For multiple reasons, it is far better to prevent than to treat diseases. This is for sure the case for the RP disease complex in dairy cows. When searching for preventive strategies against a certain disease, one should know the typical risk factors of that disease. Major risk factors for RP are: dystocia, twinning and abortion. For secondary RP, the most well known risk factor is (subclinical) hypocalcemia.

Main preventive measures against dystocia, consist of a good young stock rearing leading to well grown heifers ready to be inseminated at an age of 13 to 15 months, in combination with a well-considered bull choice to inseminate each individual cow and heifer. Especially in heifers, the use of bulls known to procreate small calves is in this context very important. Here, we also refer to the study of Potter et al (2010), which concluded that the use of sexed semen to increase the number of (smaller) female calves has the greatest potential to reduce the incidence of clinical endometritis.

To minimize the number of abortions, protocols should be applied at herd level to eradicate infectious diseases like BVD, IBR and especially Neosporosis. In many European countries, mandatory 'official' programs to eradicate the circulation of viruses like BVDv and IBR have successfully been implemented. Neosporosis however remains a problem and this protozoan is at the moment the most often diagnosed cause of bovine abortion in multiple countries. On many dairy herds, more attention should therefore be paid to minimize abortions related to Neosporosis, based on avoiding infections brought in by a dog and minimizing vertical spread (=mother to daughter) of the infection by refusing daughters from seropositive mothers.

On many present-day dairy herds, the most important risk factor for RP is twinning. Within the Holstein breed, parallel with the increase in milk yield, the number of twins has dramatically increased, raising in some herds above 15%. The birth of a twin is in most cases followed by the retention of at least one placenta. Since this twinning phenomenon seems to be associated with the increase in milk yield, it might continue to increase while further increasing milk yield. Multiple studies have been performed to study how to tackle this problem, but none have come up with a real solution applicable in the field. Hopefully, genomic testing may help in the future to reduce the number of twin births on our dairy herds.

In order to prevent secondary RP, attention should be paid to optimize peripheral Ca-levels in the peripartum cows. The latter should be based on focusing on dietary measures during the dry period. Well accepted strategies in this context are: optimizing DCAD (dietary cation anion difference), avoiding over-conditioning, and low Ca and high Mg supplementations. Furthermore, supplementation of Vit E and Selenium (to support overall immune competence) and Vit A (to support local immune response at the endometrium), have been advocated to prevent RP or to minimize its sequential diseases.

Conclusion

The seemingly simple diagnosis of 'retained placenta' hides a condition of great complexity. A large number of causal factors has been related to an increased incidence of the condition, but little is known about how the majority of these factors induce placental retention. Hence, the full pathogenesis for this disease still needs complete elucidation which impedes the search for an effective treatment. Since the most important

consequence of RP is a bacterial infection of the uterus, curative treatment is mainly based on antibacterial treatment. Main challenge here is to identify the animals that benefit from an eventual antibiotic treatment especially when attempting to obey to the principles of prudent use of antibiotics in the dairy sector. Based on the latter, a commonly advocated strategy is to take the temperature of each individual cow during the first 10 days following calving and to only treat the animals with fever (>39,5°C) via systemic antibiotic treatment. A close follow up including an appropriate treatment in an attempt to safeguard their reproductive performance in the current and next lactations should be done for all RP cows.

Comparison of cytological, microbiological and histopathological findings of genital canal in healthy cows and cows with vestibulo-vaginal sphincter dysfunction

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Vulva, vestibulo-vaginal sphincter and cervix are three main mechanical barriers preventing uterus from contamination. Vestibulo-vaginal sphincter dysfunction (VVSD) causes negative intravaginal pressure and lead to air or debris into the vagina. Here, it was aimed to compare the cytological, microbiological and histopathological findings of the vagina, cervix and uterus in healthy cows and cows with VVSD. The genital tract of cows with VVSD (n=6) and healthy cows (H) (n=6) brought to the slaughterhouse were studied. Cytological, microbiological and histopathological samples were taken from the vagina, cervix and uterus of all cows. No clinical pathology was observed in the cows of group H, but *E. coli* was cultured in the samples taken from the vagina of 2 cows and the cervix of 1 cow. In the evaluation of cytological and histopathological results of these cases, few neutrophil leukocyte infiltrations were observed only in case 2. In group VVSD, microbiological culture of all cases was positive except case 6, and *E. coli* was the main pathogen. The cytological findings (cases 4 and 5-vagina; cases 3, 4 and 5-cervix; cases 4 and 5-uterus) were compatible with histopathological findings (cases 3, 4 and 6-vagina; cases 4 and 4-cervix and uterus). There is a relationship between VVSD and genital tract contamination and pathology, and VVSD makes the genital tract susceptible to infections. It is important for clinical practice that VVSD should not be ignored in the evaluation of genital canal health of cows with infertility problems.

A new method of cytological sampling from the endometrium of dairy cows

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Subclinical endometritis (SE) is one of the most frequent diseases affecting the reproductive tract of dairy cows. It can be defined as the superficial inflammation of the endometrium extended no deeper than the stratum spongiosum. SE is difficult to diagnose because there are no visible signs in course of the disease. Cytology is considered the best technique to diagnose SE. In recent years numerous of technics were established to perform cytological sampling for eg. low-volume uterine lavage, cytobrush, cytotape, or uterine secretions. Nonetheless, there is still a lack of efficient, safe, repetitive, and easy-to-use by not experience examiner diagnostic method for SE. Therefore, we are attempting to develop a new cytological technic solving the above-mentioned imperfections. The proposed method using flock swob allows for the atraumatic collection of cytological material. This effect is achievable thanks to the flexible construction of the swobs rod, soft nylon fibers combined with an automatized rotation enabled by a developed 3D printable mechanism. Preliminary testing on a uterine obtained in the abattoir was performed in comparison to the cytobrush and biopsy. The study included parameters such as ease of implementation of the method, PMNs percentage, quality of the smears, red blood cell contamination, and suitability for molecular biology techniques. Agreement in the percentage of PMNs between cytobrush and the new method was good. The new method showed superior ease to use and was more likely to have fewer red blood cells in smear than cytobrush.

The role of microRNAs in endometritis and their potential as a diagnostic biomarker

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Although equine industry is a highly financially lucrative business, it mainly hampers due to endometritis, which is a worldwide problem. Recently in equine species, many studies revealed that serum microRNAs (miRNAs) could be used as non-invasive biomarkers either for normal physiological condition (early pregnancy) or disease condition (sarcoid disease). Nothing is known about the expression pattern of free serum miRNAs and post-transcriptional regulation of inflammatory immune response genes in mares with endometritis. Also, it was indicated that the molecular composition of extracellular vesicles (EVs) cargo (miRNAs) is reflective of physiological or pathophysiological changes in their cell or tissue of origin. Hence, cargo from EVs has significant potential as biomarkers for disease diagnosis. To date, EVs are produced from all cells, and are known to play roles in many reproductive processes. However, there is a lack of knowledge concerning role of free serum miRNAs as well as cargo from EVs in equine endometritis. Therefore, identification and quantification of some candidate free serum miRNAs as well as cargo from EVs, from mares with endometritis might serve as useful step for a better understanding the molecular regulation of endometritis in mares. Furthermore, the dysregulation of miRNAs in mares with endometritis might contribute to the aberrant expression of inflammatory immune response genes, and subsequently perturbs normal uterine function and homeostasis. This review will show how to exploit miRNAs as a new diagnostic tool for endometritis in mares.

Keywords: Equine, endometritis, miRNAs, cargo, serum, immune response genes

Endometritis in breeding mares from Spain: Microbial prevalence and antimicrobial susceptibility.

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Background: Equine endometritis is a cause of great economic losses for the equine breeding industry. A proper diagnosis, usually including bacteriological culture from uterine swabs, is essential to establish a correct treatment. A retrospective study was carried out from 2015 to 2022 in order to determine the most prevalent non-fastidious bacterial pathogens isolated from uterine swabs and their antibiotic susceptibility in breeding mares with endometritis.
Materials and methods: A total of 144 mares were included in the study. Samples were collected using double-guarded endometrial swabs and submitted to the laboratory in Amies transport medium in refrigerated conditions within 24 hours. Swabs were cultured in aerobic and microaerophilic conditions on Columbia blood agar plates and chocolate agar plates, respectively, incubated at 37ºC for 24-48 hours. Positive samples were subcultured and isolates were identified using Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Antimicrobial susceptibility test (AST) was performed using disc-diffusion assay including six antibiotics recommended by the Clinical Laboratory Standards Institute (CLSI) plus neomycin.
Results: Bacterial growth was obtained from 72/144 samples (50%), 53 showed pure growth whereas 19 showed at least two different colony types. Identification at species level detected 29 different species [18 Gram-negative (62,07%) and 11 Gram-positive (37,93%)]. The most prevalent species was *Streptococcus equi* subsp. *zooepidemicus* (29/72; 40,27%) followed by *Escherichia coli* (25/72; 34,72%), *Acinetobacter iwoffii* (3/72; 4,17%) and *Streptococcus dysgalactiae* (3/72; 4,17%). The results of the AST showed that penicillin was the antibiotic with the highest resistance rate (41/88; 46.59%) whilst ceftiofur showed the highest susceptibility rate (70/88; 87.5%).
Conclusions: The percentage of positive cultures and the most prevalent species found (*Streptococcus equi* subsp. *zooepidemicus* and *Escherichia coli*) were in accordance with previous studies. The results of our study would suggest that penicillin would not be the best option for the treatment of infectious endometritis in breeding mares.

Cows with subclinical endometritis show lower IL1RA / IL1B ratio in uterine secretions detectable *in vivo* and at the abattoir

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Worldwide, cows with subclinical endometritis (SE) show lower fertility rates, which impacts economics of the dairy industry. The adequate regulation of pro- and antiinflammatory factors, such as the proteins of the interleukin (IL) 1 family, plays a central role in the prevention and healing of endometrial inflammation. Therefore, this research project was targeted on investigating the concentration of proinflammatory IL1B, its antagonist IL1 receptor antagonist (IL1RA) and their ratio in uterine secretions of cows with (SEpos) and without SE (SEneg) sampled either at the abattoir (SEpos n=3; SEneg n=32) or *in vivo* (SEpos n=5; SEneg n=21). SE was diagnosed if cytobrush samples showed >5 % polymorphonuclear cells. To measure IL1B, a commercially available immunoassay (AlphaLISA) was used. For IL1RA analysis, an *in-house* AlphaLISA had been established previously. Independent of the type of sampling, higher concentrations of IL1B were detected in SEpos compared to SEneg cows (abattoir: p=0.027; *in vivo*: p<0.001). IL1RA concentration was higher by trend in samples from the abattoir (p=0.071; *in vivo*: p=0.325). A lower IL1RA/IL1B ratio was detected in SEpos compared to SEneg, (abattoir: p=0.082; *in vivo*: p=0.002), indicating a more proinflammatory uterine environment in cows with SE. The results highlight the importance of IL1B and IL1RA in the context of bovine uterine inflammation and suggest the potential of the IL1RA/IL1B ratio as a possible biomarker for SE. Whether the observed shift in the IL1RA/IL1B ratio reflects either a cause for or a consequence of SE has to be examined in further studies.

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