Welcome to the 6th International Conference on Uterine Disorders and Corpus Luteum Function

Dear Colleagues,

We are pleased to welcome you to Wrocław for the conference: "International Conference on Uterine Disorders and Corpus Luteum Function". This is the 6th edition of our Conference and we hope not the last. The aim of the Conference is to share new research and understandings in the area of biology and pathology of reproduction, specifically, luteal function, luteal disorders/insufficiency, endometrial pathologies such as endometritis, endometrosis, and adenomyosis as well as new strategies and possible interventions to address these pathologies.
This year's conference is taking place in a specific city at a specific time. Exactly 90 years ago, in 1934 almost simultaneously, four research teams published papers on the purification and structure of primary hormone produced by Corpus Luteum- progesterone. However, the pioneer of research on the structure and role of progesterone is indeed Dr. Ludwig Fraenkel. We will be hosting at the University, where over 100 years ago Dr. Fraenkel successfully conducted research on the role of progesterone and corpus luteum in reproductive biology and medicine. Therefore, Ludwig Fraenkel is so called the "spiritus rector" on the early progesterone research. Exactly 90 years ago, supported by Wroclaw (Breslau) university, group scientists (J. Born, K. Slotta, E. Fels, H. Ruschig) developed and successfully completed work on purification and semi-quantitative determination of progesterone. Tree decades earlier, on May 20, 1900, Ludwig Fraenkel proved that "elimination of the corpus luteum prevented the onset of pregnancy" in an experiment on rabbits, beginning a new era in reproductive endocrinology. For the details please see Frobenius (1999) Ludwig Fraenkel: "spiritus rector" of the early progesterone research. European Journal of Obstetrics & Gynecology and Reproductive Biology. 83:115–119.
Over the years we have gathered a group of regular participants in our conference and if we may say good friends with a common passion for reproductive biology and pathology. We therefore really look forward to meeting you and have many exciting discussions. We wish all of you many fruitful discussions, meetings with old friends, establishing new partnerships and having a pleasant stay in Wrocław.
We want to thank all our invited speakers who have accepted our invitation to come to Wrocław. We are very pleased to have them with us in our meeting and look forward to hearing about the interesting research.
Finally, we want to thank the organizing and scientific committees for their hard work. We are grateful to you for your work and support.

Dariusz Jan Skarżyński and Wojciech Niżański

Organizers:

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

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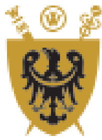

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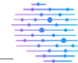

Doskonała
Nauka

POLSKIE TOWARZYSTWO
NAUK WETERYNARYJNYCH

We are grateful for the support of the Conference:

- Polish Ministry of Sciences and Higher Education "Doskonała Nauka 2" (Grant no. CONF/SP/0211/2023/01)
- Prof. Mariusz Piskuła – Director of the Institute of Animal Reproduction and Food Research of PAS in Olsztyn
- Prof. Krzysztof Kubiak – Rector of the Wrocław University of Environmental and Life Sciences
- Prof. Piotr Ponikowski – Rector of the Wrocław Medical University
- Prof. Jan Twardoń – President of the Polish Society of Veterinary Sciences

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Prof. Jan Twardoń – President of the Polish Society of Veterinary Sciences
Prof. Tomasz Janowski – University of Warmia and Mazury in Olsztyn
Prof. Bernd Hoffmann – *professor emeritus*, Justus-Liebig-Universität Gießen

GENERAL INFORMATION

The Conference Venue will be the Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences (Norwida 31 str., 50-366 Wrocław), lectures will be held in the conference room 1W (entrance from the inner courtyard, last floor).

Plus Code: 4367+VW Wrocław, GPS: 51.11227, 17.06496

Afternoon ceremonial session of the Ludwig-Fraenkel Symposium on Corpus Luteum and Progesterone Function will be held in the Collegium Anatomicum – Department of Morphology and Embryology, Medical University of Wrocław (Tytusa Chałubińskiego 6a, 50-368 Wrocław).

Plus Code: 4368+JF Wrocław, GPS: 51.11159, 17.06626

FOOD SERVICE

Meals

Lunches (lunch boxes) will be provided by organizers for all conference members according to the schedule of the meeting.

Coffee Breaks

Coffee, beverages and pastries will be available in the foyer close to the presentation room.

SOCIAL EVENTS

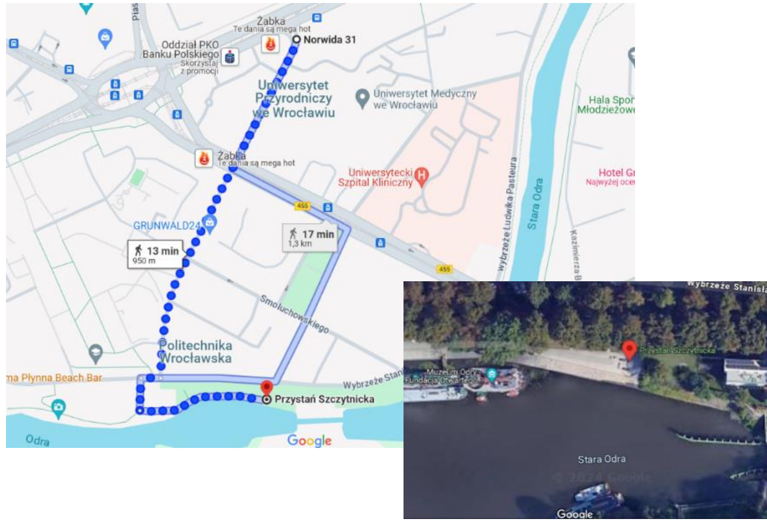
Get together

Monday, 9th September, 5:40 p.m. (Free for registrants, **Collegium Anatomicum**)
Visiting Medical University of Wroclaw – Anatomical Museum, Old Library, and places where Dr. Fraenkel and Dr. Slota worked to discover and characterize progesterone.

Gala Dinner

Tuesday, 10th September, 7:00 p.m. – 10:00 p.m. (Free for registrants) Cruise with dinner on the barge "Rusałka" along the Odra River. Dress code - Smart casual. The below pointed "Przystań Szczytnicka" is a port of ships on the river and located in walking distance from the Venue.

Plus Code: 4347+PF Wrocław, GPS: 51.10684, 17.06366

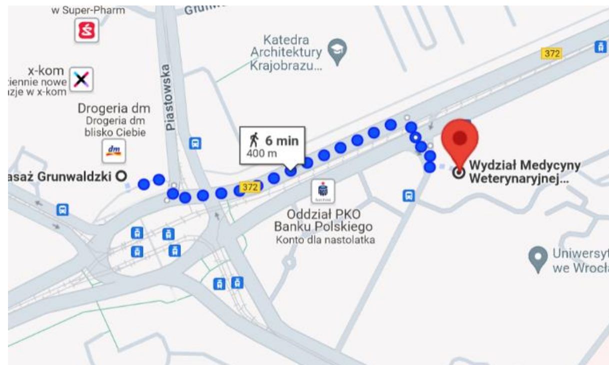


Wrocław by night

Wednesday, 11th September, 7:00 p.m. – 10:00 p.m. (Free for registrants)- guided Wroclaw sightseeing tour (in Polish and English).

Meeting point: Pasaż Grunwaldzki (Grunwald Mall), address: plac Grunwaldzki 22, 50-363 Wrocław.

Plus Code: 4365+VV Wrocław, GPS: 51.11228, 17.05984



Swidnica, Książ Castel and Książ Horse State Stud

Thursday, 12th September, 9:00 a.m. – 5:00 p.m. Scientific excursion to Horses State Stud (extra paid). The bus will stop in front of the Hotel ZOO.
Address: Zygmunta Wróblewskiego 7, 51-627 Wrocław.

Plus Code: 433J+MM Wrocław, GPS: 51.10427, 17.08184

Conference program

9th SEPTEMBER 2024

08:00 – 09:00 Registration (Lecture Room 1W, Vet. Faculty)

09:00 – 09:15 Opening remarks: *Prof. D. Skarżyński, Prof. Wojciech Niżański*

09:15 – 11:05 Session I: Formation of the CL and maintenance of the luteal function
Moderator 1: Prof. Agnieszka Rak *(Jagiellonian University, Krakow, Poland).*
Moderator 2: Prof. Mariusz Kowalewski *(Vetsuisse Faculty, University of Zurich, Zurich, Switzerland)*

Lectures:
09:15 – 09:50 Agnieszka Rak *"A new perspective on the regulation of corpus luteum: expression and role of visfatin (NAMPT) on luteal transcriptome, proteome and functioning"*
09:50 – 10:25 Mariusz Kowalewski *"Targeted and Global Insights into the Regulation of CL Function in the Dog"*

Short oral presentations:
10:25 – 10:45 Vijay Simha Baddela *"Single-cell reconstruction of the bovine corpus luteum"*
10:45 – 11:05 Karolina Pich *"Comparative analysis of expression of omentin-1 in porcine ovarian follicles vs corpus luteum and it's in vitro effects on proliferation, apoptosis and steroidogenesis"*

11:05 – 11:30 Coffee break

11:30 – 14:00 Session II: Regression of CL function and luteal dysfunctions
Moderator 1: Dr. Camilla Hughes "Luteal responsiveness to prostaglandin F2A: from development to regression" (Department of Animal Science, College of Agriculture Sciences, University Park, PA, USA),
Moderator 2: Prof. Sławomir Wołczyński *(Faculty of Medicine, Medical University of Bialystok, Poland)*

Lectures:
11:30 – 12:05 Camilla Hughes *"Luteal responsiveness to prostaglandin F2α: from development to regression"*
12:05 – 12:40 Sławomir Wołczyński "*Luteal phase defect in women"*

Short oral presentations:
12:40 – 13:00 Ewa Mlyczyńska *"Phoenixin-14 modulates angiogenesis, cell proliferation, and apoptosis in porcine corpus luteum"*
13:00 – 13:20 Muhittin Tekin *"Effect of heat stress on the mRNA expression in the corpus luteum of dairy cows"*
13:20 – 13:40 Marvin Hölper *"Corpus luteum diagnostic via ultrasound - A viable tool for treatment decisions in Ovsynch protocols"*

13:40 – 14:30 Lunch break

15:00 – 19:00 Ceremonial session of the Fraenkle's Symposium (the *Collegium Anatomicum*, Medical University of Wrocław)

15:00 – 15:20 Official opening ceremony:
– Prof. Piotr Ponikowski – *Rector of Medical University of Wroclaw*
– Prof. Krzysztof Kubiak – *Rector of Wroclaw University of Environmental and Live Sciences*
– Prof. Bernd Hoffmann – *Justus-Liebig-Universität Gießen*
– Prof. Jan Twardoń – *Wroclaw University of Environmental and Live Sciences*

15:20 – 16:10 Plenary lecture: Prof. John Davis "Control of Luteal Function by Luteinizing Hormone: Insights into multi-organelle control of steroidogenesis" *(Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE, United States)*

16:10 – 16:30 Bernd Hoffmann *"Dr Ludwig-Fraenkel – "spiritus rector on the early progesterone research"*
16:30 – 16:50 Tomasz Fuchs *"Progestagens in current human reproductive medicine"* (Wrocław Medial University, Poland)
16:50 - 17:10 Malgorzata Ochota *"Progestagens in current veterinary reproductive medicine"* (Wroclaw University of Environmental and Live Sciences, Wroclaw, Poland)

17:10 – 17:40 Coffee break

17:40 – 19:00 Visiting Medical University of Wroclaw
– Anatomical Museum &Old Library – **Prof. Marek Syrycki**
– Places where Dr. Fraenkel and Dr. Slota worked to discover and characterize progesterone (former Dep. of Reprod.) – **Prof. Tomasz Fuchs**

10th SEPTEMBER 2024 (Lecture Room 1W, Vet. Faculty)

09:00 – 09:45 Plenary lecture: Prof. Milo Wiltbank "Physiologic models and practical implications from my research on the corpus luteum" *(Department of Dairy Science, University of Wisconsin, Madison, USA)*

09:45 – 10:15 Coffee break

10:15 – 13:00 Session III: Ovarian-oviductal-uterine interactions during the estrous cycle and early pregnancy
Moderator 1: Dr. Dimitrios Rizos *(Dept. of Animal Reproduction, INIA-CSIC, Madrid, Spain),*
Moderator 2: Prof. Graca M. Ferreira-Dias *(Faculty of Veterinary Medicine, Lisbon University, Lisbon. Portugal)*

Lectures:
10:15 – 10:50 Dimitrios Rizos "*Current insights into Preimplantation Embryo-Maternal Communication in Cows"*
10:50 – 11:20 Graca M. Ferreira-Dias *"Mare endometrium disorders: Inflammation, Fibrosis, and Therapeutic approaches"*

Short oral presentation:
11:20 – 11:40 Saumya Gunasekara *"Determination of Porcine Follicular Fluid Extracellular Vesicles Effect on Boar Spermatozoa Survival In Vitro"*
11:40 – 12:00 Edith Maria Habich *"Relationship between the epithelial immune response in the uterus and oviduct in postpartum dairy cows"*

12:00 – 13:00 Poster session / Coffee break

13:00 – 14:00 Lunch

14:00 – 17:00 Session IV: Etiology and pathogenesis of uterine disorders
Moderator 1: Dr. Fidel Ovidio Castro *(Laboratory of Animal Biotechnology, Department of Animal Science, Faculty of Veterinary Science, University of Concepción, Chillán, Concepción, Chile),*
Moderator 2: Dr. Anna Szóstek-Mioduchowska *(Department of Reproductive Immunology and Pathology, IAR&FR PAS Olsztyn)*

Lectures:
14:00 – 14:35 Fidel Ovidio Castro *"The impact of TGFβ1, mesenchymal stem cells, and extracellular vesicles on equine in vitro endometrial fibrosis models"*
14:35 – 15:10 Anna Szóstek-Mioduchowska *"Fibroblasts as key regulators of endometrial fibrosis in the mare: insights from transcriptomic and functional analysis"*

15:10 – 15:40 Coffee break

Short oral presentations:
15:40 – 16:00 Lei Xie "*Transcriptomic landscapes of the endometrium in dairy cows with clinical or subclinical endometritis"*

16:00 – 16:20 Joana Alpoim-Moreira “*The effect of the demethylating agent decitabine on collagen expression in mare endometrial fibroblasts treated with elastase”*

16:20 – 16:40 Natacha Murderspach “*Mapping of the plasma and endometrial proteome of mares with healthy and fibrotic endometria”*

16:40 – 17:00 Agnieszka Sadowska “*The potential role of IL-17 in the processes associated with the development of endometrosis”*

19:00 – 22:00 Gala dinner – at the cruise on Odra River

11th SEPTEMBER 2024

09:00 – 09:45 Plenary lecture III: Prof. Stephen LeBlanc "Metabolic and reproductive health in dairy cows" *(Ontario Veterinary College, University of Guelph, Ontario, Canada)*

09:45 – 10:15 Coffee break

10:15 – 13:00 Session V: New diagnostic tools of uterine disorders in farm animals
Moderator 1: Dr. Tal Raz *(Jerusalem Hebrew Univ. Rehovot, Israel),*
Moderator 2: Dr. Mette Christoffersen *(University of Copenhagen, Department of Veterinary Clinical Sciences; Copenhagen, Denmark)*

Lectures:
10:15 – 10:50 Tal Raz "*Metagenetics of the female reproductive tract: opportunities, challenges, and lessons learned"*
10:50 – 11:25 Mette Christoffersen *"Update on diagnostic procedures for equine endometritis"*

11:25 – 12:00 Coffee break

Short oral presentations:
12:00 – 12:20 Muhammad Hussnain Rashid "*Functional metagenomics of endometrial cytology samples from dairy cows diagnosed with clinical or subclinical endometritis"*
12:20 – 12:40 Laura Vanina Madoz "*Comparative analysis of antimicrobial resistance through whole genome sequencing in healthy and metritic cows and their environment"*
12:40 – 13:00 Priit Karis *"The association of cytological endometritis with insulin resistance and gene expression in adipose tissue in transition dairy cows"*

13:00 – 14:00 Lunch

14:00 – 16:50 Session VI: Therapy of uterine disorders in farm animals
Moderator 1: Dr. Igor Canisso (*College of Veterinary Medicine, University of Illinois, Urbana, IL, USA),*
Moderator 2: Prof. Geert Opsomer (*Faculty of Veterinary Medicine, Ghent University, Belgium)*

Lectures:
14:00 – 14:35 Igor Canisso *"Endocrinological and embryo-associated parameters in PPID in mares treated with pergolide and metformin"*
14:35 – 15:10 Geert Opsomer *"New insights in the treatment and control of postpartum uterine disease in high-yielding dairy cows"*

15:10 – 15:40 Coffee break

Short oral presentations:
15:40 – 16:00 Rodolfo Luzbel de la Sota *"The addition of meloxicam to the treatment of clinical and subclinical mastitis improved the cure rate and fertility in dairy cows in Ecuador"*

16:00 - 16:20 Ahmet Cihad Gok *“Mechanisms regulating the protection of mammalian cells against damage caused by cholesterol-dependent cytolysins from pathogenic bacteria”*
16:20 - 16:40 Marta Marcinek *“Antimicrobial effects of cold plasma-activated solutions against selected bacteria causing endometritis in mares: an in vitro preliminary study”*

16:40 Closing Ceremony, Awarding of best presentation and poster

19:00 – 22:00 Guided Wroclaw sightseeing tour

12th SEPTEMBER 2024

9:00 – 17:00 Open Workshop and Discussion on animal behaviour, welfare and health: “Whether large farm animals could be alternative research models for biomedical study?” with scientific and historical excursion to Swidnica, Książ Castel and Książ Horse State Stud.

Keynote lecturers

John S. Davis, **PhD** is a Professor and the Director of Research and Development at the Olson Center for Women's Health. He also serves as the Director of the Signal Transduction Laboratory. Dr. Davis leads research efforts to understand the physiological events and molecular mechanisms that regulate ovarian function and related pathologies, such as steroid synthesis and ovarian cancer. His work focuses on how hormones and growth factors control these processes through complex intracellular signaling pathways. The Davis Lab is dedicated to unraveling these signaling events to discover therapeutic interventions to improve women's health.

https://www.unmc.edu/obgyn/research/academic-research/davis/davis-john.html

Milo Wiltbank joined the faculty at University of Wisconsin-Madison in 1991 and is currently Professor of Animal and Dairy Sciences and Endocrinology-Reproductive Physiology. He was recently named the Judge John J. Crown Chair in Dairy Physiology. He has done research in reproductive physiology throughout his career with >300 peer-reviewed publications that have been cited more than 20,000 in the scientific literature. Although the corpus luteum has been an important focus of his research he also has publications in other research areas including: understanding and reducing pregnancy loss, selection of a single dominant follicle, and interactions of nutrition and reproduction. From a practical standpoint, he is probably best known for development, validation, and modification of timed AI protocols such as Ovsynch and Double Ovsynch for lactating dairy cows.

Stephen LeBlanc is a Professor in the Department of Population Medicine at the University of Guelph and director of Dairy at Guelph – The Centre for Dairy Research and Innovation. He received a BSc(Agr) in Animal Science from McGill University in 1992, and a DVM (in 1997) and DVSc (in 2001) from the University of Guelph. His research focuses on dairy cow metabolic and reproductive health and management, precision technologies, and antimicrobial stewardship. With graduate students and collaborators, this work has resulted in 200 peer-reviewed papers and invited talks in 20 countries. He is a senior editor for the Journal of Dairy Science and serves as a member of Board of Directors of the American Dairy Science Association.

Agnieszka RAK, **PhD**, works as an Associate Professor at the Faculty of Biology in Jagiellonian University in Kraków. She received her PhD in 2008, HDR in 2016 in Poland and joined international labs in Japan, Portugal, Germany and France. Since 2024 he has been the scientific director at the Institute of Zoology and Biomedical Research. She is a leader of the research team AdipokinesTEAM investigating the role of metabolic hormones and adipokines in the regulation of the female reproduction in the hypothalamus, pituitary,

ovary and placenta cells in human, rodents and domestic pig. Additionally, her research interests focus on investigating the influence of environmental factors on female reproduction. She is an active member in international and national organizations, e.g. of European Society for Domestic Animals, Society for Reproduction and Fertility, Polish Society for Biology of Reproduction, and Polish Academy of Sciences. Dr Rak authored more than 120 papers in international journal, 180 abstracts, and 4 book chapters. Her work has been cited 2,005 time with an h-index of 25. She was PI/research supervisor of 11 projects and supervised 3 PhD theses (7 in progress) and about 65 master/bachelor's theses. Dr Rak was awarded the Foundation for Polish Science "START", Scholarship of the Minister of Science and Higher Education for Young Prominent Scientist.

Mariusz P. Kowalewski, **PhD**, obtained his Doctor of Veterinary Medicine (DVM) degree from the Faculty of Veterinary Medicine at the University of Warmia and Mazury in Olsztyn, Poland, in 2002. From 2002 to 2006, he pursued his PhD in reproductive biology at the joint graduate program of the Faculties of Medicine and Veterinary Medicine, Justus-Liebig-University (JLU) in Giessen, Germany. His PhD project was focused on understanding the luteal function in the domestic dog. Afterwards, he completed his postdoctoral training at the Institute of Veterinary Anatomy, Histology, and Embryology at JLU between 2006-2007, and at the Texas Tech University Health Sciences Center (TTUHSC), School of Medicine, Department of Cell Biology and Biochemistry, in Texas, USA, between 2007 and 2009. Since 2009, he has held the position of Principal Investigator, leading his own research group at the Institute of Veterinary Anatomy (IVA) within the Vetsuisse Faculty at the University of Zurich (VSF, UZH) in Zurich, Switzerland. In 2012, he received Venia legendi (Habilitation) in the fields of Veterinary Anatomy, Histology, and Embryology from UZH, and in 2014, became a Professor of Functional Microanatomy at VSF, UZH. As of 2019, he has been serving as a Professor of Veterinary Anatomy, Histology, and Embryology, as well as the Head of the Institute, while leading the Group of Reproductive Endocrinology at IVA, VSF, UZH. He is involved in teaching courses on veterinary gross- and microanatomy, histology, embryology, as well as developmental and reproductive biology. Currently he acts as co-Editor-in-Chief for the journal Reproductive Biology. His research interests encompass comparative reproductive biology and endocrinology of domestic animals, endocrine regulation of canine luteal and placental function, concepto-maternal communication in accompanying animals, and mechanisms of STAR-dependent and hypoxia-mediated steroidogenesis.

Camilla Hughes, PhD, is an Assistant Professor in the Department of Animal Science at Penn State University. She received a BS in Animal Science and Biology at Virginia Tech. She then completed a PhD with Dr. Joy Pate at Penn State University, focusing on mechanisms of luteal rescue in the ruminant. In 2019, she moved to the University of Montreal and conducted postdoctoral research under the supervision of Dr. Bruce Murphy, focusing on understanding transcriptional regulation of ovarian reserve establishment and depletion, using the mouse model. Current research in the Hughes laboratory primarily uses the cow as a model and focuses on

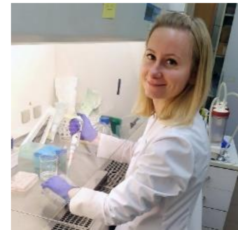
mechanisms of ovarian research establishment and depletion, luteinization, and luteal responsiveness to prostaglandins, with a focus on understanding the role of inflammatory and immune-related mechanisms in these processes. The long-term goal of her research program is to generate an improved understanding of ovarian development and function, to improve fertility in both livestock and humans.

Sławomir Wołczyński is a Professor and the Head of the Department of Reproduction and Endocrinological Gynecology at the Medical University of Bialystok, Poland. He also leads the research group on Biology and Pathology of Human Reproduction at IRZiBŻ Olsztyn PAN. He is the former President of the Polish Society of Reproductive Medicine and the past Vice-President of the Society of Reproductive Biology. Additionally, he is a member of the Committee of Bioethics of the Polish Academy of Sciences. His research focuses on infertility treatment, reproductive endocrinology, mechanisms of hormonal action, and the impact of environmental estrogens on reproduction. Prof. Sławomir Wołczyński has co-authored 264 articles, with an impact factor of 540 and an h-index of 34.

Graça Ferreira-Dias is a Full Professor of Physiology at the Faculty of Veterinary Medicine (FMV) at the University of Lisbon, Portugal. Her research area is mare reproductive physiology and pathology. She got her Veterinary Medicine degree in 1983 at the School of Veterinary Medicine, Technical University of Lisbon, Portugal. After, she got her PhD degree in Physiology, at the School of Medicine, SIU (1993), supported by research and teaching grants (Physiology). Currently, she is the Studies Coordinator of the Department of Morphology and Function and the Coordinator of the Ethics Committee for Research and Teaching. Among other tasks, she has been the coordinator of mare physiology of reproduction research team, and the principal investigator of 17 national projects and 12 projects of international cooperation between Portugal and Poland. She has participated in 12 national projects and 6 international projects (Poland, Spain, Chile). She has evaluated PhD grants and Projects final reports and as a Pannel Coordinator, at Foundation for Science (FCT), and international projects from Poland, Spain, Austria, and Canada. She is the representative member of Portugal in the European Society for Domestic Animal Reproduction (ESDAR) and served as ESDAR Vice-President (2018-2020).

Dimitrios Rizos is a Professor of Research at the National Institute for Agricultural and Food Research and Technology (INIA-CSIC), Department of Animal Reproduction, Madrid-Spain. He received his Ph.D. from University College Dublin (UCD) in 2002, where he spent a further 3 years as a post-doctoral fellow. In 2004, he got a "Ramon y Cajal" Research Fellowship and in 2006 a permanent position as "Senior Researcher" leading the "*Assisted Reproduction and Preimplantation Embryology in Cattle*" group at INIA-CSIC in Spain. His main research focus on: factors affecting embryo production in vitro and their quality; mechanisms controlling embryo-maternal interactions and the role of Extracellular Vesicles (EVs); and fertility in dairy cows. His results represent a technological improvement in reproductive biotechnology

that allows the generation of better quality embryos in vitro, capable of establishing a pregnancy after transfer to a recipient. In addition, his research provided new insights essential to solve important problems related to infertility and embryo loss, which are the main problems in the dairy industry. To date, Dr. Rizos has authored over 130 peer-reviewed scientific articles in high indexed journals (h-index 62) and has numerous invitations to give plenary lectures at International Conferences, Symposia and University Seminars. He has supervised more than 20 PhD students and postdoctoral fellows, 15 master students, mentoring >70 undergraduate students, technicians and visiting scientists and secured over €8 million in funding from the EU and the Spanish Ministry of Science and Innovation for research projects. He is an active member in various scientific committees and was elected to the Board of Governors of the Association of Embryo Technologies in Europe (AETE: 2010-2015) and International Embryo Technology Association (IETS: 2016-2020), and elected President of AETE (2015-2018).



Anna Szóstek-Mioduchowska, **PhD**, is an Assistant Professor at the Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences. Dr. Szóstek-Mioduchowska authored more than 60 papers in international journals and 130 abstracts. She was the principal investigator of 5 projects. She is interested in the molecular and metabolic processes associated with the pathogenesis of equine endometrosis. Dr. Szóstek-Mioduchowska and her collaborators found changes in the secretory function and transcriptomic alteration in fibrotic equine endometrium. Now her research focuses on the effect of immune cells and their products in processes associated with the development of endometrosis. She and her collaborators are focusing on the action of different populations of macrophages and T helper cells on endometrial fibroblast in the context of tissue remodeling, ECM deposition, fibroblast proliferation and migration, and myofibroblast differentiation in *in vitro* conditions.

Fidel Ovidio Castro, **PhD**. Professional title: Animal Husbandry Engineer at the Belarusian Agricultural Academy in Gorki, Belarus, former Soviet Union, in 1984 and a Master of Science degree at the same University. In 1986, he started as a scientist at the Centre for Genetic Engineering and Biotechnology (CIGB) in Havana, Cuba, where he was Director of the Vivarium and the Experimental Animal Models and Transgenic facilities. In 1993, he obtained his PhD in Veterinary Sciences from the University of Havana, Cuba. He continued working at the CIGB until 2004, where he became Senior Researcher, Head of the Department of Animal Biotechnology, and Deputy Director of Research of the Agricultural Division. In 2004, he moved to the Faculty of Veterinary Sciences of the University of Concepción in Chile, where he still works. At UDEC, he became a full professor and director of the Faculty of Veterinary Science graduate school. During his career, he has published more than one hundred peer-reviewed articles and hundreds of presentations at conferences and meetings and directed theses at undergraduate and postgraduate levels. He is the director of the PhD program in Veterinary Sciences. His expertise includes molecular and cell biology, animal biotechnology, and reproductive technologies. For the last ten years, he has been working on

regenerative therapies for animals, mainly horses. His postgraduate studies include four post-doctoral stays at the University of Freiburg, Germany, 1997-1998; University of Cambridge, UK, 2000-2001; Free University of Berlin, Germany, 2007-2008; and a sabbatical year at the University of Liège, Belgium. He has founded two biotech companies, TECELVET (Regenerative therapies in veterinary sciences; 2014) and Soluciones Biotecnológicas CR SpA, (Vi-Embryos, for reproductive technologies, including somatic cell nuclear transfer).

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Tal Raz, **PhD**, is a reproductive biologist and theriogenologist. He earned his DVM from the Koret School of Veterinary Medicine, Hebrew University of Jerusalem (Israel) in 2002. After completing an internship in Large Animal Medicine and Surgery, he began a three-year Theriogenology residency program in Animal Reproduction at the Western College of Veterinary Medicine, University of Saskatchewan, Canada. In 2007, he became a diplomate of the American College of Theriogenologists (Dipl. ACT). Dr. Raz completed his PhD in Animal Reproduction in 2010 at the University of Saskatchewan, Canada, focusing on the effects of equine FSH on mare fertility in an embryo transfer setting. He then conducted a post-doctoral fellowship at the Weizmann Institute of Science, Israel, using a multimodal approach to study molecular and cellular aspects of ovulation, and uterine and placental function in normal and high-risk pregnancies in murine

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Igor Frederico Canisso, **PhD**, received his veterinary training from the Federal University of Paraná, Brazil, and earned a master's degree from the Federal University of Viçosa, Brazil. After completing his master's, he had a brief but meaningful experience at Aberystwyth University in the United Kingdom. He then moved to the United States for residency training in theriogenology (Animal Reproduction) at Cornell University's College of Veterinary Medicine in Ithaca, New York. After completing his residency, he moved to the University of Kentucky, where he obtained his Ph.D. in Reproduction. He is a board-certified veterinary specialist by the American College of Theriogenologists and the European College of Animal Reproduction. His research interests include perinatology, reproductive efficiency, semen biotechnology, reproductive toxicology, and reproductive physiology. He is particularly interested in ruminants, canids, and equids, with a special focus on horses and donkeys.
https://loop.frontiersin.org/people/304902/bio

Geert R.G. Opsomer graduated as a DVM at the Ghent University (Ghent, Belgium) in 1989. After graduation, he started to work at the Department of Reproduction, Obstetrics and Herd Health at the same university. His main interest was fertility and herd health control in high yielding dairy herds.
In 1995 he obtained a masters degree (Ms) in Animal Production with a thesis entitled: 'Energy metabolism in the high yielding dairy cow'. In 1999 he successfully defended his PhD entitled: 'Postpartum anoestrus in high yielding dairy cows: a field study'. In 2002 he became diplomate of the European College of Animal Reproduction (ECAR), and in 2003 diplomate of the European College of Bovine Health Medicine (ECBHM).
At the moment he is full professor of bovine reproduction and herd health management at the Veterinary Faculty of the Ghent University and is heading the Ambulatory Clinic at the Department of Internal Medicine, Reproduction and Population Medicine. Besides educating undergraduate and graduate students, he is currently supervising multiple PhD students researching a variety of aspects of bovine herd health and reproduction.

Abstracts

A new perspective on the regulation of corpus luteum: expression and role of visfatin (NAMPT) on luteal transcriptome, proteome and functioning

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The corpus luteum (CL) is a transient gland that is formed in the mammalian ovary, and its proper functioning determines the maintenance of pregnancy as well as the cyclicity of the ovary. The CL performs its function mainly by secreting steroid hormones; progesterone (P_4), prepares the uterine wall for embryo implantation and prevents its contractions and further rejection of the fetus, estradiol (E_2) formation of blood vessels, while prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), control luteinization and luteolysis, respectively. Disturbances in the luteal function lead to numerous negative consequences, primarily luteal phase deficiency and may result in premature regression of the gland and subsequent transition to an infertile cycle. Therefore, we hypothesise that adipokine visfatin can be new regulator of luteal function. Visfatin exists in two functional isoforms, an intracellular form called nicotinamide phosphoribosyltransferase (iNAMPT) and the extracellular form of visfatin (eNAMPT) is considered an adipokine. It has a pleiotropic effect in the body, glucose uptake in myocytes and adipocytes, inhibiting hepatic glucose release, and stimulating the accumulation of triglycerides. In the present study we used porcine CL to study expression and in vitro effect of visfatin on luteal transcriptome, proteome and function.

The study demonstrated that visfatin expression in CL depends on hormonal status related to the phase of the estrous cycle. Moreover, visfatin protein abundance was increased by P_4, and decreased by both prostaglandins, while LH and insulin have modulatory effects, depending on the phase of the cycle. The obtained results from Illumina's NovaSeq 6000 RNA sequencing revealed 170 the differentially expressed genes (99 up and 71 downregulated) assigned to 45 functional annotations; we revealed 40 long non-coding RNAs, of which 3 were known and 37 were described for the first time. Furthermore, using liquid chromatography with tandem mass spectrometry, LC–MS/MS we have identified 511 diferentially regulated proteins (DRPs, 276 up and 235 down-regulated) in the presence of visfatin. Revealed DRPs were assigned to 162 gene ontology terms. We indicated that visfatin effect on the transcriptome and spliceosome of luteal cells, including the genes and proteins involved in the processes crucial for angiogenesis, steroidogenesis, inflammation, cell development, migration and proliferation and modulates the expression of proteins connected with the regulation of lipogenesis and cholesterologenesis, and, in consequence, synthesis of steroid hormones and prostaglandins' metabolism. The obtained results were confirmed in in vitro effect of visfatin on steroidogenesis, prostaglandin synthesis, angiogenesis and luteal cell proliferation/apoptosis. In conclusion, our results suggest important roles for visfatin in the regulation of porcine CL.

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Targeted and Global Insights into the Regulation of CL Function in the Dog

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The corpus luteum (CL) remains an important gland with a central role in regulating canine reproductive function. While its main function is the provision of progesterone (P4), it exhibits several species-specific features throughout its life span, providing important translational insights into the organ's functionality. Thus, apart from the cessation of luteal function, the CL of pregnant and nonpregnant animals experience similar functional dynamics throughout most of the luteal development. Histologically, the canine CL arise from the exceptionally strong luteinization of preovulatory follicles. The early onset of morpho-functional changes in the follicles is indicated by estrogens originating mostly in the theca interna cells. Responding to increasing LH pulsatility, P4 strongly rises prior to ovulation and remains of luteal origin in pregnant cycles. Its production is unaffected by the uterine presence of embryos. Notably, the early CL is largely gonadotropin-independent and remains relatively autonomous during this period, with prostaglandins (PGs) playing regulatory roles. Confirmed experimentally, including through transcriptome analysis and in clinical studies, suppressing luteal PGs, temporarily reduces luteal activity. Additional functions of translational potential include the anti-inflammatory, proangiogenic and proliferative properties of local PGs. In particular, transitioning to the mature, PRL-dependent CL appears to make it more susceptible to PGs withdrawal. Physiological pseudopregnancy typically extends the lifespan of the CL beyond that observed during pregnancy. Luteal regression is marked by slow morpho-functional degeneration. Transcriptomic analyses highlighted increased cholesterol efflux during late luteal regression, indicating substrate depletion as a possible contributor to the withdrawal of steroidogenic activity. Similarly, ongoing proteomics analysis indicates decreased enrichment scores for terms associated with the metabolism of lipids in the regressing CL. The proteomics also supports matrix remodeling with no active involvement of the immune system. Yet, the triggers of the slowly ongoing luteal regression remain unknown. The active luteolysis occuring abruptly in regressing CL of pregnancy appears to be an active immune process driven by placental PGF2α, as supported by transcriptomic data. P4 is luteotropic and controlling the function of its nuclear receptors (PGRs) offers possibilities for targeted clinical approaches. Accordingly, P4/PGR bear immunosuppressive, proliferative, and gene-activating properties. Particularly, the negative regulation of RNA biosynthetic processes and reduction of DNA-templated transcription are reflected in the respective functional terms observed in the ongoing proteome analysis. In pregnant animals, antigestagens targeting PGRs activate the placental luteolytic cascade, and in non-pregnant animals, they shorten directly the luteal lifespan. Interfering with PGR prior to ovulation does not affect the process. The local role of estrogens remains unveiled. Nevertheless, the withdrawal of local E2 production is associated with higher SULT1E1 (estrogen sulfotransferase) and lower STS (sulfatase) expression, indicating local E2 deactivation. Beyond luteal function, canine ovarian physiology still requires investigation, particularly regarding the transition from anestrus to proestrus, and the maturation of ovarian follicles and oocytes.

Single-cell reconstruction of the bovine corpus luteum

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Various cutting-edge approaches have shaped our present understanding of molecular processes that govern the stimulation or demise of corpus luteum (CL) structure and function. In particular, analyses involving mRNA and miRNA transcriptome, proteome, and metabolome of whole CL or specific luteal cell types have shed light on various previously unreported molecular regulators of CL function. In the present study, we analyzed the cellular and molecular landscape of bovine CL at mature and regressing stages using single-cell RNA sequencing to further improve the resolution of our understanding of the CL. CL collected from heat-synchronized and ultrasound-monitored German Holstein heifers by a transvaginal surgery on day 11 after ovulation were considered mature (n=3), and those collected 8 hours after a PGF2α injection (0.5 mg cloprostenol i.m., Veyx-Pharma GmbH, Schwarzenborn, Germany) on day 11 were considered regressive (n=3). Radioimmunoassay revealed a significant decrease in the plasma progesterone concentration 8 hours after the PGF2α injection. CL tissues were dissociated using collagenase, and the debris and red blood cells in the dissociated mixture were removed using Percoll gradient centrifugation. A small volume of cell suspension was stained with propidium iodide and assayed in a flow cytometer, which indicated >85% viability of cells in all six samples. Three cell samples of the mature and regressing stages each were pooled in an equal ratio to have a single mixture of cells at each stage. Two single-cell libraries were prepared for each stage using the 10X genomics reagents and sequenced on a Novaseq X Plus platform. Sequencing reads were analyzed using Cell Ranger and R software packages. Integration of mature and regressing CL cell clusters was performed using the Seurat tool kit. After quality control, 14,919 individual cells of mature CL and 13,131 cells of regressing CL remained for the data analysis. We identified 14 distinct cell clusters at both CL stages. The data revealed that 43% steroidogenic, 19% endothelial, 15% immune, and 23% fibroblast cells were present in the mid-cycle CL. In comparison, we found 35% steroidogenic, 14% endothelial, 32% immune, and 18% fibroblast cells in the regressing CL. The present single-cell transcriptome data reveals novel markers for different luteal cell types and informs early events of molecular and cellular changes that govern the luteal regression in bovine.

Comparative analysis of expression of omentin-1 in porcine ovarian follicles vs corpus luteum and its *in vitro* effects on proliferation, apoptosis and steroidogenesis

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Introduction: Ovarian follicles and corpus luteum (CL) play crucial roles in reproductive cycle. Follicles are responsible for the maturation and release of oocytes, while the CL is primarily involved in maintenance of pregnancy. The basic functions of these structures are proliferation, apoptosis and production of steroid hormones- progesterone (P_4) and 17β-estradiol (E_2). Recent studies have highlighted the role of adipokines- hormones produced by white adipose tissue in the regulation of ovarian function. Omentin-1 (ITLN1), is an adipokine predominantly expressed in visceral fat tissue. In the context of reproductive physiology, ITLN1 has been shown to play a role in modulating insulin sensitivity and has been linked to polycystic ovary syndrome.
Aims: This study aims to compare the expression of ITLN1 in ovarian follicles and the CL as well as to investigate the *in vitro* effect of ITLN1 on cell proliferation, apoptosis as well as steroidogenesis within these ovarian structures.
Materials and methods: To assess the mRNA expression and immunolocalization of ITLN1 (PCR, immunofluorescence) the ovarian follicles and CL were collected from Large White pigs on days 2-3, 10-12, and 14-16 of the estrous cycle. To determine the *in vitro* effect of ITLN1 on proliferation (AlamarBlue, BrdU; mRNA expression of cyclins and proliferating cell nuclear antigen- PCNA *via* PCR), apoptosis (mRNA expression of caspases, B-cell lymphoma 2- BCL2 and BCL-2-associated X protein *via* PCR) and steroidogenesis (mRNA expression of steroidogenic enzymes *via* PCR and P_4/E_2 levels *via* ELISA) cultures of ovarian cells and luteal cells (10-12 days of the estrous cycle) were treated with ITLN1 (10-100 ng/mL) by 24-48 h. Statistical analysis was performed using one-way ANOVA ($p < 0.05$, $n = 5$).
Results: We showed that mRNA expression of ITLN1 in ovarian follicular and luteal cells increased with the progression of the estrous cycle in pigs. What is more, ITLN1 was expressed in granulosa, theca, oocyte as well as large and small luteal cells. Additionally, ITLN1 enhanced proliferation and inhibited apoptosis of follicular and luteal cells by modulating the levels of cyclins, PCNA as well as pro- and antiapoptotic factors. Moreover, in dependence of dose ITLN1 increased or decreased steroidogenesis in ovarian follicle cells and CL.
Summary: INTL1 expression in ovarian follicles and CL depends on the estrous cycle stage. Moreover, ITLN1 independently on ovarian structures modulates proliferation, apoptosis and steroidogenesis, suggesting it's a new regulatory role of porcine reproduction.

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Luteal responsiveness to prostaglandin F2A: from development to regression

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Prior to day 5 of the bovine estrous cycle, the corpus luteum (CL) fails to regress in response to prostaglandin (PG)F2A, whereas after day 5, CL regress when exposed to PGF2A. This phenomenon has also been observed in other species has been termed "acquisition of luteolytic capacity," and provides a useful model to understand mechanisms regulating luteal regression. To evaluate global physiological changes in CL of differing capacity to regress in response to PGF2A, we compared transcriptome and proteome of CL collected on days 4 and 6 of the estrous cycle. Estrous cycles of eight cyclic Holstein cows were synchronized, with the day of behavioral estrus and ovulation induction with GnRH designated day 0. On day 4 or 6, CL were collected and frozen. miRNA, mRNA, and proteins were quantified and tested for differential abundance using Nanostring technology (Human v3 miRNA CodeSet), next-generation sequencing, and Q-Exactive HF mass spectrometry (Data Independent Acquisition mode) respectively. Targetscan, Ingenuity Pathways Analysis (Qiagen), and Metascape were used for miRNA target prediction and pathway analyses. miRNA that decreased temporally were predicted to target proteins that were associated with mitochondrial function and known PGF2A-response pathways, including AMPK and MAPK signaling. Moreover, MYC-mediated apoptosis signaling was the top pathway predicted to be regulated by targets of the 19 miRNA unique to day 4. Several proteins that are regulators of apoptosis were more abundant on day 6 relative to day 4, including BAK1, BASP1, and BCL2L13. Interesting, several nucleoporins, which regulate transport of apoptotic signals into the nucleus, were also in greater abundance on day 6. One nucleoporin, NUP43, was downregulated in culture in response to treatment with RU486, a progesterone receptor inhibitor, suggesting that upregulation of nucleoporins may be a progesterone-mediated event. Overall, these results indicate that changes in protein expression and alteration of miRNA-mediated repression between days 4 and 6 may make the CL more susceptible to PGF2A-induced cell death. On day 4, miR-125b-5p was the most abundant miRNA in the CL (~20% of all reads). This miRNA then decreased by more than 4-fold, making let-7a-5p the most abundant on day 6. Among the 31 predicted targets of miR-125b-5p that were less on day 4, 17 were associated with apoptosis, most notably, BCL2 antagonist/killer 1 (BAK1). Eleven proteins that were predicted targets of let-7a-5p were less on day 6, including ATPase plasma membrane Ca2+ transporting 4 (ATP2B4), a plasma membrane calcium transporter. These data indicate that the switch in the most abundant miRNA in the CL between days 4 and 6 may regulate luteal cell calcium homeostasis and susceptibility to apoptosis, clearly implicating miRNA in acquisition of luteolytic capacity.

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Luteal phase defect in women

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Luteal phase defect (LPD) is a condition of insufficient progesterone exposure to maintain a normal secretory endometrium and allow for normal embryo implantation and development of pregnancy. The LPD was first described as a possible cause of infertility by Georgiana Seegar Jones in 1949. Hypothetically LPD could be caused by intrinsic cellular defect in luteal steroidogenic function or a defect of the vascularization of corpus luteum. Due to the incomplete understanding of the pathogenesis and lack of an accurate method to diagnose LPD, empiric treatment of suspected LPD cannot be completely evidence-based. Progesterone supplementation is frequently used to mitigate potentially inadequate corpus luteum function, but there is no evidence that progesterone supplementation in natural cycles improves fertility and the outcome of pregnancy. Most would agree that there is no intrinsic pathology of the corpus luteum that is associated with reduced fertility in women.
True luteal defect does exist and it is iatrogenic after the induction of ovulation and controlled ovarian stimulation with subsequent oocyte retrieval in medical assisted conception. Luteal luteinizing hormone (LH) levels have been found to be reduced in gonadotropin agonist or antagonist/gonadotropin protocols. Supraphysiological steroid concentration routinely observed in stimulated cycles may adversely affect LH secretion and induce a luteal-phase defect. When hCG is used to triggering ovulation its long half-life means that early luteal progesterone production is supraphysiological but it isn’t enough to support a full luteal phase. However, in a conception cycle, the endogenous hCG will support luteal progesterone production there is a time of relative progesterone deficiency in the mid-luteal phase. Luteal phase support is mandatory in ovarian stimulation cycles in assisted reproductive technology. This is classically accomplished by means of exogenous P administration. There is currently no consensus on when to begin progesterone, in what doses, by what route, or to personalize supplementation during an IVF cycle or in FET transfer. Although luteal phase support is commonly continued for up to 10 weeks into pregnancy, there is accumulating evidence that it can be stopped after the first ultrasound or even after a positive pregnancy test.
Even more profound LPD occurs after the peak is triggered by the GnRH agonist in cycles GnRH antagonist/gonadotropins. A short LH peak is sufficient for oocyte maturation, but is not sufficient for the formation of a functional corpus luteum. This may be clinically beneficial, because it prevents hyperstimulation syndrome OHSS in patients with excessive response to stimulation. In patients who are not at risk of OHSS, in order to form a proper corpus luteum after egg retrieval, 1500 IU hCG should be administered or 150 IU hCG every other day.
In conclusion, in induced and stimulated cycles, impaired LH secretion seems to be responsible for the occurrence of luteal phase defect in woman.

Phoenixin-14 modulates angiogenesis, cell proliferation, and apoptosis in porcine corpus luteum

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Phoenixin (PNX) is a neuropeptide with a well-established role in the central regulation of reproductive processes; however, knowledge regarding its function in the ovary is limited. Our previous studies have shown that PNX-14 is a luteotropic factor and positively affects the endocrinology of the porcine corpus luteum (CL). The current study focused on the PNX-14 effect on other crucial processes in luteal cells such as angiogenesis, proliferation, and apoptosis. For this purpose, we performed *in vitro* cultures of luteal cells collected from CL in the mid-luteal phase. Cells were treated with PNX-14 (1-1000 nM). Following incubation time, we checked the mRNA level of angiogenic factors VEGFA, bFGF2, and ANG-1 (real-time PCR) and their secretion by luteal cells (ELISA). We examined the proliferation of luteal cells (alamarBlue assay); DNA fragmentation (Cell Death Detection ELISA), and caspase 3 and 7 activity (CaspaseGlo 3/7 assay). Then, we analysed the mRNA (real-time PCR) and protein levels (western blot) of PCNA and cyclin A, B and E, caspase-3, -8 and -9, and BAX, BCL2. Additionally, using pharmacological inhibitors of extracellular signal-regulated kinases 1/2 (ERK1/2), protein kinase B (AKT), 5'AMP-activated protein kinase (AMPK) as well as silencing the PNX-14 receptor - GPR173 by siRNA, we studied the molecular mechanism of PNX-14 action. The obtained results indicate that PNX-14 stimulates the level of bFGF2 and ANG-1 and protein expression of VEGFR2, FGFR1, and TIE2 while decreasing FGFR2. PNX-14 stimulated the proliferation of luteal cells, as well as the expression of the PCNA factor. However, it has various effects on the level of mRNA and protein of cyclins. PNX-14 reduced DNA fragmentation and caspase 3/7 activity. We also observed that the levels of caspase 3, 8 and 9 and BAX decreased dose-dependently, while the level of the anti-apoptotic protein BCL2 increased. Additionally, protein kinases were involved in the effect of PNX-14 on the secretion of bFGF2 - AKT, luteal cell proliferation - ERK1/2, AKT, AMPK, and caspase 3/7 activity - AKT and AMPK. Moreover, the GPR173 receptor mediated the action of PNX-14 on all of the processes mentioned above. The current study confirmed that PNX-14 is a luteotropic factor in the CL of pigs by stimulating the process of angiogenesis, proliferation, and protecting luteal cells against apoptosis.

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Effect of heat stress on the mRNA expression in the corpus luteum of dairy cows

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Heat stress (HS) adversely affects corpus luteum (CL) development and reproductive performance in dairy cows. The main aim of this study was to evaluate the effect of HS on the mRNA expression in the CL under European climatic conditions. The study was conducted on a commercial dairy farm in Slovakia. A total of 63 clinically healthy Holstein-Friesian cows were enrolled at the day of artificial insemination (AI) as part of an ovulation synchronization protocol initiated 70 days postpartum. The CL size (CLS) was assessed weekly by ultrasound from the day of the first AI until day 21 after AI. Biopsy samples of the CL were obtained using a transvaginal ultrasound-guided technique on day 21 after AI. Climate data were recorded during the study period at 15-minute intervals using data loggers placed in the barn. The time and amplitude of the temperature humidity index (THI) surpassing the threshold of 68 were calculated. From the obtained luteal tissue, the total RNA was extracted and used for real-time quantitative PCR for selected genes (*FGF1*, *IL1B*, *IL10*, *ISG15*, *MX2*, *OAS1*, *PTGS2*, and *VEGF2*). Out of the 63 study animals, 34 cows (54%) were exposed to heat stress and 20 cows (32%) became pregnant. Among the heat-stressed cows, 5 (14.3%) became pregnant, while 15 (51.7%) of the non-heat-stressed cows conceived. There was a significant difference in the mRNA expression in the CL (*ISG15*, *OAS1*, *PTGS2*, and *VEGF2*) between pregnant and nonpregnant animals (P<0.05). No significant difference, however, was observed in mRNA expression between heat stressed and non-heat stressed animals (P>0.05). This was also the case when pregnant and non-pregnant animals were analysed separately. In addition, no significant correlation was found between the mRNA expression of selected genes and THI values during 21 days after AI (P>0.05). The CLS varied between 11 and 30 mm. The CLS at the first and second week after AI differed between heat stress and non-heat stressed animals (P<0.05). Moreover, the CLS at week 3 after AI correlated positively with the mRNA expression of *VEGF2*, *OAS1*, and *ISG15* (P<0.05). We conclude that under different heat stress conditions the luteal mRNA expression of the selected genes remains consistent. However, the luteal mRNA expression varied depending on the CLS and pregnancy status. More research is required to gain a deeper understanding of the impact of HS on mRNA expression and the development of the CL.

Corpus luteum diagnostic via ultrasound - A viable tool for treatment decisions in Ovsynch protocols?

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Reproductive failure in dairy cows is often associated with the absence of a functional corpus luteum (CL) at initiation of a timed artificial insemination (TAI) protocol, e.g., Ovsynch. Circulating progesterone (P4) concentrations > 1 ng/mL are indicating the presence of a functional CL and are beneficial at the start of TAI protocols for, e.g., luteal regression after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment as part of the Ovsynch protocol and subsequent conception rate. Furthermore, sufficient P4 concentrations at the start of TAI protocols are associated with improved oocyte quality, reduced probability of double ovulations and subsequent twinning rates. The accurate diagnosis and interpretation of luteal structures in a feasible way is of utmost importance for practitioners in the field, as treatment decisions or protocol alterations may be chosen based on these diagnostic outcomes. Therefore, we compared CL size via ultrasound with circulating P4 concentrations at protocol initiation in order to evaluate if ultrasound deems as a viable diagnostic tool and to create potential thresholds for optimized CL assessment.
As part of a larger study (Hölper et al., 2023), cows subjected to different Ovsynch protocols (n = 1,056) received transrectal ultrasound (Easi-Scan:GO, IMV Imaging) assessment of the ovaries in order to determine the absence or presence and size of a CL. Blood samples were collected at protocol initiation by venipuncture of the coccygeal vessels. Serum P4 concentrations were determined by an enzyme labeled chemiluminescent competitive immunoassay (Immulite Progesterone Enzym, Siemens Healthcare). To define reference criteria for identifying cows with a functional CL based on the CL diameter, we used a receiver operating characteristic (ROC) analysis. The continuous variable was CL diameter, and the classification variable was P4 concentration > 1.0 ng/mL.
The overall accuracy to identify a functional CL using transrectal ultrasound was 87.2%. The ROC analysis provided an area under the curve (AUC) of 0.901, which can be considered a highly accurate result (Swets, 1988). The optimum cutoff was a 20 mm diameter of the CL. Sensitivity and specificity was 89.6 and 80.0%, respectively. The positive predictive value was 92.8%. The negative predictive value was 73.1%.
In conclusion, transrectal ultrasound examination is suitable for determining functional CL if a diameter of 20 mm is considered as threshold. Therefore, potential modifications to the Ovsynch protocol such as the addition of a second $PGF_{2\alpha}$ treatment or the application of an intravaginal P4 releasing device, may be considered based on ultrasound CL assessments.

Control of Luteal Function by Luteinizing Hormone: Insights into multi-organelle control of steroidogenesis

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Luteinizing hormone (LH) stimulates ovulation, luteal development, and progesterone biosynthesis. This presentation will summarize recent progress towards understanding cellular and organelle-specific changes induced by LH in steroidogenic luteal cells. LH activates multiple protein kinase-mediated signaling pathways. Using multiple approaches we examined the morphology, lipidome, metabolome, transcriptome and steroidogenesis of highly purified bovine small luteal cells in response to acute stimulation with physiologic concentrations of LH. We observed that LH via activation of protein kinase A (PKA) phosphorylates vital enzymes that contribute to increases in progesterone synthesis. LH rapidly stimulates the PKA-hormone-sensitive lipase (HSL) signaling pathway. A dynamic relationship was established among AMP Kinase, PKA, HSL, and lipid droplets (LD) in luteal progesterone synthesis. Activation of AMP Kinase inhibits both HSL activation and progesterone synthesis, whereas activation of PKA by LH stimulates HSL and cholesterol trafficking to the mitochondria, resulting in elevated progesterone synthesis. Analysis of the bovine luteal LD proteome following activation of PKA revealed increased association of the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase with the LD. LH via PKA also acutely regulates mitochondrial dynamics via phosphorylation of dynamin-related protein 1 (DRP1), which decreases the association of DRP1 with mitochondria and stimulates mitochondrial fusion. Inhibition of DRP1 association with mitochondria elevates LH-induced progesterone biosynthesis. LH also induces rapid changes in key metabolic pathways including glycolysis, tricarboxylic acid cycle, pentose phosphate pathway, de novo lipogenesis, and hydrolysis of phospholipids. LH via PKA signaling stimulates phosphorylation of ATP citrate lyase (ACLY), an enzyme involved in de novo synthesis of fatty acids. Inhibition of ACLY and fatty acid transport to mitochondria suppresses LH-stimulated progesterone production and phosphorylation of PKA substrates. In summary, multiple LH-sensitive and organelle-specific pathways are essential for maintaining signaling and steroidogenesis in ovarian luteal cells.

Physiologic models and practical implications from my research on the corpus luteum.

Milo Wiltbank

This talk will provide a historical and current perspective related to research on the corpus luteum. It will primarily focus on research that I have been part of ranging from luteal blood flow (highest blood flow per gram of tissue in the body) in the rabbit to regulation of ovine large and small luteal cells and finally to hormonal and molecular mechanisms regulating the bovine corpus luteum. One novel physiologic mechanism that will be explored relates to the two distinctive mechanisms that maintain the bovine corpus luteum of pregnancy. The first pregnancy mechanism is triggered by embryonic interferon-tau and the second is an area of active research by several laboratories, including ours. Corpus luteum research also has practical implications in animal agriculture. Initial regulation of the corpus luteum was by using prostaglandin F2a to synchronize estrus. Our laboratory developed the first program to synchronize ovulation allowing timed AI of all cows that are eligible for breeding. The newer "fertility" programs use regulation of luteal function to produce greater fertility to timed AI than breeding to estrus in lactating dairy cows. Finally, an overview will be presented of current research on the role of luteal mechanisms in pregnancy loss, particularly in lactating dairy and in recipients of in vitro produced embryos.

Current insights into Preimplantation Embryo-Maternal Communication in Cows

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Embryo-maternal communication remains a formidable challenge in reproductive biology. Understanding the intricate physiological mechanisms governing interactions between the maternal tract and developing embryo is vital for enhancing bovine reproductive efficiency. To achieve this goal, integrating in vivo and in vitro models appears to be the most suitable approach for comprehending this delicate dialogue. However, creating effective in vitro models presents a significant hurdle, primarily due to the intricate and dynamically changing nature of the maternal environment. One alternative to conventional co-culturing of embryos with oviductal or endometrial epithelial cells lies in harnessing reproductive fluids, extracellular vesicles (EVs), tissue explants, organoids or cutting-edge organ-on-a-chip technologies. Oviductal and uterine fluids are rich in a spectrum of substances including simple and complex carbohydrates, ions, lipids, phospholipids, proteins, and EVs, which collectively emulate maternal conditions to varying degrees of fidelity. Among these components, EVs stand out as pivotal mediators of intercellular communication within the complex milieu of embryo-maternal interactions. EVs play a crucial role in facilitating the essential crosstalk between the developing embryo and the maternal environment. These vesicles, secreted by various cells including oviductal and uterine epithelial cells, carry a cargo rich in bioactive molecules such as proteins, lipids, RNA, microRNA and signaling molecules. By transferring these bioactive molecules, EVs actively participate in shaping the maternal environment to support embryo growth and development. Their ability to modulate gene expression and cellular behavior further underscores their significance in orchestrating the finely tuned interactions necessary for successful pregnancy establishment. Besides, an ex vivo model has been successfully established, facilitating direct interaction between bovine embryos and oviductal or endometrial explants with promising initial outcomes. These innovative in vitro approaches hold tremendous potential to deepen our understanding of embryo-maternal interactions. Such advancements not only promise to unravel previously inaccessible nuances of this intricate relationship but also pave the way for the development of novel strategies aimed at enhancing reproductive outcomes in cattle. These models provide opportunities to enhance both the yield and quality of in vitro produced blastocysts, thus addressing longstanding challenges for advancing assisted reproductive technologies and improving outcomes in both animal and human fertility.

Mare endometrium disorders: inflammation, fibrosis, and therapeutic approaches

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In the genomics and transcriptomic era, new approaches have been developed to solve endometritis and endometrosis in the mare. The application of next generation sequencing to the analysis of mare endometrium at different physiological and pathological stages has allowed the identification of new potential pathways and regulators involved in development endometrial pathologies (i.e.: endometritis and endometrosis), and thus affecting mare fertility. An unresolved chronic endometritis may lead to endometrosis establishment, mainly in older susceptible mares. In aged mares, collagen fibers develop in the endometrium and oviduct, which might cause early embryonic death. If the embryo survives, foetal development will rely on placenta plasticity and function. Older fertile multiparous mares, develop a heavier, more vascularized functional placenta, with more collagen. Thus, even though collagen deposition is detrimental in mare reproductive tract, it appears to be structural in the placenta. While older mares are still fertile, their placentas undergo morphophysiological adaptations to carry on a healthy and heavier foal to term. Equine neutrophils are capable to form Neutrophil Extracellular Traps (NETs) in mare endometrium, when in the presence of microorganisms and/or sperm cells. Besides defeating infection, NETosis may cause pathologic endometrium collagen deposition (endometrosis). The establishment of endometrosis is very complex and besides persistent NETs formation, it involves cytokines and prostaglandins dysregulation, and epigenetics action, contributing for collagen and extracellular matrix (ECM) deposition. Some specific inhibitors of cathepsin, elastase, or myeloperoxidase, which are proteins present in NETs, as wells as a non-specific protease inhibitor (noscapine), decrease their *in vitro* pro-fibrotic effects. In severe endometrosis, the increase in collagen is epigenetically regulated by inhibiting the anti-fibrotic genes of metalloproteinases (MMP2 and MMP9), enabling the fibrotic genes and MMPs inhibitors (TIMPs) to act. As such, epigenetics might be an alternative therapeutic target for endometrosis. An epigenetic inhibitor (decitabine) used *in vitro* on equine endometrial fibroblasts, was able to reduce collagen production, and therefore fibroblast pro-fibrotic effects. The increase in collagen in the placenta of older mares, is also epigenetically regulated. Collagen deposition, mainly in the gravid horn, is associated with hypomethylation. Functional enrichment of the transcriptomic data obtained suggests that inflammation and metabolic changes may be features of both mild and moderate stages of endometrosis. Differentially expressed genes (DEGs) were annotated for organ inflammation, cellular infiltration by leukocytes, macrophages and phagocytes, as well as phagocyte and leukocyte cell movement, and cytokine levels in mild (IIA) and moderate (IIB) endometrium compared to category I endometrium. In conclusion, epigenetic modulation and transcriptomic studies may be considered as novel therapeutic approaches, for mare endometritis and endometrosis, and to explain placenta plasticity and function in older fertile mares.

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Determination of porcine follicular fluid extracellular vesicles effect on boar spermatozoa survival *in vitro*

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Follicular fluid (FF) is a dynamic biological fluid formed from blood plasma components and molecules secreted by the oocyte, mural granulosa cells, cumulus cells, and theca cells. During ovulation, FF from the ruptured follicle enters the oviduct known to have a role in ovarian and uterine functions. Previous investigations with bovine FF reported that extracellular vesicles in FF (FFEVs) capable of improving sperm survival and functionality. However, whether this phenomenon is common to other species is not yet fully investigated. Therefore, we hypothesize that FEVs isolated from different types of porcine ovarian follicles may also act as regulators of spermatozoa physiology and functions.
Porcine FF samples were collected from small (<3mm), medium (3-7mm), and large (>7mm) follicles based on their size. FFEVs were enriched using size exclusion chromatography (SEC) and characterized via nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and purity assessments assessment based on the International Society for Extracellular Vesicles guidelines. To investigate their functionality, fresh porcine spermatozoa (10 million sperm/ml) were incubated with different EV concentrations (0.1, 10, 1000 million EVs/ml) for 4 hours, then assessed for viability, capacitation, and acrosome integrity using a live/dead® viability/cytotoxicity kit and chlortetracycline (CTC) staining. The experiment was repeated three times and data were analyzed using ANOVA followed by Dunnett's post hoc analysis.
Regardless of the follicular size, SEC fractions 6, 7, and 8 provided the highest nanoparticle concentration with minimal soluble protein contamination, with the size range from 100 nm to 150 nm. The distinct extracellular vesicle morphology was conserved with the TEM. Moreover, the experiment concluded that the concentration of nanoparticles is inversely related to their follicular origin. Similar to previous studies in bovine FF, the present study demonstrated a tendency of porcine FFEVs to aid sperm survival in a dose-dependent manner. However, a significant*($p=0.0015$)* influence was observed only at the 1000 million EVs/ml treatment from medium-sized follicles. Notably, there was no significant influence on sperm capacitation and acrosome reaction by the FFEV treatment.
In summary, the present investigation indicated that FF from different sizes of porcine ovarian follicles may contain nano-sized signaling molecules capable of modulating boar sperm survival, particularly at the highest concentration with a 1:100 Sperm to EV ratio. Hence, further in-depth work will shed more light on the exact role of porcine FFEVs on sperm functions paving the way to use them under ART applications.

Relationship between the epithelial immune response in the uterus and oviduct in postpartum dairy cows

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An adequate immune response in the uterus is crucial for withstanding bacterial infections after calving. Excessive inflammation in the reproductive tract, however, can negatively impact fertilization and embryo development. The anatomical connection of the uterus and the oviduct suggests that uterine inflammation might progress to the oviduct. Therefore, this study aimed to explore the relationship between uterine and oviductal inflammatory processes in animals with and without endometritis. Cytobrush and mini-cytobrush samples were taken from the uterus and oviducts, respectively, of 31 cows at day 28 and 56 postpartum (pp) by transvaginal endoscopy (TVE). Samples were subjected to cytological examination to determine the percentage of polymorphonuclear neutrophils (PMN%). Total RNA was extracted from the brushes for real-time quantitative PCR for selected pro-inflammatory factors (*CXCL1/2*, *CXCL3*, *IL1A*, *IL1B*, *IL8*, and *PTGS2*). Based on a vaginoscopic examination and uterine PMN% at day 28 pp, cows were classified as "healthy" (HE; n=17) and "endometritic" (EN; n=14). Uterine PMN% and mRNA abundance of *CXCL1/2*, *IL1A*, *IL1B*, and *PTGS2* were significantly greater in EN than in HE (2.2 to 9.3-fold; $p<0.05$) at day 28 pp. In the oviduct, EN showed significantly greater PMN% and a greater mRNA abundance of *CXCL1/2* and *CXCL3* at day 28 pp than HE (1.4 to 1.9-fold; $p<0.05$). At day 56 pp, no significant differences were found between groups, neither in the uterus nor in the oviduct. A positive correlation was observed between the uterine and oviductal mRNA expression at day 28 pp (*CXCL1/2*, *IL1A*, and *IL1B*; $r=0.4$ to 0.5, $p<0.05$), whereas at day 56, no significant correlation was found. In addition, a positive relationship was observed between the uterine RNA abundance at day 28 pp and those of the oviduct at day 56 pp for *IL1B* ($r=0.5$, $p<0.05$). Numerous associations were found between the PMN% and the RNA abundance; most pronounced results were observed between uterine PMN% at day 28 pp and oviductal mRNA expression at day 28 pp and day 56 pp (all pro-inflammatory factors $p<0.05$, $r=0.3$ to 0.8). In addition, the PMN% and the RNA abundance in the oviduct correlated positively at day 56 pp (all pro-inflammatory factors $p<0.05$, $r=0.3$ to 0.8). By using TVE-guided repetitive sampling, we could show for the first time in vivo that inflammatory processes might progress from the uterus into the oviduct. The results are the basis for future studies on the effect of reproductive tract inflammation on fertility and early embryo development.

The impact of TGFβ1, mesenchymal stem cells, and extracellular vesicles on equine in vitro endometrial fibrosis models

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Mare endometrosis, a condition characterized by peri-glandular fibrosis induced by TGFβ1 signaling, currently lacks an effective treatment. This presents an exciting opportunity to explore the potential of using mesenchymal stem cells (MSCs) or their secreted extracellular vesicles (EVs) as a candidate. However, a key consideration is the potential impact of the fibrotic environment on the administered MSCs, which could mask their beneficial effect. We studied the role and interaction of TGFβ1, MSC, and EVs secreted by MSCs in endometrial fibrosis. First, we replicated a cellular model of induced fibrosis in vitro by exposing mare endometrial fibroblasts to a pro-inflammatory cocktail (10 ng/mL of TGFβ) combined with IL1β, IL6, or TNFα (10 ng/mL each) or all together for 24 h. This led to a fibrotic phenotype in fibroblasts from the follicular phase. Fibrotic genes (*CTGF, COL1A1, COL3A1*, and *TIMP1*) and pro-fibrotic miRNAs were elevated in EVs from the follicular phase. Anti-fibrotic miRNAs were upregulated in the mid-luteal phase.
Next, we evaluated the impact of preconditioning equine MSC cells with TGFβ1 for 4, and 24h, on the profile of fibrotic/antifibrotic cellular mRNAs and miRNAs, as well as EVs-enclosed miRNAs. Preconditioning MSC with TGFβ1 for 24 h led to a significant increase in the expression of myofibroblast gene markers *aSMA, COL1A1,* and *TGFβ1* as well as of profibrotic mir192 and mir433, while 4h preconditioning enriched the anti-fibrotic miRNAs (mir29c, mir145, and mir200) in cells and EVs. TGFβ1 significantly upregulated the expression of PGE_2-related genes, COX2, PTGES, and receptor EP4 early at 4 h of exposition and PGE_2 secretion compared to controls; conversely, at 24 h, the PGE_2 values decreased significantly). In all, preconditioning MSC for 4 h led to an anti-fibrotic secretory phenotype; a more extended period (24 h) led to a pro-fibrotic one. Finally, the resulting EVs from MSC were added for 48 h to endometrial stromal cells previously induced to fibrosis, and the expression of genes related to fibrosis was analyzed by qPCR or NGS. Preconditioning MSCs with TGFβ1 for 4 h enriched the anti-fibrotic miRNAs (mir29c, mir145, and mir200) in cells and EVs. Conversely, preconditioning the cells for 24 h leads to a pro-fibrotic phenotype overexpressing mir192 and mir433. These findings might have significant implications for developing an EV-based protocol to treat endometrial fibrosis in mares. It is tempting to propose a 4-h preconditioning of exogenous MSC with TGFβ1 to drive them towards an anti-fibrotic phenotype.

Fibroblasts as key regulators of endometrial fibrosis in the mare: insights from transcriptomic and functional analysis

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Endometrosis is a chronic degenerative condition of the mare's uterus. The term "endometrosis" encompasses a number of different endometrial changes, of which fibrosis is the most common. In general, fibrosis is characterised by excessive deposition of both collagenous and non-collagenous extracellular matrix (ECM) components due to the accumulation, proliferation and activation of fibroblasts and myofibroblasts. Fibroblasts, a heterogeneous population of stromal cells, play a central role in the synthesis of components essential for the structural integrity of the ECM. In addition, fibroblasts actively remodel the ECM microstructure through covalent cross-linking, protein glycosylation and controlled proteolysis involving the balanced secretion of enzymes such as lysyl oxidase, matrix metalloproteinases (MMPs) and MMP inhibitors. In addition to their role in structural support, fibroblasts are capable of secreting and responding to cytokines, chemokines and growth factors. They are responsible for maintaining the homeostasis of neighbouring cells and orchestrating the maintenance of inflammatory infiltrates, indicating their importance in tissue development, differentiation, remodelling and repair.

The aim of this study was to determine whether the transcriptomic signature and functional characteristic of *in vitro* cultured fibroblasts derived from non-fibrotic and fibrotic endometrium of the mare is altered. In addition, the study aimed to investigate the effect of the T helper cell secretome on the transcriptomic signature and functional characteristics of endometrial fibroblasts.

The obtained results show that the transcriptomic profile of fibroblasts is altered during the development of fibrosis, which could consequently affect their function. The gene expression profile of enzymes responsible for ECM remodelling is downregulated, suggesting that the excessive deposition of ECM in the fibrotic process cannot be degraded. The expression of immune-related genes differs between fibrotic and non-fibrotic fibroblasts, suggesting that fibroblasts from fibrotic endometrium may be more active in modulating immune responses. In turn, the functional enrichment of the transcriptomic data obtained from fibroblasts derived from non-fibrotic endometrium treated with Th1 and Th2 cell secretomes suggests a direct role for these cells in ECM remodeling, chemotaxis, fibroblast proliferation, collagen metabolism, or metallopeptidase activity.

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Transcriptomic landscapes of the endometrium in dairy cows with clinical or subclinical endometritis

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Subclinical (SCE) and clinical endometritis (CE) are distinct manifestations of reproductive tract inflammatory disease in dairy cows. The development of SCE and CE stems from the postpartum dysregulation of the inflammatory response of the endometrium or a shift in the composition of the uterine microbiome, respectively. To gain further insight into these responses, we aimed to identify changes in the endometrial transcriptomic landscape in healthy postpartum dairy cows compared to those diagnosed with SCE or CE. To do so, we assessed the uterine health status of twenty-four multiparous Holstein cows in the fifth week postpartum based on the evaluation of vaginal discharge (Metricheck) and endometrial cytology (cytobrush). Twelve cows were diagnosed as healthy (clear or absence of vaginal discharge and ≤5% endometrial polymorphonuclear cells (PMN)), six cows as SCE (clear or absence of vaginal discharge and >5% endometrial PMN), and six cows as CE (mucopurulent vaginal discharge or worse and >5% endometrial PMN). After endometrial sampling, the cytobrush was stored at −80 °C. Total RNA was isolated using the RNeasy Micro kit (Qiagen, Germantown, TN, USA) according to the manufacturer's protocol. Sequencing was performed on a high throughput Illumina NextSeq 500 flow cell generating 75 bp single reads. Differentially expressed genes (DEGs) analyses were performed with DESeq2 (p.adj < 0.05). A total of 1,091 DEGs were identified between healthy and CE, 250 DEGs between healthy and SCE, and 829 DEGs between SCE and CE. Overrepresentation analysis using the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways database (p.adj < 0.05) revealed that IL-17, TNF, chemokine, NF-kappa B, and Toll-like receptor signalling pathways were enriched in CE compared to healthy cows. When comparing healthy versus SCE cows, enriched pathways in SCE were TNF and IL-17. NOD-like receptor, IL-17, complement and coagulation cascades, and the chemokine signalling pathways were found to be enriched in CE than in SCE cows. Endometrial transcriptomic changes in SCE or CE compared to healthy dairy cows mainly involve immune-related reactions and inflammatory responses, engaging multiple biological pathways. Additionally, upregulated pathways in CE (compared to SCE) are mostly indicative of host immune response towards infection. These findings highlight the distinct pathologic mechanisms underlying CE and SCE, suggesting the need for tailored preventive and therapeutic interventions for each condition. Specifically, enhancing immune function in cases of SCE and controlling bacterial dysbiosis and its subsequent damage in cases of CE.

The effect of the demethylating agent decitabine on collagen expression in mare endometrial fibroblasts treated with elastase

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Endometrosis, a mare endometrial condition characterized by degenerative changes and excessive collagen accumulation, negatively impacts fertility. Neutrophils, part of the immune system, can form neutrophil extracellular traps (NETs), which are DNA-associated complexes of nucleic and cytoplasmic proteins (e.g. elastase, ELA) that trap and kill pathogens. While NETs have antimicrobial properties, they can also contribute to the development of fibrosis. Elastase has been shown to increase collagen expression in mare endometrial explants. The inhibition of ELA action could potentially reduce endometrial fibrosis development in mares. Epigenetic modifications, such as DNA methylation, can influence fibroblasts' behaviour and fibroproliferative disease progression. These changes are reversible, making epigenetics a promising therapeutic option. In previous research, the demethylating agent 5-aza-2′-deoxycytidine (decitabine) was shown to reduce collagen type I (COL 1) and III (COL 3) expression in TGF-β1-treated endometrial fibroblasts *in vitro*. Therefore, this study investigates whether ELA action in fibroblasts is modulated by epigenetic mechanisms and if decitabine can inhibit ELA to impair fibrogenesis. Equine endometrial fibroblasts (n=5) were treated with ELA (1µg/mL) for 48h, followed by decitabine (1 µM) for another 48h, to assess their effects on DNA methyltransferases (*DNMT1, DNMT3A* and *DNMT3B*) and *COL1A1* and *COL3A1* transcripts (qPCR) in fibroblasts, and COL concentration in conditioned medium (EIA). As controls, fibroblasts were incubated in culture medium, or with decitabine or ELA, for 96h. ELA increased *DNMT3A* and decreased *DNMT3B* transcripts, while also increasing *COL1A1* mRNA and protein levels ($P<0.05$). Decitabine alone reduced *DNMT3B* transcripts ($P<0.001$). When used in combination with ELA, decitabine decreased *COL1A1* ($P<0.01$) and *COL3A1* ($P<0.05$) mRNA and COL1 protein ($P<0.01$), as well as *DNMT3A* and *DNMT3B* transcripts ($P<0.01$). It also decreased COL3 protein abundance when compared to non-treated fibroblasts ($P<0.05$). The study suggests that ELA treatment leads to an increase in *DNMT3A* expression, which may cause hypermethylation and increased collagen production by inhibiting antifibrotic genes, like *MMP2* and *MMP9*. When decitabine is added to ELA-treated fibroblasts, it reduces *DNMT3A* and *DNMT3B* transcripts, leading to hypomethylation and a decrease in collagen. This could be due to the activation of antifibrotic genes that impair collagen production. The decrease in *DNMT3B* after ELA treatment is further reduced by decitabine, potentially activating the transcription of antifibrotic genes, such as MMPs or PGE2, which diminish collagen production. These data, together with the downregulation of the profibrotic effect of ELA in decitabine-treated endometrial fibroblasts, suggest an epigenetic involvement in the pathogenesis of endometrosis. Further studies are needed to better understand how epigenetics modulate processes associated to the development of endometrosis.

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Mapping of the plasma and endometrial proteome of mares with healthy and fibrotic endometria

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Diagnosis of equine endometrial fibrosis (EF) can only be achieved by histopathological evaluation of an endometrial biopsy. When the condition is diagnosed, it is already advanced and irreversible. Proteins are integral in cellular functions, and while many proteins have been identified in relation to an accumulation of extracellular matrix, a proteomic profiling of endometrial tissue could be the key to gain better understanding of the pathogenic pathways leading to EF. Through evaluation of hematoxylin and eosin stained endometrial biopsy sections from mares in diestrus, 46 mares were selected with no, moderate, or severe EF. Immunostaining for α-smooth muscle actin confirmed the presence and intensity of EF in control group (no lesions; n=9), moderate (n=9) or severe EF (n=9). Proteins were extracted from endometrial tissue and plasma and analyzed by data independent acquisition mass spectrometry. Only proteins present >75% of the samples in a single group or >60% of all samples were included. The data was log2-transformed and tested for normality with Shapiro-Wilk test. Limma test was used for proteins with normal distribution and Wilcoxon-Mann-Whitney test for proteins without normal distribution. The Benjamini-Hochberg method was used to adjust for multiple comparisons. Over-representative analyses (ORA) were performed on proteins with adjusted p-value <0.05 (tissue) and non-adjusted p-value <0.05 (plasma). A total of 10,052 proteins were identified in tissue, and a total of 510 proteins were identified in plasma. Between moderate EF cases and controls there were 310 differentially expressed proteins (DEPs) in tissue, and 75 DEPs in plasma. ORA showed pathways involved in cell adhesion, extracellular matrix and innate immune system. Between severe EF cases and controls there were 585 DEPs in tissue, and 71 DEPs in plasma. ORA showed more pathways involved in cell adhesion and extracellular matrix, and also pathways involved in cell differentiation, regulation and repair processes, and metabolism. Between severe and moderate EF cases there were 391 DEPs in tissue and 31 DEPs in plasma. ORA showed pathways involved with cell adhesion, extracellular matrix, regulation and metabolism. In conclusion, we have identified several DEPs between mares with no, moderate and severe EF in endometrial tissue and plasma. Based on over-represented pathways, it appeared that the fibrotic processes progressed from activation of the innate immune system and an increasing involvement of cell adhesion and extracellular matrix, to regulatory, repair and metabolic processes.

The potential role of IL-17 in the processes associated with the development of equine endometrosis

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Equine endometrosis is characterized by endometrial stromal fibrosis with degenerative changes in the adjacent tissue structures. Tissue fibrosis generally arises from chronic inflammation caused by various stimuli and is marked by the excessive production and deposition of extracellular matrix (ECM) components. This process is further complicated by the disrupted balance of ECM degradation, regulated by matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). A growing body of evidence indicates the critical role of interleukin (IL)-17 in fibrotic disorders. However, the exact mechanism of its action on endometrial fibroblasts during mare endometrosis remains to be discovered and explained. Therefore, the main aim of the current study was to establish 1/ the expression of IL-17 and its receptor (IL-17R) in equine non-fibrotic and fibrotic endometrium (qPCR, Western-blot, ELISA), and 2/ the effects of IL-17 on a/ endometrial fibroblast proliferation (BrdU assay), b/ the expression of extracellular matrix (ECM)-related genes (qPCR) and c/ the changes in the transcriptome of fibroblasts isolated from non-fibrotic and fibrotic equine endometrium (RNA-seq).

The endometrial expression of both, IL-17 and IL-17R transcripts did not differ between non-fibrotic and fibrotic endometria, while protein abundance of IL-17R was up-regulated in fibrotic endometrium when compared to non-fibrotic. IL-17 did not affect equine endometrial fibroblast proliferation and gene expression of ECM components. However, it influenced gene expression of *MMP3* and *MMP9*. Moreover, RNA-seq results revealed that IL-17 altered the expression of 176 and 97 genes in fibroblasts derived from non-fibrotic and fibrotic equine endometrium, respectively, with 79 common genes. Genes with expression altered by IL-17 (i.a. *CXCL3*, *CXCL6*, *CXCL8*, *CXCL12*, *PTGS2*, *PTGES*, *IL-1α*, *IL-6*, *IL-11* and *LIF*) were involved in the immune cell chemotaxis, migration and activation, prostaglandin secretion, transport and metabolism, as well as toll-like receptor and TNF signaling pathways – immune-related processes with evidenced role in fibrotic disorders.

Our findings indicate that the response to IL-17 differs between fibroblast derived from non-fibrotic and fibrotic endometrial tissue. Moreover, IL-17 may be involved in the changes of course of equine endometrial fibrosis, potentially through its effects on the expression of MMPs and immunomodulatory properties of endometrial fibroblasts.

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Postpartum Metabolic and Reproductive Health in Dairy Cows

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Inflammation is a necessary part of the physiologic process of uterine involution after calving. Inadequate immune response, trauma to the reproductive tract, and changes in the uterine microbiome soon after calving combine to cause metritis or, later, purulent vaginal discharge (PVD). Metritis and PVD are associated with uterine bacterial dysbiosis: changes in the microbiota to lesser diversity and greater abundance of pathogens, especially Gram-negative anaerobic bacteria, and *Trueperella pyogenes* in the case of PVD. Metritis is justifiably treated with approved antibiotics but criteria for more selective treatment without loss of performance are emerging. In contrast, endometritis (inflammation diagnosed by cytology that associated with impaired fertility i.e., > 5% neutrophils at 4 to 6 weeks postpartum) is associated with excessive or persistent uterine inflammation but generally not with bacterial infection. Endometritis seems in many cases to reflect persistent, dysregulated inflammation, for which the inciting cause is unclear. It may relate to systemic inflammation, with roots before calving. However, clarity and progress are limited by a lack of validated criteria to quantify systemic inflammation and to identify its sources. Our recent experiments in early postpartum cows were not able to induce uterine from acute systemic inflammation or vice versa. Current evidence suggests that endometritis is associated with metabolic and inflammatory dysfunction that impairs innate immune function, most importantly, resolution of inflammation. Adaptation to systemic metabolic stressors may be as important for the development of uterine disease as the local host interactions with bacteria and the inflammatory response in the reproductive tract. Prolonged or excessive hypocalcemia, limited availability of glucose, and upregulated lipolysis and sterile inflammation may contribute to dysregulated inflammation in postpartum dairy cows. Current evidence supports the hypothesis that endometritis is the result of transition period maladaptation including excessive metabolic stress, immune dysfunction, and failure to resolve uterine inflammation. Postpartum uterine infection and inflammation have harmful effects on oocytes, embryo development, and the endometrium for at least three months, even if the disease is apparently resolved. Emerging concepts of the resolution and regulation of inflammation are promising for the improvement of prevention and therapy of endometritis.

Metagenetics of the female reproductive tract: opportunities, challenges, and lessons learned

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Recent studies highlight the crucial role of reproductive tract microbial communities in shaping mammalian health and reproductive outcomes. Notably, the transition from late pregnancy to early postpartum, marked by the opening of the cervix, often results in the most extensive contamination event of the female reproductive tract, which may be even more substantial for primigravid females experiencing their first parturition. Interestingly, in many species, postpartum uterine inflammatory diseases are more prevalent among primiparous females. Traditional culture-dependent methods have isolated various microbial species in postpartum cow uteri (e.g., *E.coli, T.pyogenes, F.necrophorum, P.melaninogenica*), some linked to metritis. However, recent culture-independent methods, particularly next-generation sequencing of 16S-rDNA amplicon libraries (i.e., metagenetics), have provided new opportunities and insights. These techniques have identified bacteria not detectable by traditional methods and have also shown that Koch's postulates are not fully fulfilled in postpartum uterine diseases. However, metagenetics studies face challenges such as sample sensitivity to ecosystem conditions, contamination, technical confines in sample preparation and sequencing, and the need for robust computational tools and comprehensive bioinformatics skills.

We and others have conducted several metagenetics studies exploring the reproductive tract of Holstein-Friesian cows. Our recent results revealed distinct spatio-temporal microbial transitions between the prepartum vagina (258±4 days gestation) and postpartum vagina and uterus (7±2DIM) in first-pregnancy heifers. The prepartum vagina and postpartum uterus were most distinct, with the postpartum vagina showing intermediate profiles. Significant reductions in bacterial richness and diversity in the postpartum uterus indicated a selective niche shaped by parturition-related endocrine-physiologic changes. Our findings suggest that vaginal microbiota may seed the postpartum uterine microbiome, with unique signatures in the postpartum uterus implying environmental influences.

In studies at 5-12 DIM, we found no difference in uterine bacterial load between healthy and metritis cows. However, metagenomics revealed that metritis cows had a distinct bacterial community with higher relative abundances of Bacteroidetes and Fusobacteria, mainly comprising *Bacteroides*, *Porphyromonas*, *Fusobacterium*, and *Tissierellaceae* spp. In contrast, healthy cows had more diverse communities, although some exhibited a 'metritic-like' community, suggesting differences in uterine immune responses. In another cohort study, we measured a decrease in uterine bacterial load over time (5-12DIM→30-40DIM→60-70DIM). Metagenomic analysis did not clearly distinguish bacterial communities between endometritis (cytobrush diagnosis) and healthy cows, but RNA-seq analysis indicated differences in uterine transcriptomics. Future studies leveraging advanced bioinformatics and refined disease definitions promise deeper insights into microbial dynamics and their impact on reproductive health, presenting exciting opportunities for improved prevention, diagnosis, and therapies.

Update on diagnostic procedures for equine endometritis

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Endometritis is a leading cause of reproductive inefficiency with a prevalence ranging from 25–60% in barren mares. It reduces conception rates and elevates the risk of various complications including early embryonic death, abortion, placentitis and delivery of septic foals. Intensive research for decades has emphasized multiple levels of failure of the uterine defense mechanisms in mares susceptible to persistent breeding induced endometritis (PBIE). Given that optimizing antibiotic treatment is crucial in combating rising antimicrobial resistance, accurately diagnosis and identification of associated pathogens in mares intended for breeding is more important than ever. The primary microorganisms associated with endometritis in mares are *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Many pathogens have the ability to create bacterial persistence by formation of slowly growing persister cells with reduced antibiotic susceptibility due to dormancy. This feature increases the likelihood of resistance development and potential chronic infections. Until recently, it was believed that the uterine lumen of the normal fertile mare was bacteriologically sterile, but the use of new generation sequencing techniques has revealed that the equine uterus harbors a commensal microbiota. Any shift in this population may lead to an increased risk of PBIE and bacterial endometritis. Currently, endometritis is diagnosed via ultrasonography of the reproductive tract, endometrial cytology and traditional aerobic culture. Ultrasound is used to detect clinical signs of endometritis, such as intrauterine fluid and excessive edema, however these signs may not always be present. Samples for diagnosis can be obtained using double-guarded uterine culture swab, cytobrush, biopsy and low-volume lavage. Although these techniques are used in clinical settings, they are prone to false positives and false negatives, which can result in incorrect diagnosis or unnecessary treatments. In addition, endometrial tissue samples should undergo histological assessment for signs of inflammation. Unexplained inflammation may suggest focal infection and require further diagnostic work-up. Adding a broth-enrichment culture before plating the diagnostic sample on solid media has been proven to increase the sensitivity [1]. Mass-spectrometry-based proteomics is an extraordinary tool to accurately detect and quantify thousands of proteins, which can be used for the discovery of potential biomarkers. Most recently, proteomic profiling of plasma [2] and uterine fluid [3,4] revealed differentially expressed proteins in mares with bacterial endometritis compared to healthy mares. The proteins were associated with the innate immune system, cell proliferation and cellular energy metabolism or groups of proteins with antimicrobial properties. Continued investigation into differentially expressed proteins and associated pathways may identify promising targets for new diagnostic and therapeutic strategies.

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Functional metagenomics of endometrial cytology samples from dairy cows diagnosed with clinical or subclinical endometritis

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Potentially pathogenic bacteria can be found in the uterus of both healthy cows and those diagnosed with uterine disease. Little is known, however, about the functionality of bacteria in cows with clinical (CE) and subclinical endometritis (SCE). We performed functional metagenomic analyses of endometrial samples collected from Holstein cows diagnosed as healthy, CE, or SCE. Uterine cytobrush samples were collected from multiparous cows (n = 22) at 21 days postpartum and the uterine health diagnosis was performed at 36 days postpartum. Cows were classified as healthy (n = 8; clear or absence of vaginal discharge and ≤5% endometrial polymorphonuclear cells (ePMN)), SCE (n = 7; clear or absence of vaginal discharge and >5% ePMN), or CE (n = 7; mucopurulent vaginal discharge or worse and >5% ePMN). The endometrial cytobrush samples collected at 21 days postpartum were sequenced using deep shotgun metagenomic sequencing on Illumina MiSeq platform, yielding over 20 million sequencing reads per sample. To extract the functional profile from the raw data, the HUMAnN pipeline (v. 3.8) was employed. Adapter and host genome (bosTau9) sequences were removed using Trimmomatic (v. 0.39) and Bowtie2 (v. 2.5). Microbial reads were processed by identification of microbial species and their predicted functions. The aligned genes were mapped against gene ontology (GO) database to obtain GO terms. These data were fitted for differential abundance among uterine health status using Microbiome Multivariate Association with Linear Models (MaAsLin2) with a significance threshold of FDR < 0.25. Twelve functions were different between CE and healthy, three differed between SCE and healthy, and only 2 differed between CE and SCE cows. Crucial functions for the maintenance of uterine integrity and damage recovery, namely Wnt signaling pathway (LFC = 0.95), response to estrogen (LFC = 0.67), and positive regulation of cell population proliferation (LFC = 0.67) had greater abundance in healthy compared to CE cows. These functions were attributed to Staphylococcus, Lactococcus, and Lactobacillus. Compared to SCE, the translation elongation factor activity involved in bacterial protein synthesis was greater in CE (LFC = 3.0) and healthy cows (LFC = 3.1). Moreover, deoxyribose-phosphate aldolase activity, involved in bacterial metabolic pathways, was greater in CE (LFC = 6.3) and SCE (LFC = 5.3) than healthy cows. Our findings underline the importance of shotgun metagenomics for studying microbial function in distinct manifestations of endometritis. Understanding the function of bacterial communities is critical for the development of preventive and therapeutic strategies against uterine disease.

Comparative analysis of antimicrobial resistance through whole genome sequencing in healthy and metritic cows and their environment

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Antimicrobial resistance (AMR) is a critical public health challenge. AMR surveillance in production environments is crucial, as they can serve as reservoirs for resistant pathogens. Metritis is a postpartum disease that usually requires antibiotic treatment and affects animal welfare and profitability. Our study used whole genome sequencing to compare AMR patterns in *Escherichia coli* from healthy and metritic cows and their environment. The research was conducted over six months in a commercial dairy farm in Castelli, Argentina, which housed 1800 Holstein cows. The study involved 63 cows that were 8±3 days postpartum and had not been treated with antibiotics after calving. Vaginal discharge (VD) was assessed using Metricheck, and cows with VD3 (reddish-brown discharge and foul odor) were considered to have metritis, while cows with VD0 (normal clear discharge) were considered healthy. Uterine samples were collected using cytobrush technique with double protection and placed in a Stuart transport medium. Environmental samples (milking parlor and parturition pen) were obtained using absorptive boot covers every two months. Samples were cultured on EMB agar for *E. coli* isolation and confirmed by Gram staining and IMViC biochemical tests. *E. coli* was isolated in 57.1% (36/63) of samples. A single, isolated colony was chosen at random from healthy (n= 13) and metritic cows (n= 18) as well as environmental samples (n= 5) to identify resistance genes by shot-gun sequencing (Microbes, NG). Only 5.5% of the isolates from metritic cows (1/18) were resistant and carried genes encoding for beta-lactams (*blaTEM-1B* gene), tetracyclines (*tetA* gene), nalidixic acid (*gyrB* mutation), aminoglycosides (*aadA1, strA,* and *strB* genes), and sulfa-trimethoprim (*sul2* and *dfrA5* genes). In contrast, remaining isolates (17/18) were susceptible to the antimicrobials tested. Only 7.7% of isolates from healthy cows (1/13) were resistant, containing genes for resistance to tetracyclines (tetB gene). Regarding environmental isolates, 20% (1/5) were resistant to fosfomycin, containing the fosA7 gene. No differences between metritic and healthy cows were found (P = 0.8). Extended-spectrum beta-lactamase (ESBL) was not detected in any of the samples analyzed. Despite finding a multidrug-resistant strain in one metritic sample, the resistance levels were lower than expected for sick animals. Although resistance levels were relatively low, it is important to emphasize the need for rational use of antimicrobials. Surveillance and prudent use of antimicrobials are essential for sustainable dairy farming and to reduce the risks associated with AMR in veterinary settings.

The association of cytological endometritis with insulin resistance and gene expression in adipose tissue in transition dairy cows

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Key players in the development of cytological endometritis (CYTO), a disease hindering the reproductive success of dairy cows, are immune function and metabolic stress during the transition period. Insulin resistance (IR) is associated with both of them, so we hypothesized that IR could be a contributing factor to CYTO.

This research, funded by IUT8-1 and PUTJD1217, was carried out on multiparous Estonian Holstein cows of one herd. Cows were assigned into two groups based on the proportion of polymorphonuclear neutrophils (PMNs) in the endometrium on day 40 after calving: CYTO-negative (PMNs $<5\%$; n=29) or CYTO (PMNs $>5\%$; n=10). Cytology samples were taken from the base of the larger uterine horn using an endometrial brush. Numbers of epithelial cells and PMNs were counted from 100 cells per slide in two replicates. Insulin and fatty acids (NEFA) concentrations were analysed from coccygeal vein blood samples taken weekly from d-21 to 28, and on d42. Intravenous glucose tolerance tests (IVGTT) were performed on d-21 and 21 in relation to calving. Insulin was quantified from IVGTT blood samples and the insulin area under the curve (AUC) between 5 to 30 min above the basal level (-5 min) was used as IR marker. Subcutaneous adipose tissue (SAT) biopsies were taken on d-21 and d21 from the pin bone region and analysed with RT qPCR for mRNAs coding for glucose transporter 4 (GLUT4), insulin receptor (INSR), and hormone sensitive lipase (HSL). *GADPH* was used as a reference gene. Relative gene expression levels were calculated using the ΔCt method. Mixed linear models were fitted in R, significance was declared at $P \leq 0.05$, tendency at $P \leq 0.10$.

CYTO cows tended to have higher insulin AUC on d-21 ($P=0.09$), approximately 60 d before the assessment of CYTO, but this relationship was not evident postpartum (d21). The opposite was true for gene expression levels in SAT. No difference was found prepartum, but postpartum *GLUT4* expression tended to be ($P=0.10$) and *INSR* was higher ($P=0.03$) in CYTO cows, which is probably due to the lower ($P=0.04$) circulating insulin levels in CYTO cows. In addition, lower insulin increased the lipolytic potential in adipose tissue in CYTO cows, evidenced by their higher *HSL* ($P=0.03$) expression, which in turn resulted in higher postpartum circulating NEFA ($P=0.05$) concentrations for CYTO cows.

It is plausible that IR development already before parturition sets the scene for increased metabolic stress after parturition thereby increasing the risk of CYTO.

Platelet-rich plasma as therapy to improve the fertility of mares

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Platelet-rich plasma (PRP) is widely used in sports medicine to modulate inflammation and in recent years has been used in equine reproduction to mitigate persistent-breeding endometritis (PBIE) in mares, a condition associated with poor fertility and excessive inflammation post-breeding and infection. A series of studies conducted in our laboratory we were able to demonstrate that embryo donor mares susceptible to PBIE benefited from serial infusions of PRP pre- and post-breeding. The studies showed a dose-dependent effect with regards to the uterine inflammatory response. In addition, embryo donor mares receiving PRP had higher progesterone concentration, greater embryo recovery and lower chance of developing post-breeding uterine infections. Embryos recovered from mares treated with PRP had distinct cellular pathways giving suggesting that they came from a favorable uterine environment. This could also be due to the distinct inflammatory pathways activated in the endometrium of mares treated with PRP. In conclusion, PRP is a highly practical and effective therapy for mares with PBIE, its use is growing in equine practice.

New insights in the treatment and control of postpartum uterine disease in high-yielding dairy cows

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Postpartum uterine disease represents a significant challenge in high-yielding dairy cows, affecting their reproductive performance and therefore overall productivity. In dairy cows, up to 100% experience bacterial contamination of the uterus immediately after calving, necessitating a robust immune response to prevent the progression to clinical or subclinical disease states. The innate immune system plays a critical role in the postpartum uterine disease complex by controlling the overgrowth of potentially pathogenic bacteria inside the uterine cavity.
The inflammatory response is a normal part of uterine involution; however, in some cows, this response becomes dysregulated, leading to persistent inflammation and subsequent uterine disease. While cows suffering from metritis and clinical endometritis have been shown to harbor a different uterine microbiome in comparison to healthy cows, while this has not been the case for subclinical endometritis. The latter is characterized by an elevated percentage of polymorphonuclear cells (PMNs) in the endometrium without overt clinical signs but leading to a impaired fertility. This condition affects approximately 20-30% of postpartum cows and is diagnosed through cytological examination. The role of the innate immune system is pivotal in this context as it involves the initial detection and response to pathogens by endometrial cells, which results in the migration of PMNs to the endometrial lining.
High-yielding dairy cows are particularly susceptible to postpartum uterine diseases due to metabolic and environmental stresses that may compromise their immune function. Especially the negative energy balance which is a hallmark of high-yielding dairy cows, has been shown to be associated with a higher prevalence of SCE, indicating a link between metabolic status and immune function.
Management practices that enhance the innate immune response during the peripartum period are therefore crucial for preventing postpartum uterine disease. This includes nutritional strategies that minimize metabolic stress and support immune function, as well as timely and accurate diagnosis of all pathologic conditions.
In conclusion, the innate immune system is integral to managing the postpartum uterine disease complex in dairy cows. Effective modulation of this system through managerial and nutritional interventions can help mitigate the adverse effects of these diseases, thereby enhancing reproductive efficiency and overall herd productivity.

The addition of meloxicam to the treatment of clinical and subclinical mastitis improved the cure rate and fertility in dairy cows in Ecuador

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The objective was to study the addition of meloxicam to the treatment of clinical and subclinical mastitis to improve the clinical cure rate and fertility of dairy cows with 35-150 days in milk (DIM). All cows had a California Mastitis Test (CMT) done every two weeks and cows with a CMT score 2 (CMT2), score 3 (CMT3), or with clinical mastitis (CM) were enrolled in the study. For a cow to be enrolled in the study, the CMT2-3 and CM event had to take place 30 d before to 45 d post-AI. Cows were assigned to the control group (CON, n=204) or to the treatment group (TRT, n=189). Cows with CMT2 in the TRT group had meloxicam (MEL; 0.5 mg/kg BW, Metacam), whereas cows in the CON group remained untreated. Cows with CMT3 and CM in the TRT group had an intramammary antibiotic (IMA, Mastijet Fort) and MEL, whereas cows in the CON group only the IMA. After treatment, cows had a CMT done every two weeks to determine the clinical cure rate (CCR). The CCR to CMT2 and CMT3+CM at 30 d post-treatment were examined. Pregnancy rate at 30d (PREGR), pregnancy losses at 60 d (PLOSS), calving to conception interval-days open (CCI), number of services per conception (SPC), and culling rate (CULLR) were analyzed with logistic regression. The CCR for CMT3+CM in the TRT group was higher compared to the CON group (72.5% vs. 57.3%, P=0.036) but was similar between CMT2 TRT and CON groups (62.5%, P=0.73). The PREGR and PLOSS were similar in TRT and CON groups for CMT3+CM (41.9%, P=0.44; 36.76%, P=0.53), and CMT2 (47.22%, P=0.56; 32.35%, P=0.35). The CCI for CMT3+CM in the TRT group was shorter than the CON group (100.66±11.08 vs. 133.23±11.79 d, P=0.04). Also, the SPC for CMT3+CM in the TRT group was lower than the CON group (1.69±0.32 vs. 2.62±0.34, P=0.05). Conversely, the CCI and the SPC were similar between CMT2 TRT and CON groups (118.50±10.95 d, P=0.46; 2.19±0.24, P=0.21). The CULLR was reduced in CMT2 TRT cows compared to CON cows (5.50% vs. 15.5%, P=0.01), but was similar in CMT3+CM TRT and CON cows (14.19%, P=0.52). Cows with CMT3+CM in the TRT group had a higher CCR, shorter CCI and fewer SPC than CON cows. Cows with CMT2 had no benefit of meloxicam treatment on CCR, CCI and SPC, but had a lower culling rate during lactation.

Mechanisms regulating the protection of mammalian cells against damage caused by cholesterol-dependent cytolysins from pathogenic bacteria

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Fertility depends on a healthy uterus in cattle, but postpartum bacterial infections often cause damage to the endometrium lining the uterus (Sheldon et al., 2019). These uterine infections cause disease in approximately 40% of dairy cows each year. The cost of treatment, lost milk production, and infertility is $2 billion dollars per year across the EU and US (Sheldon et al., 2019). Although multiple species of bacteria are associated with uterine disease, *Trueperella pyogenes* is the bacterium that causes most damage in the endometrium, during the postpartum period, by releasing the cholesterol-dependent cytolysin, pyolysin (PLO). The effect of treatment on cellular cholesterol was explored as manipulating cholesterol homeostasis is an effective method of protecting cells against cholesterol-dependent cytolysins (Amos et al., 2014, Statt et al., 2015). This study utilized a consistent approach to examine the effect of pre-treating cells with oxysterols, LXR agonists such as GW3965 and TO901317, antagonists like SR9243 or steroids, such as progesterone and oestradiol before cells treated were treated with pyolysin (PLO), to investigate their effect on cell tolerance to PLO.
The consequences of pore formation by PLO were measured by the MTT assay and the leakage of lactate dehydrogenase (LDH). We demonstrate that PLO damages the plasma membrane and kills HeLa cells. Oxysterol protects cells against damage caused by PLO; whereas Progesterone or oestradiol have little effect on cell damage caused by PLO. Indeed, progesterone and oestradiol may reduce the cytoprotective effects of oxysterols, and GW3965 and T0901317, against PLO.

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Antimicrobial effects of cold plasma-activated solutions against selected bacteria causing endometritis in mares: an in vitro preliminary study

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Inflammation of the endometrium *(endometritis)* is one of the main causes of reduced fertility in mares, mostly common bacterial infections are the crucial background and still antibiotics treatment is main therapeutic method. It is well known that cold air plasma has a bactericidal effect, however, there are not many studies focusing on effect of plasma activated fluids. Therefore, the objective of this study was to examine the effects of plasma-activated solution (Ringer or NaCl) on main bacteria causing endometritis: *Escherichia Coli, Streptococcus equi sub. zooepidermiticus, Pseudomonas Aeruginosa, Staphylococcus spp.* For the production of plasma-activated solution (Ringer or NaCl) cold atmospheric pressure plasma (CAPP) was utilized. The CAPP was generated in an open-to-air atmosphere between the solid (pin type) anode and the flowing liquid solution cathode with 3 mm gap between electrodes. The CAPP was powered by high voltage (HV) dc power generator (DSC Electronics).

Bacteria samples were obtained using two methods: 1) in vivo - an intrauterine brush samples were taken from mares suspected of having *Endometritis* (n=25*)*, and 2) ex vivo - endometrial samples were taken from slaughter material using a sterile swab (n=33). The material was stored in sterile tubes with PBS fluid at -80 degrees Celsius until transferred to the microbiological laboratory. After thawing, culture was performed on Mcconkey agar, blood agar, tryptone soya agar and, after incubation at 37 degrees Celsius, colony appearance was assessed, gram staining, catalase test and, for selected strains, API test was performed.

Freshly cold plasma activated fluids were mixed 1:1 with a previously prepared bacterial suspension and, using the MIC method, 50ul of the suspension was inoculated on the plates. After 24 h, the number of colonies was counted. Statistical analyses were performed using the R statistical programme (version 4.1.2).

In the case of *Streptococcus zooepidemicus* in a mixture with cold plasma-activated Ringer's fluid or NaCl, a 94% decrease in the number of bacteria was found. In the case of *Staphylococcus Haemolyticus*, a percentage decrease in the number of bacteria after one hour in the mixture with Ringer active and NaCl active in was 99%. In the case of *Pseudomonas aeruginosa* and *Escherichia coli* in a mixture with active Ringer and active NaCl was 100%. For each bacterium, an antibiogram was performed.

Concluding, cold plasma has a greater effect on Gram (-) than Gram (+) bacteria. The antibacterial effect of the cold plasma activated fluids on bacterial cultures isolated from the mare's uterus was observed dependently on bacteria species and CAAP-activated solutions could be in the future an alternative method to the use of antibiotics in endometritis therapy.

Recent insights into the molecular correlation aspects among corpus luteum, oviduct, and uterus during the first trimester in dromedary camel

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Coordinated embryo-maternal crosstalk is the main player to achieve a successful pregnancy. The expression patterns of genes are the main forces that induce biochemical and functional changes in the oviduct, uterine environment, and embryo. Therefore, studying the molecular signals associated with corpus luteum (CL), oviductal, endometrial receptivity, and embryonic establishment is critical to improve pregnancy outcomes in she-camel. For this purpose, 40 genital tracts of first-stage pregnancy and blood samples were collected from abattoirs during the breeding season (November 2020 to March 2021). For instance, all pregnancies were in the left uterine horn (LUH) with contralateral CL. Total RNA was isolated from different uterine segments: uterine body (UB), right uterine horn (RUH) & LUH, right & left fallopian tube (RFT & LFT), fetal membrane (FM). RT-PCR was conducted on candidate genes related to endometrium receptivity like JUN proto-oncogene, solute carrier organic anion transporter family member 2A1, and integrin subunit beta 4 (JUN, SLCO2A1, and ITGB4, respectively), in addition to vascularization & placental formation like vascular endothelial growth factor A (VEGFA). Furthermore, histological examination was performed. Data analysis of gene expression was performed by Kruskal-Wallis one-way ANOVA test followed by Dunn's multiple comparisons test. The peak of JUN expression was observed at B, RUH, LUH, and CL compared to other segments. JUN mRNA was significantly increased at FM but less than the previous segments. SLCO2A1 mRNA was highly expressed at FM, RUH, and CL. The same pattern was detected at UB and LUH but lower than the previous segments. Additionally, JUN and SLCO2A1 showed the lowest expression at both oviducts. ITGB4 mRNA was significantly increased at FM, UB, RUH &LUH, respectively. The slightest expression pattern of ITGB4 was observed at both oviducts and CL. VEGFA gene was highly expressed at FM. Moreover, VEGFA was significantly upregulated at UB and RUH with a lower pattern than FM. Nevertheless, VEGFA showed no significant differences among LUH, RFT, and CL. On the other hand, the least expression of VEGFA was noted at LFT. Concerning histological examination, showed normal granulosa lutein cells of CL and normal amnion, chorion, and decidua of FM. Progesterone concentration was 3.12 ± 0.93 ng/ml. It is proposed that hormonal regulation and the site of CL might implicate positively and negatively on dynamic gene expression patterns among the different uterine segments to promote proper endometrial milieu and a synchronized embryo-maternal interface during camel pregnancy.

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Validation of estrus synchronization method combining short-term CIDR treatment and PG in Japanese Black Cows

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Estrus synchronization using a controlled intravaginal drug releasing device (CIDR) is effective in improving the reproductive efficiency in cows. In Japan, a single CIDR treatment for 12-15 days is commonly used for estrus synchronization. However, its effectiveness depends on the estrous cycle stage of the treated cows. Therefore, it is desirable to develop a method that can induce estrus at any stages of the estrous cycle. In this study, we investigated the effects on luteal function and estrus by injection prostaglandin F2□ (PG) into Japanese Black Cows in various stages of the estrous cycle after a short-term CIDR treatment, with the aim of developing an estrus synchronization method that can induce estrus with less labor, regardless of the estrous cycle stage.

CIDR was implanted for 5 days in Japanese Black Cows with normal estrous cycles of Day 2 or 3 (Group 1: n=10), Day 10 or 11 (Group 2: n=8), and Day 16 or 17 (Group 3: n=8), with estrus as the reference (Day 0). PG was injected at the same time CIDR removal. The luteal diameter and luteal periprostatic blood flow area (LBFA), which reflect steroidogenesis, were examined during the CIDR treatment period and up to 3 days after the completion of the treatment. LBFA was also measured immediately after PG injection (15 and 30 minutes later) to confirm the presence of sensitivity to PG in the corpus luteum (CL). Furthermore, the number of days from the completion of the treatment to estrus was measured.

During the CIDR treatment period, the diameter of the CL and LBFA increased in Group 1, whereas they were maintained or decreased slowly in Group 2 and 3, suggesting that the structure and function of the CL change depending on the estrous cycle in all groups. In all groups, the diameter of the CL decreased after PG injection. In all groups, LBFA increased temporarily immediately after PG injection and then decreased, suggesting that CLs have sensitivity to PG in all groups and this treatment causes the luteolysis regardless of the estrous cycle stage. Although the time to estrus was significantly longer ($P<0.05$) in Group 1 (3.2±0.6 days) and 2 (3.4±1.0 days) than in Group 3 (2.5±0.6 days), estrus developed within 4 days after treatment in all cows. These results suggest that this protocol can be used to stably induce luteolysis and estrus by the same treatment in early, mid, and late luteal phase cows.

The effect of a scavenger receptor-B1 inhibitor on the function of bovine luteal steroidogenic cells

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Understanding the mechanism of luteolysis is helpful in controlling bovine estrus. The corpus luteum (CL) synthesizes and secretes progesterone (P4), which is essential for the establishment and maintenance of pregnancy in mammals. In the CL, P4 is synthesized using cholesterol in lipoproteins, such as high-density lipoprotein (HDL), as a precursor. We previously found that scavenger receptor-B1 (SR-BI), an HDL receptor, is expressed in bovine CL, and its expression is reduced during luteolysis and after injection of prostaglandin F2α in cows. These findings suggest that a decline in SR-BI expression plays an important role in functional luteolysis in cows. However, the roles of SR-BI in P4 production and luteolysis mechanisms in the bovine CL are unknown. In the present study, to determine if SR-BI is involved in luteolysis, we investigated the effects of an SR-BI inhibitor (BLT-1) on P4 production, cell viability and gene expressions involved in steroidogenesis in cultured bovine luteal steroidogenic cells (LSCs).

Isolated LSCs from bovine CLs at the mid-luteal stage were treated with 50 µg/mL human HDL (hHDL) in combination with 1 µM BLT-1 for 18 hours. After culture, P4 concentration in the supernatant and cell viability were measured by ELISA and alamarBlue, respectively. Furthermore, the effects of BLT-1 on gene expressions of *SR-BI*, *StAR*, *P450scc* and *3βHSD* were analyzed by RT-qPCR.

While a single treatment with BLT-1 did not affect P4 production, BLT-1 prevented the increase in P4 production induced by hHDL in the LSCs. Cell viability was increased by hHDL and was not affected by BLT-1. Neither hHDL nor BLT-1 affected *SR-BI* mRNA expression. Human HDL decreased *StAR* and *P450scc* mRNA expression with or without BLT-1 treatment. *3βHSD* mRNA expression decreased with hHDL but not in combination with BLT-1. In conclusion, these findings suggest that bovine LSCs synthesize and secrete P4 from HDL via SR-BI. SR-BI may be involved in the regulation of luteolysis by decreasing its own expression and attenuating HDL-mediated P4 production during luteolysis.

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A new prospective insight into therapeutic approaches of endometritis in she-camel using *in vitro* model

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Endometritis is the most common clinical sign in female camels suffering from infertility. Antibiotic residues increased antimicrobial resistance of pathogens and the abuse of antibiotics causes serious problems. Plant extracts and essential oils may become promising alternatives for controlling infectious endometritis. The purpose of current study was to evaluate the therapeutic efficacy of OLEMO-20/20® and/or OHAMO-20/02® in the treatment of endometritis, with an emphasis on changes in histopathological, gene expression and inflammatory mediators. The purchased natural plant extracts (OLEMO-20/20® & OHAMO-20/02®) were subjected to analysis by High-Performance Liquid Chromatography (HPLC) and Gas Chromatography–Mass Spectrometry (GC-MS). The antibacterial as well as antifungal activities were evaluated against *Escherichia coli* (*E. coli*) (ATCC 43888) and *Candida albicans* (*C. albicans*) (NRRL-Y 477) using well diffusion and broth microdilution methods, respectively. Uteri (n = 50) of cyclic, non-pregnant healthy mature she-camel were collected from local abattoirs, during the breeding season. Uterine explants were randomly divided into seven groups as follows: 1st was served as control, 2nd was challenged with *E. coli*, 3rd was challenged *C. albicans*, 4th was challenged with *E. coli* + OLEMO-20/20®, 5th was challenged with *E. coli* + OHAMO-20/02®, 6th was challenged with *C. albicans* + OLEMO-20/20®, and 7th was challenged with *C. albicans* + OHAMO-20/02®. All groups were cultured at 38.5°C under 5% CO_2, for 48 h. Supernatant and tissues were collected at 24 and 48 h for the detection of inflammatory mediators (IL-6 & IL-10) and gene expression, as well as for histopathological examination. Data were analyzed using two-way ANOVA. GC-MS revealed the presence of 25 constituents. Geranial and Neral were the major compounds, with percentages of 29.85% and 26.47%, respectively. Findings of HPLC showed 10 phenolics, 8 phenolic acids, and 2 flavonoids. The antibacterial susceptibility of OLEMO-20/20® and OHAMO-20/02® against *E. coli* was 12.5 mg/mL and 6.25 mg/mL, respectively. The antifungal susceptibility of OLEMO-20/20® and OHAMO-20/02® against *C. albicans* was 3.125 mg/mL and 0.78 mg/mL, respectively. Histological examination revealed that co-culture of plant extracts with tissues challenged either with *E. coli* or *C. albicans* exhibited extreme improved tissue reaction, where the inflammatory reaction was minimal and dense stroma was evident. At the molecular level, treatment of the challenged tissues with plant extracts significantly ($P < 0.001$) modulated the mRNA expression patterns of IL-8, IL-6, IL-1β, TNF_{α}, IL-10 and NFκB1. Furthermore, we found that the level of IL-6 in the culture medium decreased dramatically, while the concentration of IL-10 increased. Taken together, both natural plant extracts (OLEMO-20/20® and OHAMO-20/02®) are effective in the treatment of infectious endometritis in she-camel.

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Effect of nano-formulated prostaglandin $F_{2α}$ (PG-T50® or PG-S50®) treatments during Ovsynch protocol on luteal regression and pregnancy outcomes in dairy Egyptian buffaloes

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It is well documented that nanotechnology is an important tool that can offer new strategies for drug delivery. The current study aimed to investigate the effect of nano prostaglandin $F_{2α}$ ($PGF_{2α}$) on the mechanisms of corpus luteum (CL) sensitivity and the acquisition of luteolytic capacity in buffaloes compared to bulk $PGF_{2α}$. A total of two experiments were conducted. In the 1st one (*in vitro*), around 40 mature corpora lutea were collected from buffaloes at a local abattoir. CL explants were incubated with the following factors: (1) medium alone, (2) bulk $PGF_{2α}$, (3) nano-$PGF_{2α}$ (PG-T50®), and (4) nano-$PGF_{2α}$ (PG-S50®) for 48 h. Supernatant and explants were collected at 24 & 48 h for measurements of progesterone (P4) concentration and gene expression, and histological examination. The 2nd experiment (*in vivo*), Egyptian buffaloes (n = 120) were randomly divided into 6 groups (20/group); the 1st served as control, the 2nd was injected with bulk $PGF_{2α}$, the 3rd was injected with 3 ml of PG-T50®; the 4th was injected with 2 ml of PG-T50®, the 5th was injected with 3 ml of PG-S50®, and the 6th was injected with 2 ml of PG-S50®. CL parameters at time of PG administration of the GPG program were investigated using a B-mode and color doppler ultrasonography. Serum was collected for determination of P4 level. P4 concentrations in supernatant and gene expression were analyzed using two-way ANOVA. Doppler measurements and P4 levels in serum were analyzed using three-way ANOVA. Also, Receiver Operating Characteristic (ROC) curves were used. Zeta potential of bulk $PGF_{2α}$, PG-T50® and PG-S50® were 37.20, -53.6, and -44.3 mV, respectively. Histological examination showed proliferating fibroblasts with vacuolation in luteal cells, degenerating luteal cells in bulk $PGF_{2α}$ group as well as in nano-$PGF_{2α}$ groups at 24 & 48 h compared with control one. Compared to the control group at 24 or 48 h, PGR as well as steroidogenic genes (LHCGR & STAR) in the treatment groups were significantly down-regulated, while apoptosis-related genes ($TNF_{2α}$, FASLG, BAX, and CASP3) were significantly up-regulated. ROC analyses indicated that P4 concentration and CL parameters would be used to predict the pregnancy rates at the time of PG. P4 concentrations were significantly reduced in a time-dependent manner either with bulk $PGF_{2α}$ or nano-$PGF_{2α}$ was used compared to the control group. Nano-formulated PG might be an effective drug delivery system. The lower concentrations of nano PG were used during Ovsynch protocol. This strategy is characterized by increased drug absorption, solubility, bioavailability, without adverse effects on pregnancy outcomes.

Nano-formulated prostaglandin $F_{2α}$ was submitted into Egyptian patent office (EGPO/20240319000048).

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Regulation of spexin expression, and its direct role in the luteal steroidogenesis of porcine corpus luteum. *In vitro* study

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Spexin (SPX) is a neuropeptide and adipokine linked with regulation of energy homeostasis, appetite control, metabolism and food intake. Previous studies showed negative impact of SPX on granulosa cells steroidogenesis and proliferation suggesting its role in female reproduction. However, SPX expression and function in corpus luteum (CL) are still unknown. The aim of study was firstly to identify and then investigate the regulation of the expression of SPX and its receptors galanin receptor 2 and 3 (GALR2/3) in the porcine CL. Secondly, we analysed the effect of SPX on luteal steroidogenesis and signaling pathways.

Porcine CLs were collected at the early (day 2-3), middle (day 10-12) and late (day 14-16 of estrous cycle) luteal phase to investigate mRNA and protein expression of the SPX/GALR2/3, as well as its immunolocalization. For *in vitro* experiments luteal cells at the middle luteal phase were cultured with: *i)* insulin (INS, 10 ng/mL), luteinizing hormone (LH, 50–150 ng/mL), progesterone (P_4, 10-1000 nM), prostaglandin PGE2 and PGF2 (10–250 ng/mL) for 24 h to determine the expression regulation; *ii)* SPX (0.1–100 nM) for 24 h to investigate its effect on steroidogenesis; *iii)* SPX (1 nM) for 1- 60 min to measure kinase phosphorylation; *iv)* pharmacological inhibitors of GALR2, mitogen activated (MAP3/1) and protein kinase A (PKA) and subsequently SPX (1 nM) for 24 h to investigate molecular mechanism of SPX action on steroidogenesis.

We demonstrated that *SPX* mRNA was higher in the middle and late compared with the early luteal phase, opposite to *GALR2*, which was confirmed at the protein level, while GALR3 protein was decreased with luteal phase progression. Additionally, we observed cytoplasmic localization of SPX and both receptors in small and large luteal cells. We found that INS, LH and P_4 stimulated *SPX* and *GALR2* mRNA levels, while prostaglandin F2 decreased *GALR2* transcript. Moreover, SPX directly decreased P_4 secretion by inhibition of HSD3B expression and *via* GALR2 and PKA. While it increased STAR and CYP11A1, as well as aromatase protein expression with no effect on estradiol secretion. Additionally, SPX increased GALR2 protein level, MAP3/1 phosphorylation and inhibited both PKA and protein kinase B phosphorylation.

To sum up, SPX is a new important factor in the luteal cells function by direct effects on steroid synthesis and may be an important player related to prolongation of the function of the CL in metabolic related fertility regulation in farm animals.

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The role of microRNAs in regulating on progesterone production in cultured porcine granulosa cells

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Progesterone is a major gonadal hormone synthetized in the corpus luteum involved in the female reproductive cycle, prepares the endometrium for the potential of pregnancy after ovulation in the pig and other species. The process of steroidogenesis, where the synthesis of progesterone takes place, is very complicated. Several studies and our earlier results suggested that one of the regulating mechanisms of steroidogenesis may take place with the participation of short, non-coding miRNAs, involved in the negative regulation of gene expression. The primary aim of this research project is to gain a deeper understanding of progesterone synthesis. To achieve this, in the study focused on determining the influence of miRNAs on the expression profiles of genes and proteins related to progesterone synthesis in granulosa cells *in vitro* model.
After transfection of granulosa cells with miRNA, cells were treated with luteinizing hormone (24, 48, 72 hours) to stimulate expression of enzymes and factors regulating steroidogenesis. The experiment was conducted using granulosa cells obtained from preovulatory follicles (<6 mm; P4/E2 <10) collected from mature gilts (n=6).

The *in vitro* experiment on granulosa cells revealed that progesterone concentration was higher in the medium with cells transfected with miR-21, miR-34, miR-132, and miR-503 ($p<0.05$) after 48 and 72 hours of incubation with LH, compared to the medium with non-transfected cells. We observed an increasing expression of *CYP11A1* and *STAR* mRNA in granulosa cells transfected with miR-21, miR-34, miR-132, and miR-503, as well as increasing protein expression of HSD3B1, STAR, and CYP11A1 in granulosa cell transfected with miR-21, miR-34, and miR-132 after 48 hours. The results also showed that the mRNA and protein expression levels of CYP11A1, HSD3B1, and STAR were increased in granulosa cells transfected with miR-21, miR-34, and miR-132 after 72 hours. Additionally, we noted that after 72 hours, the mRNA and protein levels of ovarian steroidogenesis transcription factors CREB1 and ATF4 were elevated in granulosa cells transfected with miR-21, miR-34, miR-132, and miR-503. There were no changes in the expression of analyzed transcript or protein in 24 hours incubation.

To sum up, the data presented suggests that miR-21, miR-34, miR-132, and miR-503 may serves an essential role in the progesterone synthesis during steroidogenesis.

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Efficacy of induction of parturition by dexamethasone alone or in combination with aglepristone and misoprostol in rabbits

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Induction of parturition (IP) is essential to manipulate the pregnancy for both physiological and pathological reasons. The aim of this study is to investigate the effectiveness of the drug combinations (aglepristone, misoprostol, and dexamethasone) to induce parturition in rabbits.
Totally 30 New Zealand, female rabbits were studied. The rabbits divided into six groups equally, and IP's were performed at 27th gestation day of parturition as follows; group I (control), group II (sham; 0.1 ml 0.9% NaCl im.), group III (dexamethasone, 1 mg im.), group IV (dexamethasone 1 mg im. + aglepristone 10 mg/kg sc.), group V (dexamethasone 1 mg im. + misoprostol 200 mcg intravaginally until parturition begins), group VI (dexamethasone 1 mg im. + aglepristone 10 mg/kg sc. + misoprostol 200 mcg intravaginally until parturition begins). In the groups, the daily vital parameters of the rabbits from the 27th pregnancy day to birth, total pregnancy length (TPL) and the time between IP and parturition (TIPP) were recorded. The mean and standard deviation values of TPL and TIPP of the rabbits in each group were calculated and then these values of the groups were analyzed comparatively with Kruskal-Wallis test. In case of significance, The Mann-Whitney U test was used to compare the values of groups in pairs. In control and sham groups, TPL and TIPP were longer than induction groups. Among the induction groups, TPL and TIPP was determined to be longer at group V. There was a significant difference between all groups in terms of TPL ($p=0.006$) and TIPP ($p=0.006$). In the pair comparisons of groups for TPL, there was a significant difference between groups I and III ($p=0.016$); groups I and IV ($p=0.009$); groups I and VI ($p=0.016$); groups II and III ($p=0.047$); groups II and IV ($p=0.047$); groups III and V ($p=0.047$); groups IV and V ($p=0.009$) and groups V and VI ($p=0.047$). In the pair comparisons of the groups for the TIPP, there was a significant difference between groups I and III ($p=0.016$); groups I and IV ($p=0.009$); groups I and V ($p=0.016$); groups I and VI ($p=0.009$); groups II and IV ($p=0.028$) and groups II and VI ($p=0.028$). As conclusion, a single dose of dexamethasone alone or in combination with aglepristone or misoprostol induce to the parturition. However, on the basis of TPL and TIPP values and statistical analyzes, it is seen that IP with combination of dexamethasone and misoprostol takes longer.

Progesterone has the ability to control the process of DNA methylation in the corpus luteum of cows through the estrous cycle

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DNA methylation is a biological process that involves the addition of methyl groups to the DNA molecule. This process can alter the activity of a gene without modifying its sequence. When it occurs in a gene promoter, it suppresses gene transcription. DNA methylation is catalyzed by DNMT methyltransferases, and the main ones are DNMT1, DNMT3a, and DNMT3b. The reverse process is DNA demethylation and involves the return of gene expression that has been suppressed. This is achieved through the activity of dioxygenases, specifically TET1, TET2, and TET3 which oxidize methylated cytosine to hydroxymethylated cytosine. Modifications in the expression and function of DNMTs and TETs may impact the corpus luteum (CL) functionality. This study was to investigate whether changes in DNMT and TET activities, including DNMT1, DNMT3a, DNMT3b, and TET1, TET2, and TET3 mRNA expression, could be detected in the cows' cyclic CL. If there is a correlation between the changes mentioned and progesterone (P4) levels throughout the cycle. The material included CLs from days 2-4, 6-10, 11-16, and 17-20. Gene expression was quantified using real-time PCR, while DNMT and TET activities were assessed using luminescent and fluorescent ELISA kits, respectively. The expression levels of all DNMTs increased between days 2–10 and subsequently declined. In contrast, the highest expression levels of TET1 and TET2 were observed between days 6–16, while TET3 reached its peak expression at the end of the cycle. The activity of DNMT was minimal from days 2–16 and significantly enhanced towards the final stage of the cycle. On the contrary, TET exhibited the highest activity on days 2–16 and subsequently declined to the end of the cycle. All enzymes, except DNMT1 and TET3, revealed a positive correlation with P4 levels. Moreover, we found a correlation only between the activity of TET and the P4 level released by the CL during the estrous cycle. Thus, the variability in DNMT and TET activities, gene expression levels for individual enzymes involved in both DNA methylation and demethylation and their correlation with P4 may suggest that this hormone may have a contribution to controlling DNA methylation.

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Changes in equine endometrial fibroblasts induced by long-term exposure to the secretome of macrophages

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To date, endometrosis remains one of the main causes of infertility in mares. As its etiology remains unknown, it is essential to search for potential pathways that drive its pathogenesis. Active or inactive stromal endometrial fibrosis is one of the main feature of this condition. It has been demonstrated in different species and tissues that macrophages (MΦ) play a major role in the progression of fibrosis. A polarization of MΦ that occurs in the presence of IFNγ and LPS appears to be the classical activation of M1 macrophages (MΦ1) and the presence of IL-4 and IL-13 leads to an alternative activation of MΦ2. The two populations are also distinguished by their distinct functions – inflammatory and reparative, respectively. Taking this into account, the aim of the study was to determine the impact of prolonged exposure to the secretome of $M\Phi^{IFN\gamma/LPS}$ and $M\Phi^{IL\text{-}4/IL\text{-}13}$ on mare endometrial fibroblasts in the context of the development of fibrosis in the course of endometrosis.

Fibroblasts were isolated post-mortem from category I endometria of mares during the mid-luteal phase of the estrous cycle (n=6) and treated with secretome of $M\Phi^{IFN\gamma/LPS}$ and $M\Phi^{IL\text{-}4/IL\text{-}13}$ for 96 hours. mRNA transcription of ECM-associated compounds (*Col1a1,-3a1, Fn1, Mmp2,-9, Timp1,-2, Loxl2*) and proinflammatory cytokines (*Tnfα, IL-6, IL-1β, Tgf-β1, Ctgf, Areg*) was analyzed by qPCR. The expression of selected factors was analyzed using immunofluorescence. Changes in fibroblasts' properties were determined by scratch assay, proliferation and viability assays.

Secretome of $M\Phi^{IFN\gamma/LPS}$ increased the expression of *Col3a1* ($P < 0{,}05$), *Loxl2* ($P < 0{,}05$), *Timp2* ($P < 0{,}01$), *IL-6* ($P < 0{,}05$) and decreased the expression of *Fn1* ($P < 0{,}01$), *Mmp9* ($P < 0{,}05$), *Tgf-β1* ($P < 0{,}001$), *Ctgf* ($P < 0{,}01$) in fibroblasts after 96h exposure. Simultaneously, the secretome of $M\Phi^{IL\text{-}4/IL\text{-}13}$ increased the expression of *Col3a1* ($P < 0{,}05$), *Loxl2* ($P < 0{,}01$), *Timp1* ($P < 0{,}05$), *IL-6* ($P < 0{,}05$), *Tnfα* ($P < 0{,}05$) and decreased the expression of *Col1a1* ($P < 0{,}01$), *Tgf-β1* ($P < 0{,}01$), *IL-1β* ($P < 0{,}05$), *Areg* ($P < 0{,}05$) in fibroblasts after 96h exposure. The secretome of both MΦ populations led to gradual increase in cell migration over time and significantly increased migration at 36h and 48h ($P < 0{,}05$). After 96h, an increase in both proliferation and viability of fibroblasts was observed ($P < 0{,}05$ for $M\Phi^{IFN\gamma/LPS}$ and $P < 0{,}01$ for $M\Phi^{IL\text{-}4/IL\text{-}13}$).

The results indicate that prolonged exposure to the secretome of MΦ upregulates the expression of genes associated with fibrosis in endometrial fibroblasts, and the effect varies depending on their population. Meanwhile, both MΦ populations contribute to increased potency of fibroblasts properties.

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Insights into uterine and fetal fluid extracellular vesicles: Implantation window in Arabian camel (*Camelus Dromedarius*)

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Camels, known for their remarkable adaptability to harsh environments, are increasingly vital under climate change conditions. However, there is a significant shortage of studies focusing on their reproductive biology, which is essential for optimizing their management and conservation. Investigating the role of extracellular vesicles (EVs) in camel reproduction offers a promising avenue to understand the molecular crosstalk between maternal and embryonic tissue. This study aimed to investigate the role of EVs in maternal-embryonic communication in camels, exploring how these vesicles mediate crucial signals that influence embryonic implantation and pregnancy maintenance. To achieve that, 100 she-camel genital tracts (50 pregnant and 50 non-pregnant) and blood samples were collected from the slaughterhouse. To estimate the stage of pregnancy in gravid uteri, the crown-vertebral rump length (CVRL) of the fetuses was measured by caliber to select the early stage of pregnancy. Samples from the corpus luteum, uterine tissue, and fetal membrane were collected and fixed in 10% formalin for histological examination. Furthermore, the EVs were isolated and purified from non-pregnant (serum & uterine fluid) and pregnant (serum, uterine fluid, and fetal fluid), using differential ultracentrifugation followed by characterization by flow cytometry, transmission electron microscopy (TEM), and particle sizing system. Upon EVs confirmation, the mRNA was isolated and converted to cDNA for transcription analysis of candidate genes associated with implantation and pregnancy maintenance (IGF-1, VEGFA, FOS, PGF, and BMP6). The EVs showed significant differences in concentration and morphology, with higher concentrations and larger sizes found in the serum of pregnant she-camels and fetal fluids compared to non-pregnant, indicating dynamic physiological changes and potential roles in pregnancy. The expression of candidate genes was significantly higher in the EVs derived from the serum of pregnant she-camel. Interestingly, the expression patterns of candidate genes in EVs derived from fetal fluids closely mirror those in the serum of pregnant and non-pregnant she-camels, with significant upregulation in pregnant she-camels, including up to 22-fold increases in FOS and PGF. Moreover, the changes in mRNA transcript levels during pregnancy resulted in histological modifications in tissues critical for a successful pregnancy, such as the corpus luteum and uterine tissue. In conclusion, this study sheds light on the importance of EVs cargo in facilitating maternal-embryonic communication to enhance embryo implantation during the early stage of pregnancy (35 days). This research opens doors for further exploration of their potential in optimizing embryo implantation strategies in *Camelus Dromedarius*.

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Effect of interleukin (IL)-4 and IL-13 on the production of reactive oxygen species

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Oxidative stress plays a pivotal role in the development and progression of tissue fibrosis. This occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the cellular ability to neutralize them with antioxidants. Oxidative stress acts as an important mediator in the fibrotic process, being involved in fibroblast activation, promotion of pro-fibrotic signaling, sustained inflammation, alteration of extracellular matrix (ECM) dynamics, and contribution to cell death and senescence. IL-4 and IL-13 are known as proinflammatory and profibrotic factors. Nevertheless, their precise role in the development of mare endometrial fibrosis in the course of endometrosis remains unknown. Our recent transcriptomic study revealed that both IL-4 and IL-13 induce changes in the expression of genes involved in ROS metabolism in equine endometrial fibroblasts. However, the direct effect of these Th2-secreted interleukins on ROS production in mare endometrial fibroblasts remains unknown. Thus, we aimed to determination of the effect of IL-4 and IL-13 (10 ng/ml, 48 hours) on cytoplasmic as well as mitochondrial and nuclear ROS production in fibroblasts derived from non-fibrotic (category I) and fibrotic (category IIB) endometrium using flow cytometry.
Results of our research indicate that IL-4 increased mitochondrial, nuclear, and cytoplasmic ROS levels in fibroblasts derived from category I endometria ($p < 0.05$). In turn, IL-13 increased cytoplasmic ROS levels in fibroblasts derived from category IIB endometria ($p < 0.01$).
The findings of this study indicate that cytokine secreted by Th2 cells, namely IL-4 and IL-13, may play a pivotal role in metabolic shifts in cells derived from non-fibrotic and fibrotic endometria. We demonstrated that IL-4 and IL-13 differentially affect cytoplasmic as well as nuclear and mitochondrial ROS production. Moreover, these results indicate that the cellular responses to IL-4 and IL-13 treatment may vary depending on the degree of fibrosis present in the tissue of origin.

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Silencing of progestin and adipoQ receptor (PAQR) 7 and PAQR8 affects the expression of selected genes related to prostaglandin synthesis, proliferation, and apoptosis in bovine endometrial endothelial cells

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In our previous research, we found fluctuations in mRNA and protein levels of membrane P4 receptors: PAQR7 and 8 in the endometrium during the estrous cycle and the first trimester of pregnancy in cows. These data also showed higher protein expression of the receptors in the luminal and glandular epithelial cells and the endothelial cells of blood vessels. These may indicate that PAQR7 and 8 participate in the regulation of the proliferatory and secretory function of the uterus. Therefore, the aim of this study was to investigate the involvement of PAQR7 and PAQR8 in proliferation and secretion processes in bovine endometrial endothelial cells (bEECs). Bovine EECs (10-12 days of the estrous cycle) were transfected with small interfering RNA (siRNA) for PAQR7 or PAQR8 and next cultured for gene expression (real-time PCR), proliferation (CellTiter96 Cell Proliferation Assay) and secretion (EIA) analysis. The research concerned mRNA expression of selected genes including prostaglandins synthesis-related genes (COX1, COX2, PGES, PGFS), proliferation (TEK), and apoptosis-related genes (BCL2, BAX, CASP3, CAS8) and growth factors genes (FGF2, IGF1, TNFα, VEGF) in cultured endometrial endothelial cells before and after PAQR7 and PAQR8 silencing. The results showed that the silencing of PAQR7 and PAQR8 led to the down-regulation of COX1, IGF1, BAX, CASP8, and TEK mRNA expressions and the up-regulation of FGF2 mRNA expression. No significant changes were detected in the expression of the PGES, PGFS, BCL2, CASP3, and VEGF genes in cells treated by siRNA specific to PAQR7 and PAQR8. Additionally, the silencing of PAQR7 and PAQR8 mRNA increased PGE2 and PGI2 secretion. Therefore, it can be assumed that PAQR7 and 8 can regulate the expression of genes involved in the synthesis of prostaglandins, cell proliferation, and apoptosis in the bovine endothelial cells, and this way they may control endometrium function.

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Changes in *Thy-1* mRNA expression in histological features of equine endometrosis

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Endometrosis is a crucial factor affecting the fertility of mares associated with age. main changes involve fibrosis of the extracellular matrix (ECM) and glandular degeneration, resulting in early embryonic death caused by undernutrition. While the disease is of interest among researchers, most features involved in its pathogenesis remain unknown. As transforming growth factor β (TGF-β) is recognized as an important factor in the development of endometrosis, Thy-1 (cell surface antigen) may be considered as a possible component of pathogenesis, especially regarding association with fibroblast transformation into myofibroblasts and deposition of smooth muscle alpha-2 actin (SMα2). Thus, this study aims to compare the *Thy-1* mRNA expression with histological features found in equine endometrium with endometrosis.
47 full-thickness uterine sections were collected from the slaughterhouse for fixation in formaldehyde, along with endometrial specimens stored at -80°C. Histological samples were cut into 4µm sections and stained with hematoxylin and eosin. Slides were graded according to Kenney&Doig categories (I, IIA, IIB, III), and individual alterations were marked: continuity of luminal epithelium (CLE), degeneration of luminal epithelium (DLE), fibroblast activity (FA), foci of periglandular fibrosis (FPF), basal lamina destruction (BLD), glandular degeneration (GD), perivascular fibrosis (PF), vascular ectasia (VE), and inflammation (absent, mild, moderate, severe). Frozen specimens were homogenized and used for qPCR analysis of *Thy-1* and *ACTA2* with TaqMan probes, with *SDHA* and *GADPH* as reference genes. Category I served as control for ΔΔCt method. Results were compared with the Mann-Whitney test, the Kruskall-Wallis test, and the Spearman correlation.
Among Kenney&Doig categories, *Thy1* expression was significantly different only in IIA and IIB (Q1, Q2, Q3: 5.97, 170.38, 601.16 and 0.05, 0.27, 5.33, respectively, $p<0.05$), reaching its highest values in IIA. While evaluating proposed histological alterations, significant differences were found only in DLE, with higher expression when epithelium was degenerated (Q1, Q2, Q3: 0.07, 1.62, 16.63 for normal and 1.18, 23.71, 204.28 for degenerated epithelium, $p<0.05$). Interestingly, we have found a strong, positive correlation (rho 0.87, p<0,01) between *Thy-1* and *ACTA2* expression.
While role of Thy-1 in equine endometrosis is not recognized, it may be associated with initial changes and not take part in the exacerbation of alterations. Variability of expression in DLE may indicate poorer cross-talk between the uterine lumen and endometrium, although it remains rather vague. Strong positive correlation between *Thy-1* and *ACTA2* expression may show an influence of Thy-1 in TGF-β activity on fibroblasts, however, studies on gene methylation shown contradictory results.

Understanding the impact of hypoxia on the effect of distinct macrophage population on endometrial fibroblasts in equine endometrosis – an *in vitro* study

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Endometrosis is a degenerative, chronic condition characterized by excessive extracellular matrix (ECM) deposition leading to fibrosis. Macrophages (Mϕs) and fibroblasts are key cellular players in the development of fibrosis, and hypoxia is a major contributor to the development of fibrotic diseases. However, there is a lack of knowledge regarding the potential impact of hypoxia on the response of equine endometrial fibroblasts to distinct populations of Mϕs.

We aimed to determine the influence of hypoxia on the fibroblast response to the secrotome of $M\phi^{(IFN\text{-}\gamma+LPS)}$ (Mϕ1) and $M\phi^{(IL\text{-}4+IL\text{-}13)}$ (Mϕ2a) in processes associated with the development of endometrosis. Firstly, we investigated the *HIF-1α* mRNA expression and its immunolocalization in the mare *endometrium* at each stage of endometrosis (n=3), using qPCR and IHC, respectively. Subsequently, mare endometrial fibroblasts (n=5, category IIA) were cultured with $M\phi^{(IFN\text{-}\gamma+LPS)}$-, $M\phi^{(IL\text{-}4+IL\text{-}13)}$-conditioned medium ($M\phi^{(IFN\text{-}\gamma+LPS)}$-, $M\phi^{(IL\text{-}4+IL\text{-}13)}$-CM; Mϕ generated from equine peripheral blood monocytes) for 48h under hypoxic (H; 1% O_2) or normoxic (N; 21% O_2) conditions. The transcriptional profile and ECM-associated genes were determined by RNA-seq and qPCR, respectively.

HIF-1α immunolocalization and gene expression depend on the stage of endometrosis. In endometrial fibroblasts, 1981 differentially expressed genes (DEGs) were identified in $M\phi^{(IFN\text{-}\gamma+LPS)}$-N *vs* Ctr-N; 1448 DEGs in $M\phi^{(IL\text{-}4+IL\text{-}13)}$-N *vs* Ctr-N; 112 DEGs in $M\phi^{(IFN\text{-}\gamma+LPS)}$-N *vs* $M\phi^{(IFN\text{-}\gamma+LPS)}$-H and 332 DEGs in $M\phi^{(IL\text{-}4+IL\text{-}13)}$-N *vs* $M\phi^{(IL\text{-}4+IL\text{-}13)}$-H. Depending on Mϕs population and conditions, the functional analysis of DEGs was annotated to processes, such as ECM organization, chemotaxis, cell-substrate adhesion, protein oxidation and response to hypoxia, which are significant in the development of fibrosis. The expression of ECM-related genes changed depending on oxygen conditions and Mϕs population.

The results indicate that Mϕs play a role in regulating gene expression which is crucial in the development of fibrosis. The effects varies according to the Mϕs population. Also, their impact on fibroblasts is modulated by hypoxia, which suggest that this process is important in in the development fibrosis related to progression of endometrosis.

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Endocrinological and embryo-associated parameters in PPID mares treated with pergolide and metformin.

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Twenty-two Quarter horse mares aged 16 to 23 years old (18.4 ± 4.73 years) were enrolled in the study. The study was carried out during two foaling seasons (2022-2023 and 2023-2024) involving client-owned animals presented for breeding. Mares received a free choice mix of alfalfa grass hay, 1 kg/per animal/day of concentrate, and water ad libitum. All the mares were evaluated by the same operator and were bred by the same stallion in both seasons. All the stallions had proven fertility with multiple viable foals. The study compared the endocrinological panel and embryo-associated parameters from PPID/metabolic mares (ACTH >30 pg/mL, Nov- July) before and after treatment. All the mares underwent one breeding season as a control and, in the subsequent breeding season, the mares were treated with pergolide (1 mg/day, PO) and metformin (3 g/day, PO). Blood was collected once for control and treated mares (Cortisol, ACTH, and, T4). To evaluate the glycaemic curve (glucose and, insulin) mares had blood collected at hour 0 (12 hrs fasting period) then 60 and 90 min (post-prandial period) after administration of 120 mL of corn syrup (Karo®). The mares were ultrasound daily until the detection of a 35 mm follicle for induction. The data was analyzed by Mixed Model and expressed in mean ± SD and, significance was set as $P < 0.05$. The mean ± SD for ACTH, cortisol, T4, Insulin, and Glucose were: 36 ± 11.6, 16.5 ± 3.3 pg/ml; 50.4 ± 4.1, 38.4± 6.8 ng/mL; 22.2 ± 2.5, 25.2 ± 4.02 ng/mL; 20.1 ± 15.3, 25.1 ± 18.1 UI/dL; 120.5 ± 28.9 and, 111.5 ± 13.3 mg/dL for control and treated mares, respectively. There was an effect of the group for ACTH, T4, and cortisol ($P < 0.05$), and an effect of the group and time for Glucose ($p < 0.05$). No interaction group: time was appreciated. Control mares had an ACTH two-fold higher than treated mares. The overall recovery embryo/flush was 0.15 ± 0.4 and, 0.73 ± 0.5 for the control and treated group ($P < 0.05$). The overall embryo recovery rate per oocyte retrieved, fertilization rate, and, embryo development rate for ICSI mares were: 0.17 ± 0.1, 0.27 ± 0.1 embryos; 0.28± 0.2, 0.61 ± 0.15 fertilized oocytes; 0.70 ± 0.34 and, 0.55 ± 0.2 embryos for control vs treated mares. Treated mares presented a superior embryo recovery rate per oocyte retrieved than control mares ($P < 0.05$). The overall embryo for ICSI and ET was significant for the control mares but not for treated mares ($P > 0.05$). There was no difference in the follicle's diameter at 24h after induction for control vs. treated mares for ET and ICSI ($P < 0.05$). In conclusion, the embryo recovery for ET was 3-fold higher in treated mares while ICSI mares presented a superior embryo rate per oocyte retrieval and fertilization rate than control mares.

Progesterone reduced TGF-β1-induced collagen deposition in mid-luteal phase equine endometrium: an in vitro study

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Transforming growth factor (TGF)-β1 is recognized as a key pro-fibrotic factor that plays a crucial role in the excessive accumulation of extracellular matrix components within the equine endometrium. Equine endometrosis, a leading cause of infertility in mares, is marked by degenerative, functional, and fibrotic alterations in the endometrium, accompanied by an increase in collagen (COL) deposition. The ovarian steroid progesterone (P4), which regulate the estrous cycle, have been identified as a potential modulator of fibrosis, acting as either anti-fibrotic or pro-fibrotic agent. The objective was to evaluate the effect of P4 on TGF- β1-induced COL1 expression in equine endometrial explants from mid-luteal phase (MLP). Equine endometrial explants from MLP were treated with TGF-β1 (10 ng/mL), P4 (10^{-7}M), or TGF-β1+P4, for 24h and 48h. The mRNA transcription of *COL1A2* (qPCR) and COL1 protein relative abundance (western blot) were determined. The TGF-β1 treatment increased *COL1A2* mRNA transcription at 48h ($p<0.05$), and TGF-β1 + P4 treatment at both 24 and 48h ($p < 0.01$), regarding the control group. The TGF-β1 + P4 treatment increased *COL1A2* mRNA transcription comparing to TGF-β1 treatment at 24h, and to P4 treatment at both 24h and 48h ($p<0.05$). The TGF-β1 treatment increased COL1 protein relative abundance, at 48h, regarding the control group, TGF-β1 + P4 and P4 treatment groups ($p<0.05$). The cytokine TGF-β1 seems to act as a pro-fibrotic agent in MLP equine endometrium in a prolonged stimulus (48h). However, the estrous cycle hormonal environment must be taken into consideration when studying equine endometrial fibrosis, because P4 seems to modulate the fibrotic response to TGF-β1. Progesterone appears to attenuate the pro-fibrotic action of TGF-β1 in the MLP of the estrous cycle.

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