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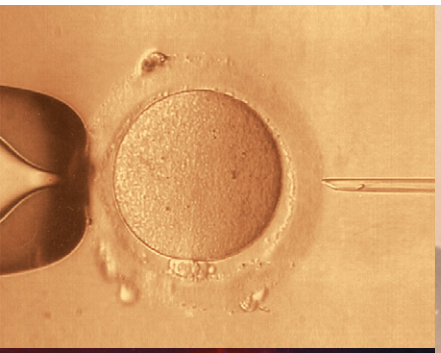

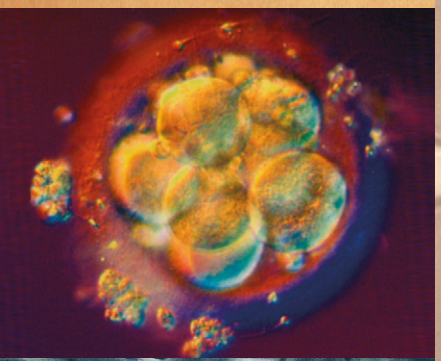

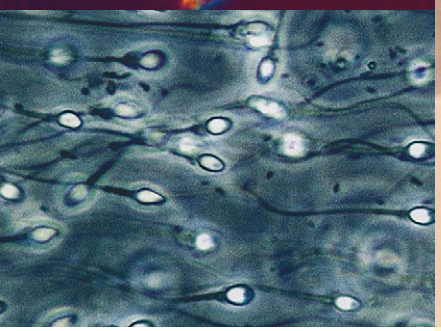

57th Annual Conference of Physiology and Pathology of Reproduction and 49th Joint Conference of Veterinary and Human Reproductive Medicine February 28th–March 1st, 2024, Berlin

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57th Annual Conference of Physiology and Pathology of Reproduction
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Welcome Note

Dear colleagues,
It is with great pleasure that we welcome you to the "Februartagung/February Conference" 2024 at the Veterinary campus of the Freie Universität Berlin. The FU Berlin Unit for Reproduction Medicine and Udder Health is this year's main organizer of the conference. The unit was just established in 2023 and its new team is very excited to welcome the reproductive medicine community on site. We were able to take on this task, because we did not have to carry out the project on our own, but received uncomplicated and cooperative support from our "reproduction colleagues" in Berlin (especially FU Institute of Veterinary Biochemistry, IFN Schönow, IZW Berlin) and Giessen.
In line with the tradition of our annual conference, and thanks to the keynote speakers and abstract contributors we could once again put together a wide-ranging program covering all disciplines of human and veterinary reproductive medicine as well as basic and clinical research. With current topics like artificial intelligence, stem cell-based reproductive biotechnologies, and the promotion of young researchers in reproductive biology and medicine we hope to stimulate lively discussions.
We thank the DVG (Deutsche Veterinärmedizinische Gesellschaft, German Veterinary Medicine Society), the DGRM (Deutsche Gesellschaft für Reproduktionsmedizin, German Society for Reproductive Medicine) and the FBF (Förderverein Bioökonomieforschung e.V.) for their invaluable support in organizing and executing the conference.
The vibrant city of Berlin with its many historical and cultural highlights awaits you and is (as you know) always worth a visit. We look forward to seeing you soon!

Marc Drillich, Christine Wrenzycki, Christoph Gabler, and Jennifer Schön (on behalf of the organizing and scientific committee)

Abstracts*

Expression of Growth Differentiation Factor 9 (GDF9) is related to follicular maturation, steroidogenesis and cell proliferation in horses

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The mechanisms regulating the growth of equine ovarian follicles and oocyte maturation remain largely unknown. GDF9 and bone morphogenetic protein 15 (BMP15) are oocyte-secreted factors that interact with granulosa cells (GC) to regulate follicle development. The aim of this study was to analyze GDF9 expression and its relationship with processes occurring in GC throughout follicle maturation in the equine ovary. Tissue samples for histology, follicular fluid (FF) and GC were collected from ovaries retrieved at a slaughterhouse. Localization of GDF9 in the equine ovary was determined by immunofluorescence using a species-specific custom-made antibody. Concentrations of both oocyte-derived factors in FF, as well as GDF9 expression in GC were determined in small (< 1 0mm), medium (10–30 mm) and large (> 30 mm) follicles by ELISA and RT-qPCR, respectively. The cytoplasm of the oocyte stained positive for GDF9 from the primary follicle stage onwards. Concentration of BMP15 was lower in FF from large follicles compared to small and medium follicles ($P < 0.05$ for both); GDF9 was decreased in FF from medium and large follicles compared to small follicles ($P < 0.001$ for both). In GC, transcript levels of *GDF9* did not differ among the analysed follicle classes but correlated negatively to *STAR* and *PCNA* expression ($P < 0.007$ for both). Taken together, these results demonstrate a dynamic expression of GDF9 in the equine ovary and its involvement in oocyte maturation by relating to essential processes such as steroidogenesis and cell proliferation.

Grants: Funded by Forschungskredit, University of Zurich, grant no. FK-21-059.

Exploring the significance of Caspase-8 and cell contacts in human ovarian cancer; in vitro and in vivo Study

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Due to the frequent chemotherapy relapses, novel therapies are eagerly needed addressing ovarian cancer (OC) biology with a focus on Caspase-8 as a key protein in the apoptotic cascade. We aimed to analyze the impact of Caspase-8 depletion in an orthotopic mouse model as well as in cell-based assays using different chemotherapy regimes (Carboplatin (CPT), Paclitaxel (PTX)). Wildtype (WT) and Caspase-8 knockout (KO) OVCAR8-Luciferase cells were injected into the bursa ovarica of n = 4 NMRI nu/nu mice (n = 2 WT, n = 2 KO), monitoring tumour growth via IVIS. *In vitro*, WT and KO cells were treated with CPT and PTX at different concentrations and time points and cell contact protein expression checked by western blot and immunofluorescence. CellTiter-Blue was performed for proliferation assay. A significant rise in IVIS signal was observed in the KO group compared to WT, suggesting a substantial increase in tumour growth after Caspase-8 depletion. In addition, proliferation assays showed that Caspase 8 KO cells are more resistant to CPT, leading to a three times higher survival compared to WT. Furthermore, while the treatment of OVCAR-8 WT with CPT demonstrated a substantial and dose-dependent rise in both early and late apoptosis, Caspase-8 KO cells showed less sensitivity, indicating a strong resistance against CPT. Moreover, preliminary results demonstrated the upregulated expression of cell contact protein Connexin-43 in the KO group. Transcriptomics showed a dysregulated expression of several genes involved in

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With permission of Wiley, the abstracts of this conference will be jointly published in the Journal of Reproduction of Domestic Animals (RDA) and the Journal of Reproductive Medicine and Endocrinology (JRE). Peer-reviewed and compiled by the scientific committee. Index of authors (only primary authors) see page 34.

biological processes exceeding the apoptotic functions of Caspase-8.

Isolation of Fusobacterium gastrosuis from the vagina of pregnant female dromedary camels: first report

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Fusobacterium (F.) gastrosuis is a novel pathogen with the ability to cause cell death and gastric ulceration. Previous research has revealed the presence of *F. gastrosuis* in the oral and nasal microbiota of pigs and dogs, the stomach of wild boars, and the stool of humans, indicating that F. gastrosuis can live in a wide range of mammalian hosts. Despite being the first line of defense against pathogens that enter the genital system, vagina contains a variety of bacteria that can cause genital infection. Furthermore, the uterus is not sterile during pregnancy, so cows and mares can become pregnant despite the presence of potentially harmful microbes. In this study, *F. gastrosuis* was found in high concentrations in vaginal swabs when compared to fecal, nasal, and ocular swabs (Cq/swab type: 28.91 ± 4.4, 34.87 ± 1.9, 37.49 ± 2.9, and 37.11 ± 1.2, respectively, P = 0.0003). Sequence data from camel vaginal swabs was submitted to GenBank as CAMSA16, accession number OQ824900. Environmental stress, immunodeficiency, mucus abrasion, unsanitary obstetrical care, or the presence of other supporting microbes are all risk factors for *Fusobacterium* infection. In conclusion, this study provides illumination on a novel and naturally occurring microbe in the camel vagina that, under certain conditions, can cause serious diseases. More research is needed to determine whether *F. gastrosuis* is a uterine microbiota member and/or whether it causes genital pathologies in dromedaries. *F. gastrosuis* may also be zoonotic.

Grants: This study was funded by Ministry of Education, Saudi Arabia through the project number (QU -IF-1-1-2).

Uterine infection in female dromedary camels

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Uterine infection is the main cause of infertility in female dromedaries. Major risk factors include hazardous reproductive management, unsanitary dealings during parturition, and postpartum problems. Predisposing factors comprise environmental stress, immunodeficiency, mucus abrasion, and the presence of other opportunistic microbes. *Trueperella pyogenes, Escherichia coli, Pseudomonas aeruginosa, Streptococcus* spp., and *Staphylococcus* spp. are the major uterine isolates obtained from females with clinical endometritis (CE, uterine inflammation with abnormal vaginal discharges), while *Bacillus* spp., *Staphylococcus* spp., and *Candida albicans* are the most frequent isolates of subclinical endometritis (SCE, uterine inflammation with no clinical signs and is described as the infiltration of polymorphnuclear cells into the endometrium). A treatment regimen consisting of intrauterine lotagen and administration of PGF2α during infusion and hCG during mating has been found to be effective in treating female dromedaries with severe endometritis. Acriflavin and gentamicin were both equally effective in treating mild endometritis. For SCE, two treatment protocols have been proposed: intrauterine infusion of 500 mg cephapirin benzathine and 1200 ml intrauterine flushing of 10% povidone-iodine. There was no statistically significant difference in the percentage of recovered females between the two protocols. Finally, this information can serve as a compilation for those working in the field of camel reproduction.

Grants: This study was funded by Ministry of Education, Saudi Arabia through the project number (QU-IF-1-1-2).

Cervical ectopic pregnancy of a nulliparous woman

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Cervical ectopic pregnancy is rare. The embryo implants and grows inside the endocervical canal. This study describes noninvasive and invasive strategies for the treatment of cervical ectopic pregnancy. A 32-year-old patient applied to the obstetrics clinic due to delayed menstruation. On ultrasound imaging (US), the endometrial cavity was empty and a cervical-located gestational sac was observed under the internal cervical ostium. Endometrial thickness was 8.2 mm, and a gestational sac and an embryo with a fetal heart rate (FHR) compatible with 6 weeks and 4 days were seen. A single dose of systemic Methotrexate (MTX; 50 mg/m², im) was administered to the patient. After the β-hCG value was 32824 mIU/ml on the 2nd day of MTX administration, a second dose of MTX intracavitary was administered under US on the 4th day of the treatment, and no complications developed. Since the FHR (+) and β-hCG value of 36233 mIU/ml on the 4th day of MTX administration and the 8th day of systemic MTX administration, liver and kidney functions were evaluated again and a third dose of systemic MTX was administered. On the 11th day, the β-hCG value was 28202 mIU/ml and FHR (–). It was decided to apply dilatation/curettage to the patient because of FHR (–) and a decrease of βhCG value by less than 35 %. In the controls examined at the end of the first month, the β-hCG value was negative, and the uterus was clear on US. Cervical ectopic pregnancies are life-threatening diseases. Systemic MTX or combined systemic MTX, MTX or KCl can be applied into the gestational sac and dilatation and curettage should be performed before the complications occur.

Ovarian steroidogenesis is regulated by the ERK1/2-SOX9/FOXL2 signaling axis thus favoring corpus luteum formation

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The ovarian steroids estradiol and progesterone play a pivotal role in female reproduction. Estradiol is primarily synthesized by the ovarian granulosa cells during follicle development under the influence of follicle-stimulating hormone and insulin-like growth factor 1. Granulosa cell steroidogenesis shifts from predominant estradiol production to predominant progesterone production in pre-ovulatory follicles by the action of luteinizing hormone, which eventually induces ovulation and corpus luteum formation. However, the underlying signaling pathways and transcriptional regulators for the cessation of estradiol and simultaneous stimulation of progesterone production are not clearly established. In the present study, we deciphered that the ERK1/2 signaling regulates steroidogenesis by inhibiting estradiol production and inducing progesterone production in granulosa cells. We identified that the downregulation of transcription factor FOXL2 and upregulation of SOX9 by ERK1/2 underpins its differential steroidogenic function in cultured granulosa cells. Interestingly, SOX9, whose function is largely uncovered in ovarian cells, was found to regulate FOXL2 along with the STAR and CYP19A1 genes, rate limiting for steroidogenesis, by acting as a transcriptional regulator. We propose that the identified ERK1/2-SOX9/FOXL2 axis in granulosa cells is an important modulator of ovarian steroidogenesis and may be considered when addressing the pathophysiologies associated with ovarian steroidogenesis in humans and animals.

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Fertility outcomes in cows with subclinical endometritis after clinical cure of clinical endometritis

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Clinical endometritis (CE) is common in postpartum dairy cows and associated with impaired reproductive performance. The aim of this study was to evaluate the effect of subclinical endometritis (SE) in cows clinically cured from CE on their fertility. The study was performed with 215 Holstein Friesian cows with CE, diagnosed by vaginoscopy

and ultrasound between 21 and 28 days after parturition. Animals were divided into three groups depending on treatments: Group 1 (n = 72): intrauterine cephapirin infusion; Group 2 (n = 73): single intramuscular injection of dinoprost; and Group 3 (n = 77): untreated control. All cows were clinically examined three times at an interval of 2 weeks. Cows without signs of CE were considered as cured, and endometrial samples from the uteri were collected by cytobrush to diagnose SE by cytological evaluation of polymorphonuclear neutrophils (PMN). The threshold for SE was set at ≥ 5% PMN. Pregnancy rate 200 days postpartum, interval from calving to conception and culling rate were calculated. On average, SE was diagnosed in 40.9% of clinically cured cows. There were no statistically significant differences in SE incidence between groups ($p > 0.05$). Cows with SE after clinical cure from CE showed a tendency toward lower fertility compared to cows without SE. Pregnancy rate 200 days postpartum was 61.2% and 66.9% ($p < 0.118$), interval from calving to conception 147 ± 66.3 and 130 ± 59.6 days ($p < 0.058$), culling rate 38.8% and 33.1% ($p < 0.155$), respectively. In conclusion, SE occurs frequently in cows clinically cured from CE regardless of treatment methods and may impair fertility.

Enhanced co-culture model of bovine granulosa and theca cells for investigating cell interactions in the ovarian follicle

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Earlier, we outlined a co-culture system involving granulosa (GC) and theca cells (TC) from bovine ovarian follicles. This model aims to enhance our understanding of the interactions, signaling pathways, and substrate exchange that occur between these two cell compartments, providing a more comprehensive insight. To account for the prolonged attachment time required for primary TC and GC onto the insert surface, we have adjusted our cell culture conditions. In brief, we initially seeded the TC onto the inverted insert membrane, employing a truncated tube to prevent medium leakage. After four days of TC culture, we inverted the insert and cultured GC on the opposite side of the membrane for an additional four days. At the end of the cell culture, we collected media for hormone analysis via RIA, and separately harvested granulosa and theca cells for gene expression analysis. The expression of marker genes indicated cell-type-specific patterns, with *CYP17A1* being highly abundant in TC and not expressed in GC. Conversely, *CYP19A1* showed high levels in GC and only low levels in TCs. The hormone analysis supported the presence of a physiological co-culture system, as evidenced by testosterone levels in the co-culture being approximately 10 times higher than in theca cells cultured alone. Estradiol concentrations were lower in the co-culture compared to GC cultured alone, possibly due to the shorter culture period for GC. This adapted co-culture model may allow a reliable basis for studying paracrine communication and substrate transport between somatic cells within the follicle.

Insights into the gene networks regulating cAMP-dependent steroidogenesis in Leydig cells under modified oxygenation conditions

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Hypoxia-Inducible Factor (HIF) 1α plays an important role as a transcription factor in the control of steroid production. It shows both direct and indirect effects on cAMP-mediated, STAR-dependent steroidogenesis. Since Leydig cells constitutively express HIF1α and continuously function under lower oxygen (O_2) tension, we conducted transcriptomic analysis (RNA-Seq) to evaluate the cAMP-mediated effects under lowering O_2 (20%, 10% and 1%) in two immortalized murine Leydig cell lines (MLTC1 and MA-10). Differentially expressed genes (DEGs) were determined, followed by downstream analyses ($p < 0.05$, FDR < 0.01). Of the 879 DEGs observed in cAMP stimulation at 20% O_2, 71 were common across all O_2 concentrations and seemed indicative of steroidogenic signature, being mainly associated with steroidogenesis and cholesterol metabolic process. Besides cAMP/PKA, the prevailing pathways included MAPK and IGF1 signaling. Lowered (10%) O_2 content yielded 113 DEGs, linked to pathways involving cellular response to hypoxia (also suggesting cellular control over HIF1a availability), DNA replication, and regulation of translation. The severely lowered O_2 levels led to 1569 DEGs associated with functional terms related to HIF1α signaling, angiogenesis, DNA damage, and oxidative stress. In targeted studies the effects of functional withdrawal of HIFα were shown. This study highlights the influence of lowering O_2 levels on Leydig cells and indicates their metabolic and biological targets. These insights hold translational value for both normal steroidogenesis and conditions like tumors associated with steroid production.

Influence of intrauterine application of *Lactobacillus buchneri* on first service conception rate in high yielding dairy cows with or without subclinical endometritis

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Most commercial dairy farms try to accomplish a calving interval of 365 to 380 days to have a constant milk yield throughout the year, presupposing that a cow has to be pregnant by day 85 to 100 postpartum (p.p.). The aim of this study was to investigate the influence of an intrauterine administration of *Lactobacillus (L.)* buchneri DSM 32407 (LAC) on the first service conception rate (FSCR) of dairy cows with clinically healthy uteri and ovaries but without first service up until day 70 ± 3 p.p. Therefore, Holstein Friesians were examined on day 70 ± 3 p.p. and cows with ovarian cysts or signs of clinical endometritis such as purulent vaginal discharge, purulent uterine filling or diffused hyperechogenic endometrial areas were excluded from the study. An endometrial swab was taken to detect subclinical endometritis (SCE, ≥ 5% polymorphonuclear neutrophils) in retrospect before randomized applying either LAC (n = 70, thereof n = 10 SCE) or a placebo (PLA; n = 87, thereof n = 11 SCE) into the uterus. The median days to first service did not differ between the groups. A pregnancy check was performed on day 32 ± 3 *post inseminationem*. Interestingly, independent of the treatment, cows diagnosed with SCE tend to have a higher FSCR than cows without SCE (47.6% vs 27.2%; p = 0.057). However, the FSCR was not different comparing the LAC to PLA group, with or without SCE. In conclusion, an intrauterine administration of L. buchneri on day 70 ± 3 p.p. to clinically healthy cows does not seem to improve the FSCR.

Grants: Funded by LfULG and MLUK Brandenburg, Germany.

Interferon tau (IFN_τ) alone does not alter the polarisation of bovine endometrial caruncular epithelial cells (BCEC) *in vitro*

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Early embryonic mortality in cattle represents a major cause of economic losses for dairy farmers, with most embryonic death occurring prior to implantation. Interferon-tau (IFN_τ) serves as the maternal pregnancy recognition signal in ruminant species and is secreted by the elongating trophoblast. It promotes conceptus development and influences the uterine gene expression. We hypothesise that IFN_τ alters the polarisation of uterine epithelial cells prior to implantation in order to enable the successful attachment of the trophoblast. Therefore, bovine caruncular epithelial cells (BCEC) grown in 60 mm cell culture dishes were stimulated with IFN_τ (10 ng/ml) for 24 h. Serum reduced medium, full medium and PBS served as controls. The mRNA expression of the interferon-stimulated genes myxovirus resistance 1 and 2 *(MX1, MX2)* as well as of the polarisation markers Ezrin *(EZR)*, Cytokeratin 18 *(CK18)*, E-Cadherin *(CDH1)*, N-Cadherin *(CDH2)* and Vimentin *(VIM)* was examined via quantitative real-time PCR. Additionally, the protein expression of *EZR, CK18, CDH1* and *VIM* was investigated using immunofluorescence. While *MX1* and *MX2* mRNA was significantly upregulated by IFN_τ, no differences in the mRNA expression of *EZR, CK18, CDH1,*

CDH2 and *VIM* were observed. Immunofluorescence staining did not show any differences in protein expression and localisation between IFN_τ and control groups. In conclusion, IFN_τ alone does not seem to influence the polarisation of the BCEC in 2-dimensional *in vitro* cultures. Further investigations are planned regarding the preincubation with steroid hormones and in 3-dimensional culture systems.

In vitro adsorption and retention ability of ultrapure Carbon-sorbent microporous beads vs *Escherichia coli* CSH26 K12: Preliminary results

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Dairy cows are frequently affected by postpartum metritis and thus treated with antimicrobials However, extensive use of antibiotics could lead to antimicrobial resistance and to an increased risk of residues in food and environment. Therefore, alternative therapies are desirable. The objectives of this study were to estimate the ability of ultrapure carbon microporous beads (VNIITU) to absorb and retain bacteria (*E. coli* CSH26 K12) on their surface compared with glass beads, together with evaluating the release of any inhibiting compound into the solution. Both experiments were conducted *in vitro*. Sorbent or glass beads were suspended with cultured *E. coli* and MDR and incubated at 37 °C for 24 hours. Pellet was recovered, vortexed for 0, 15, 30, 60 and 90 s and then re-suspended for microbial count. Supernatant was challenged with cultured *E. coli* to assess inference with microbial growth. No migration of inhibiting compounds was detected for both carbon and glass beads. Total CFU and CFU/ml counts were lower in carbon compared to glass beads, both from supernatant and from vortexed re-suspended pellets, thus confirming the absorption ability. After vortexing, the number of bacteria released from carbon remained constant while glass beads progressively released bacteria. These preliminary results indicate that carbon and porous beads do not release toxic compounds, can retain bacteria even after shaking, and can be used to limit uterine bacterial infection in postpartum dairy cows.

Study of liver development in the neonatal calf in the first 21 days postnatum by computed tomography

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To investigate the physiological-topographical conditions of the liver of the neonatal calf during its course, computed tomographic examinations were performed in 15 clinically healthy calves of the German Holstein breed on the 1st, 7th, 14th and 21st day of life. On the first day of life, three examinations were done at an interval of six hours. The first examination took place immediately after birth. The liver of the neonatal calf presents on CT between the 8th thoracic and 5th lumbar vertebrae. Over the first 21 days of life, the length of the liver remains constant and averages 7.48 ± 0.78 vertebral lengths. Liver expansions in transverse section showed average values in the vertical axis of 14.9–17.15 cm over the overall course of the study. On average, the liver presented with a height of 16.07 ± 0.92 cm. In the horizontal measurement, average values of 8.13–9.13 cm could be determined in the examined calves over the entire study period. A change in the volume of the liver on the first day of life is evident. Hepatic tissue volume decreased significantly in all calves during the first hours of life. One hour p. n. the measurements showed an average volume of 1003.4 cm^3, which decreased to 892.3 cm^3 at the second examination and to 887.1 cm^3 at the third. The data are not only basis for the evaluation of cross-sectional imaging of the newborn calf, but also provide a deeper insight into the development of the liver during the first 3 weeks of life.

Serum trace elements associated with different forms of infertility in rams

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The main objective of the present study was to investigate the association between serum trace elements and forms of infertility in rams in central Saudi Arabia. Total of 54 infertile and 18 fertile rams were used in this study. Infertile rams were admitted for breeding soundness examination. Data including owner's complaint, breeding history and signalment was recorded. The animals were bled immediately on admission and examined clinically. Sera of the studied animals were used for the estimation of serum trace elements and testosterone levels. The animals were categorized according to form of infertility (impotentia generandi, n = 40 vs impotentia coeundi, n = 8 vs lack of sexual desire, n = 6) and affected genital organs (testicular, n = 22 vs epididymal, n = 8 vs spermatic cord, n = 3 vs penile/preputial, n = 4 vs idiopathic, n = 17). Results revealed that serum Mn, Se and Zn were lower in infertile rams with different forms of infertility than in the fertile group (2.88 ± 2.28 vs 0.35 ± 0.09 mg/L, p = 0.0001, 0.76 ± 0.37 vs 0.07 ± 0.01 mg/L, p = 0.0001, 18.12 ± 2.26 vs 16.84 ± 8.46 mg/L, p = 0.02, resp.) and various genital affections (3.70 ± 3.68 vs 0.35 ± 0.08 mg/L, p = 0.0001, 0.65 ± 0.61 vs 0.11 ± 0.02 mg/L, p = 0.001, 8.04 ± 3.42 vs 4.36 ± 1.03 mg/L, p = 0.02, resp.). Serum testosterone level correlated negatively with trace elements in infertile rams. It can be concluded that trace elements may be implicated in the pathogenesis of the genital affections of infertile rams.

Influence of transportrelated vibrations on the quality of liquid-preserved AI doses in stallions

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The demand from equine breeders for liquid-preserved artificial insemination (AI) doses of high-class stallions housed throughout Europe necessitates the transport of semen doses. In this study, the influence of transport-related vibration on the quality of stallion AI doses, obtained from 30 stallion ejaculates, was investigated. Each ejaculate was split in half and diluted with the two semen extenders EquiPlus and Gent (Minitüb GmbH). Thereafter, all samples were divided into four subsamples, three of which were subjected to vibration caused by an orbital shaker (IKA MTS 4, Laborgeräte München) for 3 h, 6 h, and 9 h at 5 °C and one served as the control group (0 h). The vibration intensity applied to the experimental groups reflected roads with rough asphalt (displacement index D_i = 3.0 ± 0.1). After simulated transport, all samples were stored for four days at 5 °C and analyzed for total sperm motility, complemented by a thermo-resistance test and a flow cytometric assessment of sperm viability and mitochondrial activity as well as pH-measurements. The calculation of generalized linear mixed models revealed a negative effect of vibration on total sperm motility ($p = 0.001$), thermo-resistance ($p = 0.030$) and pH-value ($p = 0.001$) whereas viability and mitochondrial activity were not impaired ($p > 0.05$). Simulated transport for 3 h did not alter sperm quality while 6 h and 9 h diminished total sperm motility ($p < 0.01$), thermo-resistance ($p < 0.05$) and pH ($p < 0.01$). We therefore recommend keeping shipping time for stallion AI doses as short as possible in order to prevent transport-related sperm quality loss.

Establishment of 16S ribosomal RNA V3/V4 amplicon generation for microbiome analysis of uterine cytobrush samples in the mare

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Studying the uterine microbiome is challenging due to the low bacterial biomass and high host background in the samples. Uterine cytobrush samples were collected from mares during fertility assessment in estrus and DNA was extracted. A 16S rRNA fragment containing the V3/V4 hypervariable regions was amplified using prokaryotic universal primers Pro341F and Pro805R. In addition to the expected 16S rRNA product, an unspecific product was obtained due to the unfavorable host DNA to bacterial DNA ratio and similarities between 16S rRNA

and mitochondrial 12S rRNA. Furthermore, many PCR reagents contain bacterial DNA contaminations, introducing additional bias. Sequencing confirmed the specificity of the obtained 16S rRNA gene fragments but identified the additional 346 bp product as a fragment derived from the equine mitochondrial 12S rRNA gene. To overcome these problems, different PCR enzymes, reagents, and conditions were tested. Moreover, a peptide nucleic acid clamp (PNA) and a 3'-end modified oligonucleotide were designed to suppress amplification of the unspecific 12S rRNA product. From five PCR kits tested, only two did not produce detectable products in the non-template control. The unspecific 12S rRNA product could be partially suppressed with the PNA. More efficient suppression was found for the oligonucleotide blocking the binding of Pro805R to the 12S rRNA gene, which depended on the concentration of the oligonucleotide. These experiments led to the establishment of a sensitive and specific PCR protocol for the generation of 16S rRNA V3/V4 amplicons for Illumina sequencing from uterine cytobrush samples.

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Effect of the toll-like receptor 7/8 (TLR 7/8) ligand Resiquimod on bovine sperm motility characteristics in modified synthetic human tubal fluid medium and commercial sperm extender

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In recent studies, Resiquimod (R848) was used for separating X- and Y-chromosome bearing sperm. Following this approach, we performed detailed computer assisted analysis of bovine single sperm and population motility characteristics in response to treatment with R848 (30–60 min at 37 °C, 0–2.4 µg/mL), for defining conditions for future use and testing separation techniques. Six ejaculates from different bulls were included in the studies, and the dimethyl sulfoxide (DMSO) concentration for dissolving R848 was kept constant for all cases. Cryopreserved sperm was tested in modified synthetic human tubal fluid as incubation medium (mHTF). With 30 min incubation time, the percentage of rapid moving sperm decreased in a R848-dose dependent manner, while the population of slow-moving sperm increasing and exhibiting an optimum with exposure to 0.01–0.05 µg/mL R848. When analyzing separately recovered layers under the same conditions, minor differences were found between top and bottom layers in sperm recovery and motility behaviors. When using freshly diluted ejaculates (60×10^6 sperm/mL, in Andromed), higher R848-concentrations and incubation times were needed to see similar effects on sperm motility characteristics as seen when using cryopreserved sperm in mHTF. In this case, a prominent slow-moving sperm population appeared with 60 min exposure to 0.40–0.80 µg/mL R848. In conclusion, when treating sperm with R848 to modulate sperm motility characteristics, the needed incubation times and R848-concentrations are noticeably affected by the sperm processing procedures and incubation media used.

Inter- and intra-boar variability of bacterial count in boar semen

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In this study the inter- and intra-boar variability of bacterial cell count (BCC), potential reasons for that, and the relation between BCC and sperm quality were investigated. For 13 months, semen was collected at 4-week intervals by hand or automatically (Collectis, IMV Technologies, l'Aigle, France) from 92 boars of different breeds kept under different housing systems (straw vs. wood chip bedding; with vs. without cooling system). The mean temperature-humidity index (THI) was calculated for the 7-day period prior to semen collection. Semen was diluted to $20–60 \times 10^6$ sperm/mL with gentamicin-supplemented Beltsville Thawing Solution (Minitube, Tiefenbach, Germany). Sperm progressive motility (PM%) was assessed on the collection day (D0) using computer-assisted sperm analysis. Then, sperm was snap-frozen in liquid nitrogen until SYBR Green-based flow cytometric assessment of the BCC per mL. Mixed-effects models were fit to explore the effects of boar, age, breed, method of semen collection, duration of sperm storage, THI and housing system on PM% and BCC. Between-boar variability of BCC was 99.76% ± 49.45%. BCC decreased with progressing age ($b = -92.44$, $p = 0.007$) and increasing THI ($b = -3.27$, $p = 0.012$). Water-based cooling had an adverse effect on BCC compared to the negative-pressure ventilation system ($b = 5.53$, $p = 0.004$). The effect of boar, breed and collection method on BCC and PM% was not significant. Elevated BCC was linked to decreased PM% ($b = -0.67$, $p = 0.002$). In conclusion, there is a high variability in BCC between as well as within boars; boar age, housing system and microclimate contribute to the inter- and intra-boar variability of semen bacterial count.

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The effect of progesterone on the expression of SERPINA14 in bovine cumulus complexes during in vitro maturation of oocytes

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Belonging to a superfamily of proteins sharing protease inhibitory properties, SERPINA14 has been detected in bovine ovarian granulosa cells *in vivo*, and its increased expression during *in vitro* maturation (IVM) of cumulus oocyte complexes (COCs) has been demonstrated. Although proteome studies suggested a connection between SERPINA14 and the oocytes maturational competence, its regulation remains unclear. It is also secreted in uterine glandular epithelia, being regulated by progesterone (P4) and estrogen. Here, we investigated the impact of P4 on SERPINA14 expression in COCs. Oocytes obtained from ovaries collected at a local abattoir were incubated in maturation media (MM) containing a P4 synthesis inhibitor (3bHSD blocker, trilostane, 10 µM) or a nuclear P4 receptor blocker (PGR) (mifepristone, 1 µM), or no additive as control. For immunohistochemistry, oocytes after IVM from 2 replicates were used. For determination of P4 concentration in MM and cleavage and blastocyst rates, 3 replicates with 50–55 COCs per group were run. The addition of trilostane and mifepristone to the MM resulted in reduction of the immunohistochemical staining of SERPINA14, with trilostane presenting a more pronounced effect. It also led to a lower blastocyst rate compared to the control, while the cleavage rate of the 3 groups did not differ. In conclusion, our findings: 1) demonstrate that SERPINA14 expression in bovine COCs depends on *in vitro* exposure to P4, and 2) confirm that inhibiting P4 results in a reduced blastocyst rate, implying a possible role of SERPINA14 during oocyte maturation.

Characterization of bovine oocytes during in vitro maturation using microaspiration-assisted Electrical Impedance Spectroscopy

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Microaspiration-assisted electrical impedance spectroscopy (MAEIS) is a label-free and non-invasive tool to characterize different materials. Electrical impedance (EI) of small objects, trapped in a microaperture on a microfluidic chip, is measured as impedance magnitude change ΔZ/Z0 (impedance change ΔZ by trapping an object divided by impedance of an empty aperture Z0). The aim was to evaluate the electrical changes during in vitro maturation (IVM) of bovine oocytes via MAEIS. Abattoir-derived cumulus-oocyte-complexes (COCs) were matured for 24 h and at different time points (0/4/18/20/22/24 h) 4 COCs were denuded and pipetted individually on the microfluidic chip. Measurement of EI of each oocyte took 60 s. Then they were stained with Hoechst 33342 to determine their maturational stages. In total 24 oocytes (4 per time point) were analyzed. At the beginning (0 h) 2 oocytes were at the GV (Germinal Vesicle) and 2 at GVBD (Germinal Vesicle Break Down) stage. At time point 4 h, all oocytes were in GVBD. MI (meiosis I) was reached of all 4 oocytes after 18 h, 3 did so at

20 h, and one being already in MII (meiosis II). At 22 h, 2 oocytes were in MI and 2 in MII, and at the end of maturation (24 h) 3 of 4 oocytes were in MII. The magnitude change of oocytes in GV stage was 7.23 ± 0.73 % (mean ± standard deviation), in GVBD an increase up to 10.02 ± 0.81 % was detected, in contrast ΔZ/Z0 6.60 ± 0.78 % and 6.56 ± 0.97 % were shown by oocytes in MI and MII, respectively. We conclude that a non-invasive detection of maturational stages of oocytes is possible via MAEIS with further work to be done.

Effect of Lactobacillus-conditioned medium supplemented during in vitro maturation of bovine oocytes on mRNA expression patterns

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Lipopolysaccharide (LPS) has been detected in the follicular fluid of cows with infections. LPS administered during in vitro maturation (IVM) of bovine oocytes impairs their developmental competence; whereas, Lactobacillus (L.) spp. have been associated with increased oocyte quality when in follicular fluid. The aim of this study was to determine whether the negative effect of LPS could be overcome via L.-conditioned medium. Cumulus-oocyte-complexes from slaughterhouse ovaries were isolated via slicing. Five % of the IVM medium (5:95) were replaced by L.-buchneri-conditioned medium (cond.; group 1), LPS was supplemented during IVM at a concentration of 10 µg/mL (group 2). In a third group, a combination of both supplements (combi) was employed. As controls, immature oocytes and oocytes after standard IVM were included. Maturation rate was significantly reduced in oocytes of the LPS group compared to all other groups [Granacher et al. Reprod Dom Anim 2023; 58: 13]. Messenger RNA (mRNA) from single denuded oocytes (n = 5 for each group) was directly used for RT-qPCR to determine the relative abundance of gene transcripts known to play important roles during oocyte maturation (PGR, PGRMC1, PGRMC2, STAR, ZAR1, BMP15, GDF9, G6PD). Statistically significant differences were only detected for PGR transcripts (Immature: 0.01 ± 0.01a; Mature: 0.29 ± 0.10c; LPS 0.04 ± 0.03a; cond: 0.11 ± 0.05b; combi: 0.13 ± 0.02b; a: b: c $p \leq 0.05$, ANOVA followed by Tuckey test). These results suggest that L. buchneri-conditioned medium partly helps to overcome the negative LPS effect on PGR expression.

Canine uterine myocytes of dystocic bitches form contractile filaments in adherent cell culture

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Dystocia in parturient bitches requires instant veterinary obstetrical emergency care to assure the survival of the dam and the puppies. However, conservative treatment options are alarmingly limited and ineffective, especially for uterine inertia, the most common maternal reason for canine dystocia. Despite its frequent occurrence, the aetiology and pathophysiology of canine uterine inertia remain unclear. For investigations of myometrial contractions at cellular and molecular level, we established a protocol for culturing canine uterine myocytes of the longitudinal and circular layer in adherent cell cultures from dystocic bitches of different breeds and ages undergoing caesarean section. Using immunofluorescence, we detected the contractile filament smooth muscle actin alpha in cells of both myometrial muscle layers (n = 5), demonstrating that canine uterine myocytes retain their contractility in primary culture up to passage 6, as well as the expression of Oxytocin receptors. These successfully established cell cultures of canine uterine myocytes can now be used for a variety of preclinical

experiments in biomedical research, testing the impact of uterotonic substances on cell contractility as a substitute for experiments on living animals and to further investigate the role of contraction-associated hormone receptors and proteins.

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Heat stress during maturation has long lasting effects on development, gene expression and mitochondrial metabolism of bovine pre-implantation embryos

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Hot spells, today also affecting temperate climates, have a major impact on fertility, follicular development, oocyte maturation, developmental capacity and mitochondrial properties. Therefore, the present study aimed to gain deeper insights into the detrimental effect of elevated temperature during bovine oocyte maturation on mitochondrial function and health by analyzing the bioenergetic profile and the expression level of selected candidate genes in subsequent blastocysts. A total of 688 immature oocytes were subjected to in vitro maturation under elevated temperature (41 °C) whereas a total of 646 oocytes matured under routine procedures (38.8 °C). Subsequently, COCs were fertilized and cultured in vitro for 7 days. Beside significantly ($p < 0.05$) elevated accumulation of reactive oxygen species (ROS) in heat stressed oocytes (HS) after maturation, significantly lower cleavage- (77.1% vs 89.2%, $p < 0.05$) and blastocyst rates on day 7 (25.6% vs 40.3% $p < 0.05$) were observed. Blastocysts resulting from HS oocytes, displayed significantly lower basal respiration rates, maximal respiration and spare capacity rates. Furthermore, HS embryos revealed a significant higher abundance of genes regulating oxidative stress response (GPX1, SOD, NRF2 and PRDX1) or being related to apoptosis (BAX and CASP3) whereas the apoptosis inhibitor BLC2 was almost 2-fold downregulated in this group. Conclusively, a sustainable effect of heat stress during maturation on embryonic development as well as on mitochondrial function and health could be detected.

AI center management factors influencing the reproductive lifetime and sperm parameters of European breeding boars

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Artificial insemination (AI) is the primary biotechnology for pig breeding. A prerequisite for AI is an effective reproductive lifetime of boars and the production of high-quality semen doses. The aim of this study was to determine management factors that influence the reproductive lifetime and sperm quality traits of boars at 54 European AI centers. Therefore, the pedigree data of 6,496 Pietrain boars and sperm traits of 40,765 ejaculates were analyzed. A hazard model showed that the probability of a long reproductive lifetime was reduced with lower boar age at time of selection process, quarantine entry, AI center entry, and decreasing age at first semen collection. The removal risk increased with decreasing quarantine period, monthly semen collection frequency, and differed between European countries. A generalized linear mixed model revealed that the boar age, the breed, the semen collection technician, the season, and the $PGF_{2\alpha}$ treatment significantly influenced sperm traits. We found higher ejaculate volume, total sperm number, progressive sperm motility, and morphologically normal sperm for untreated boars in comparison to $PGF_{2\alpha}$-treated boars. While the $PGF_{2\alpha}$ treatment had no influence on the length of reproductive lifetime of boars at AI centers, the risk of ejaculates being rejected due to low sperm quality was increased after $PGF_{2\alpha}$ treatment. In conclusion, more attention should be paid to the age of prospective AI boars at the start of semen production and to the routine use of $PGF_{2\alpha}$.

Low-temperature transport allows superior maintenance of boar sperm quality compared to standard transport conditions

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Thanks to scientific progress, novel, antibiotic-free 5 °C preservation of boar sperm is on the verge of being introduced into practice. Transport of insemination doses to the customer is an important yet scarcely monitored cornerstone of production. It is known that transport-related vibrations (TV) have deteriorating effects on boar sperm stored at 17 °C, whereas knowledge of 5 °C transport is still insufficient. This study compared effects of TV on sperm stored at 17 °C and 5 °C to gain knowledge on the impact of low-temperature transport. Ejaculates of 18 Piétrain boars were diluted in a split sample procedure to a concentration of 2.0×10^7 sperm/mL using *Beltsville Thawing Solution* (BTS) or *Androstar Premium* (AP) extender (Minitüb, Germany). For BTS samples, transport simulation at 17°C (for 0h/3h or 6h, using laboratory shaker IKA MTS 4, displacement index = 3 was performed on the day of collection (d0). AP samples were exposed to TV on d1, following completion of the standard cooling curve to 5 °C. A generalized linear mixed model revealed a TV ($p = 0.021$) and storage time ($p < 0.001$) dependent decline in progressive motility (PM). Overall PM was slightly lower in 5 °C samples ($p = 0.027$), however, pairwise comparisons showed superior quality maintenance after exposure to TV (0h vs 6h: p (17 °C) < 0.001, p (5 °C) = 1.0). The same quality-preserving effect was observed in mitochondrial activity and membrane integrity (p [17 °C] < 0.001, p [5 °C] = 1.0). Concluding, 5 °C transport is possible without significant quality loss and with better quality maintenance than standard transport.

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Proteomics reveals substantial differences between *in vivo* matured bovine oocytes and *in vitro* matured oocytes retrieved from slaughterhouse material

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In most studies on reproductive physiology of cattle, abattoir material is used. Bovine ovaries can be obtained in large quantities several times a week without the need for animal experiments. However, little to none is known about the donor animals, such as age, reproduction status or potential health issues. All of these can have a great influence on oocyte quality. The aim of this study was to answer the question how representative experiments with oocytes obtained from slaughterhouse ovaries can be for the actual *in vivo* situation. For this purpose, immature oocytes were retrieved from slaughterhouse ovaries. Half of these oocytes were decumulated and frozen directly. The other half was matured for 22 h in the laboratory to obtain *in vitro* matured oocytes. A third group, *in vivo* matured oocytes, was retrieved by ovum pick-up after superovulation from six heifers. The proteome of these three oocyte groups (four biological replicates á 10 oocytes per group) was analyzed via mass spectrometry. A total of 1208 different proteins were identified in all oocytes. Comparing *in vitro* with *in vivo* matured oocytes, 73 proteins were differentially expressed. Of these proteins 69 were higher expressed in the *in vitro* matured oocytes. Only 4 proteins had a higher abundance in the *in vivo* group. Interestingly, unsupervised clustering revealed that *in vivo* matured oocytes clustered closer to the immature oocytes than to *in vitro* matured oocytes. Furthermore, *in vivo* matured oocytes and immature oocytes displayed homogeneous protein expression profiles, whereas the profiles of the in vitro matured oocytes were rather heterogenous.

Osteopontin gene expression is not affected by steroid hormones in bovine endometrial gland cells *in vitro*

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During early pregnancy, failure in embryo-maternal communication leads to embryonic loss and economic damages for dairy farmers. Within this time, endometrial glands play an important role, as their secretions, named histotrophe, are essential for embryo devel-

opment and successful placentation. A major component of the histotrophe is osteopontin (OPN) and in sheep its secretion is regulated by progesterone (P4) through downregulation of its own receptor (PGR). The aim of our study was to evaluate the regulation of PGR and OPN in bovine endometrial gland cells (BEGC) *in vitro*. As P4 alone did not have any effects in a former study, BEGC were now stimulated with combinations of P4 and interferon tau (IFN_τ) as well as P4, estrogen (E2) and IFN_τ. Additionally, an E2 stimulation with subsequent P4 incubation was conducted. Cells incubated in serum-reduced medium served as controls. The mRNA expression of PGR and OPN was analysed by quantitative real-time PCR (n = 4). Stimulation with P4 and IFN_τ had no effect on the PGR expression, but the combination of P4, E2 and IFN_τ led to a significant increase of the PGR expression ($p < 0.001$). Successive stimulation with E2 and P4 led to a slight decrease of PGR, but this was not statistically significant. None of the stimulations affected the expression of OPN. It could be possible that other combinations of the hormones are necessary to induce changes in OPN gene expression or that OPN is only regulated on protein level. Therefore, further stimulations and investigations on protein level are planned.

Impact of vaginal discharge on dairy cows' fertility subjected to an Ovsynch protocol with an intravaginal progesterone-releasing device

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Ovsynch protocols combined with progesterone-releasing intravaginal devices (PRID) can improve fertility, but muco-purulent vaginal discharge (VD) is often observed at removal of the device. This study evaluates the impact of VD on progesterone (P4) absorption and pregnancy per artificial insemination (P/AI) in lactating Holstein cows submitted to an Ovsynch+PRID protocol. A total of 1,056 cows were randomly assigned to 3 modifications of the protocol, with removal of PRID at d 8 and TAI 16 h after 2nd GnRH. Ovaries were scanned via ultrasonography at d 0, blood samples for P4 measurement were collected at d 0, 7 and 9 of the protocol, and VD was scored at PRID removal (VDS 0 = no debris, 1 = small flecks of pus, 2 = abundant amount of pus). No interaction was found between the three Ovsynch protocols and VDS. Overall, 6.5%, 73.9% and 19.5% of cows had VDS 0, 1 and 2, resp. VDS was significantly associated with P4 concentrations on d 7. Cows with VDS 2 had greater (4.3 ± 0.2 ng/mL) serum P4 concentrations than cows assigned to score 0 (3.0 ± 0.3 ng/mL) and 1 (3.7 ± 0.1 ng/mL). P4 concentration on d 7 was greater for cows with VDS 1 compared with VDS 0. VDS at PRID removal tended to have an association with P/AI ($p = 0.072$). P/AI did not differ among cows with VDS 0 and 1 (36.5% vs 41.3%), but was significantly greater (49.7%) for cows with VDS 2 vs. VDS 1, and tended to be greater in VDS 2 cows compared with VDS 0 ($p = 0.066$). Pregnancy loss was not affected by VDS. Further research is required to elucidate the positive association between vaginal discharge and P4 concentrations and P/AI more in detail.

Sonographic examination of testicular diameters in the European shorthair cat

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The basis of an andrological examination is the investigation of sex organ anatomy by clinical examination and ultrasound. However, systematic studies on the feline testis in larger numbers of animals are not yet available. Therefore, this study described the physiological sonomorphology of the cat testis and aimed to confirm the findings through histology and sperm production analysis. A clinical examination of the testicle was carried out in 33 European shorthair male cats as part of the preoperative examination before castration. The testes were sonographically examined (L 14-5 w) and measured in the longitudinal-transverse axis, and sagittal direction. In addition, the texture, echogenicity, and homogeneity of the testicular tissue were assessed. This was followed by castration and histological examination. The testes were weighed and measured. The cat testicles show a characteristic sonomorphology. The parenchyma is homogeneous with a distinct echogenic mediastinum. The histological examination verified that it is active testicular tissue. The arithmetic mean for the testicle parameters, measured sonographically, is: length of right testicle 1.3 ± 0.2 cm, length of left testicle 1.4 ± 0.2 cm, width of right testicle 1.2 ± 0.1cm; width of left testicle 1.2 ± 0.1 cm, depth of right testicle 1.0 ± 0.2 cm, depth of left testicle 1.0 ± 0.2 cm. The testicle volume of the right testicle is 0.93 ± 0.4 cm^3 and of the left testicle 0.97 cm^3 ± 0.4 cm^3. The presence of sperm suggests the animals were sexually adult. This study provides detailed testicular data for domestic cats, which has been verified by histological examinations, and can be used in the context of andrological examination.

Use of isolated bacteriophages specific to selected equine genital pathogens in a biofilm model

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Bacterial endometritis is one of the major problems in equine reproduction. Among the most important pathogens are *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA). Due to increasing prevalence of bacterial resistance towards antimicrobial drugs and the capability of KP and PA to produce biofilms, alternative treatment options against these pathogens are needed. Bacteriophages (phages) offer bactericidal capacities and show a species- and strain-specific lytic activity, making them a promising alternative treatment option for bacterial infections. The aim of the study was to determine the *in vitro* bactericidal effect of selected phages on clinical isolates of equine genital pathogens. The activity of phages specific to *KP* (n = 2) and *PA* (n = 1) to eradicate 48 h old biofilm was assessed in a single phage-assay. Bacterial suspensions of KP- and PA-isolates from endometrial samples were added to a microtiter plate and incubated (37 °C, 48 h) to form mature biofilms. Phage preparations were added at two different multiplicities of infection (MOIs 1 and 10). After 6 and 24 h viable cells were counted. To determine the total biofilm biomass, crystal violet staining in the microplate wells was performed and the optical density was measured at 600 nm (OD600). Preliminary results indicate that the phages specific to PA reduced mature biofilm biomass by reduction of OD600 after 24 h, while KP-specific phages showed a reduction of viable cell concentration after 6 h after single phage application. In conclusion, phage application shows promising results that have to be confirmed by additional studies *in vitro, ex vivo* and *in vivo*.

Profile of adhesive proteins in bovine pregnant endometrium

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Placentation period is crucial for physiological course of pregnancy in cattle. Adhesive proteins from extracellular matrix play important role in this process. The aim of our study was to examine the profile of selected adhesive proteins in pregnant endometrium between 2nd and 4th month of pregnancy. Endometrial tissues were isolated from pregnant uteri in slaughterhouse from 6 HF cows. The presence of protein of decorin (DCN), dermatopontin (DPT), glycodelin (Gd) were confirmed by WB. The concentrations of examined proteins were determined by ELISA tests in tissues and cell lysates. In addition adhesion test to fibronectin with and without the presence of progesterone and $PGF_{2\alpha}$ was performed. DCN concentrations in early pregnancy in maternal part reached 67.54 ± 15.64 pg/mg protein and increased together with pregnancy age. The highest concentration of DPT in maternal tissue in the 2nd month was 7.85 ± 4.93 ng/mg protein with a tendency to decrease as pregnancy progressed. Gd concentrations in cell lysates decreased significantly together with examined time, 2nd 2.10 ± 0.80 vs 4th 1.10 ± 0.60 ng/mg. Both progesterone and $PGF_{2\alpha}$ expressed an inhibitory effect on anti-adhesive properties of DCN. The adhesion of the cells treated with DPT in all tested concentrations (5, 50 or 100 ng/ml) for 24h was significantly enhanced both in the 2nd and 4th month of pregnancy and in the 4th month DPT-treated cells exhibited more than 2 times higher adhesive capacity. Cell adhesion in bovine endometrium during early pregnancy requires further studies.

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Time recording during caesarean section of the bitch

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Conflicts between the veterinarian and the dog owner often arise, if a bitch dies during a caesarean section. In this context, it is occasionally asked for an average duration of this surgery. Therefore, the aim of this study was to analyse the duration of the single steps during a caesarean section in 63 bitches. In each case, the duration from the skin incision to the exteriorization of the uterus, from the exteriorization of the uterus to the development of the first puppy, from the development of the first puppy to the development of the last puppy and from the development of the last puppy to the last stitch of skin suture were documented. The arithmetic mean value for the total surgery was 50.1 ± 17 minutes (24 to 98 minutes). The duration of development of all puppies was 3.1 ± 3.0 minutes (0.5 to 16 minutes). It increased with number of puppies. The number of puppies developed in a caesarean section was between one and 14. The average time per developed puppy was less than one minute (calculated value 46.8 seconds). The times determined can be used as references for duration of surgery.

Effects of semen collection frequency and age on sperm quality of young bulls

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The use of genomic selection in cattle breeding has led to an increased demand for the collection of ejaculates from selected bulls before or around puberty. However, it is not known how frequency of semen collection and age affect functional morphology, plasma membrane integrity, motility, sperm kinematics and oviductal sperm reservoir formation. To this end, we examined frozen-thawed ejaculates from 4 bulls (breeds: Holstein, Friesian and Red Holstein) aged 37 ± 11 days using phase contrast microscopy, light microscopy, CASA and digital live cell imaging and compared them with sperm parameters from 4 adult bulls. Results showed that in young bulls, the percentage of slowly progressing spermatozoa was significantly reduced and the number of immotile spermatozoa was increased. The percentage of sperm with intact plasma membrane was higher in young bulls than in old bulls, the percentage of sperm with intact plasma membrane decreased significantly in the 2nd and 3rd ejaculate in young bulls. When three ejaculates per week were collected, the kinematics of the spermatozoa were altered, resulting in significantly reduced amplitudes of lateral head movement (ALH) and wobble. Interestingly, in young bulls, the occurrence of proximal and distal droplets significantly increased and the percentage of slow progressive spermatozoa significantly decreased in the 3rd week of collection, irrespective of collection frequency. Results suggest that sperm quality in young bulls can be optimised by a collection frequency of 1–2/week and a break every 3 weeks.

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Congenital enlargement of the testicles in a Simmental bull calf – a case report

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A 5-day-old male Simmental calf was presented to the Clinic for Cattle with "severely enlarged testicles from birth and an umbilical hernia". The general clinical examination revealed a moderately inflamed navel, an umbilical hernia approximately 2 cm in diameter and significantly enlarged testicles. The andrological examination revealed asymmetric enlarged testicles (left: 22.0 × 6.5 × 5.5 cm; right: 21.0 × 6.5 × 5.5 cm). They were firm, turgid, sensitive to pressure, movable in the scrotum and a slight fluctuation in the scrotum was noticed in the distal part. The sonographic examination showed an inhomogeneity of the testicles, which were interspersed with isoechogenic to hyperechogenic bands. The rete testis was not visible on both sides. The ventral abdomen showed a small amount of anechogenic fluid. Biopsies of both testicles examined at the Institute of Pathology revealed a malignant blastoma with a slight purulent inflammation. After castration, the animal showed a good general condition and the castration wound healed well. As it progressed, however, the ascites continued to increase and the general condition worsened. Sonographic examination revealed larger amounts of anechoic fluid with masses of fibrin in the abdominal cavity and hyperechoic, compact structures originating from the liver and abomasum were visible. Therefore, the animal was euthanized after 3 weeks with suspected metastases. Necropsy revealed 10 L of ascites and multiple nodular masses originating from the serosa on all abdominal serosal surfaces, especially of the liver and forestomachs. Histopathologically a mesothelioma was diagnosed.

Maternal vaccination affects uterine health and milk yield postpartum

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Maternal vaccinations against newborn calf diarrhea pathogens are carried out in the last weeks of pregnancy, with the intention to induce an adaptive humoral immune response and to protect calves in the first weeks of life. There is evidence that vaccination-related innate immune responses not only lead to the generation of maternal antibodies, but also modulate the immune system of the mother towards other stressors. Whether such hypothetical effects alter the disease susceptibility of the dairy cow in the very sensitive peripartal phase has not been addressed so far. In a retrospective cross-sectional study, we investigated the influence of prepartal maternal vaccination on uterine health and fertility of the periparturient dairy cow. Herd record data from 73,379 dairy cows from 21 farms located in Eastern Germany, together with on-site-collected survey data, were analyzed via (generalized) linear mixed effects regression models and random-forest machine-learning algorithms. A total of 57,166 peripartal periods without prior vaccination, distributed along 16 farms, and 63,228 peripartal periods on 13 farms with prior vaccination are included. Farm management-related factors proved to be most influential for uterine health. Vaccinated cows have higher milk yields, but also higher rates of postpartal uterine diseases (retained placenta, acute metritis), as well as lower non-return rates compared to non-vaccinated cows. In sum, we provide evidence that prepartal maternal vaccination can be regarded as a potential immunomodulatory measure to modulate physiological processes and the response of cows towards threads.

Evaluation of simulators for training of artificial insemination in cattle

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In Germany, the first practical training in artificial insemination (AI) must take place on suitable phantoms in courses for own stock-inseminators and insemination officers [TierZDV, 2021]. The aim of the ongoing study was to evaluate the simulators "Bovine Theriogenology Model" (VSI; Canada), "Bovine Breeder™ artificial insemination simulator" (BBS; Realityworks; USA) and "Smart Repro Cow" (SRC; Germany) by course participants. Fifteen participants were divided into three groups (n = 5 each) according to their experience level. Each group received AI training on one simulator. Thereafter, they practiced on live animals over two days. After each insemination intent, the course instructor confirmed the location of the insemination device in the animal by transrectal palpation. Subsequently, the participants practiced AI on all three simulators and evaluated them using a questionnaire. In 30.9% of all cases (26/84), the course instructor confirmed that the insemination device was in the uterine body or uterine horn (= success). The SRC simulator group accounted for 57.7% of the successes (15/26), VSI = 15.4% (4/26), and BBS = 26.9% (7/26). On a scale of 1–6 (1 = no realistic feeling at all; 6 = very realistic), the haptics of the internal organs were rated as follow: SRC = 5.3 points, VSI = 4.5 points, and BBS = 3.8 points. Most respondents (13/15) preferred the SRC

simulator as a training model. Due to training on the SRC simulator, participants were more successful on live animals than others. Concluding, based on the results of this study, the SRC simulator was evaluated with the best haptics of the internal organs and rated as the preferred training model for AI.

Studies on the applicability of sex steroid measurement in milk and vaginal cytology for non-invasive monitoring of ovarian function in New World camelids

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Determining a suitable time for mating is difficult in New World Camelids because females ready to conceive do not show specific external estrous symptoms or unequivocal behavioral changes. Thus, the utility of measuring sex steroids in milk and vaginal cytology as practical, non-invasive ways to monitor ovarian activity were investigated. A total of 10 alpacas (Al) and 9 llamas (Ll) were sampled from the time of birth to 4 weeks postpartum. Concentrations of progesterone (P4) and estradiol-17ß (E2) in blood and milk were measured using radioimmunoassays (RIA) after sample extraction with organic solvents. For the determination of total estrogens (TE), samples were hydrolyzed with β-glucuronidase/arylsulfatase prior to extraction. In TE-RIA, the antiserum used exhibited a high cross-reactivity against endogenous estrogens. Vaginal swabs were evaluated following procedures routinely practiced in bitches. In Al and Ll, estrogen concentrations were significantly lower in milk compared to blood (E2 in blood: Al: 11.1 ± 2.9 pg/ml, Ll: 14.6 ± 5.0 pg/ml; E2 in milk: Al: 6.2 ± 3.1 pg/ml, Ll: 7.9 ± 5.8 pg/ml; TE in blood: Al: 120.2 ± 26.9 pg/ml, Ll: 143.7 ± 45 pg/ml; TE in milk: Al: 57.1 ± 16.1, Ll: 75.9 ± 34.6 pg/ml) whereas the differences between Al and Ll were not significant. Significant correlations between estrogen concentration in blood and milk were found in Ll (E2: $p < 0.0001$; $r = 0.475$; TE $p < 0.0001$, $r = 0.526$) but not in Al. Results from correlation analyses between blood estrogen concentrations and cytological parameters suggest that vaginal cytology may be a potentially useful method in the breeding management in Ll but not in Al. However, further evaluation is needed to reach a definite conclusion.

Shotgun proteomics in blood plasma: the search for readily accessible candidate biomarkers of sperm quality in Holstein Friesian bulls

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Early and sustainable selection of breeding bull calves until their first semen collection is an important tool on artificial insemination (AI) centers. Individual biomarkers are needed for early and successful semen production ability and subsequent high and continuous semen production capability. Aim of the study was a non-targeted serum proteomics analysis in Holstein Friesian bulls. Bull calves (n = 102) selected by AI centers based on their genomic breeding value and semen production performance were followed for two years. Plasma samples of bulls showing a low performance persistency (LP, n = 10) and bulls with a very good performance persistency (HP, n = 14) were randomly selected for proteomics. Samples were prepared and analyzed by a UltiMate 3000 RSLC Nano HPLC system + Orbitrap Exploris 240 mass spectrometer. MaxQuant was used for the bioinformatic analysis. The reference database used was Bos taurus UniProtKB unreviewed. Seven plasma proteins were upregulated (Coagulation factor XIII A chain, Matrix metallopeptidase 3, Olfactomedin like 1, Alpha-1-antitrypsin, Apolipoprotein B, Transforming growth factor beta, Serotransferrin) and ten downregulated (Elastin, MBL associated serine protease 1, Nephronectin, Golgi membrane protein 1, G protein subunit beta 1, Peptidase D, Glutathione peroxidase, Peptidoglycan-recognition protein, Pentraxin family member, Apolipoprotein B). Most interesting candidate is the antioxidant glutathione peroxidase because it is essential around sperm cells balancing ROS production.

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Prevalence of antimicrobial resistance in the canine vaginal flora pre and post TÄHAV legislative amendment (preliminary data)

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Microbiological sampling of the canine vaginal flora is a routine part in breeding soundness examination. As many breeders fear infectious infertility, they often demand prescription of antimicrobials. The rising number of nosocomial infections makes responsible use of antimicrobials more important than ever. Antimicrobial susceptibility testing results from sent-in samples from a diagnostic laboratory were retrospectively analyzed. Two periods, 2015 to February 2018 (P1; pre TÄHAV amendment), and March 2018 to 2021 (P2; post TÄHAV amendment) were compared. Results for *E. coli,* β-haemolytic Streptococci, *Staph. intermedius* group (SIG) and *Enterococcus spp.* were analyzed. Antimicrobial susceptibility against penicillin (P), ampicillin/amoxicillin (AM), amoxicillin-clavulanic acid (AMC), cefalexin (CX), cefovecin (CV), enrofloxacin (ENR), marbofloxacin (MAR), pradofloxacin (PDF), gentamicin, tetracyclines (TC) and trimethoprim-sulfamethoxazole (SXT) were evaluated. For *E. coli* (n = 5209), a decrease in resistance was found for CV, ENR, MAR, PDF, TC and SXT ($p < 0.001$) but an increase for CX ($p < 0.0001$) between P1 and P2. For SIG (n = 4157), a decrease was identified for P, AMC, CX, CV, MAR, PDF ($p < 0.05$), an increase for AM and TC ($p < 0.01$). β-haemolytic Streptococci (n = 4009) showed decreased resistance for TC ($p < 0.05$), but increased resistance for PDF ($p < 0.05$). In *Enterococcus spp.* (n = 818), an increase was found for P ($p < 0.001$) and a decrease for PDF ($p < 0.005$). Continuous monitoring of resistance development and correlation with prescription practices is needed to develop strategies to further reduce antimicrobial resistance of bacteria.

Insights into the canine vaginal microbiome in proestrus stage: a next-generation sequencing approach

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The vaginal microbiome of bitches and its correlation with fertility is poorly understood. Among dog breeders and veterinarians, *Mycoplasma* spp. are often considered as species responsible for infertility. However, scientific evidence is scarce. Knowledge of the vaginal microbiome composition is crucial to avoid misuse of antimicrobials. Previous studies investigating the canine vaginal microbiota mainly relied on standard cultivation. We examined the vaginal microbiota of 79 healthy breeding bitches presented in proestrus for pre-breeding examination using Next-Generation Sequencing (NGS) and assessed fertility data. NGS analysis revealed numerous microbiota in all samples, belonging to 32 different phyla, some of which were found in every sample (Bacteroidota, Firmicutes and Proteobacteria). Additionally, Fusobacteria (96%) and Actinobacteria (96%) were regular findings. Alpha-Diversity (Shannon-Index) and Species Evenness (Pilou-Index) ranged from 1.14 to 5.64 and from 0.22 to 0.89, respectively. On the genus level, taxa detected using NGS approach were e.g. *Escherichia* spp. (100%), *Bacillus* spp. (100%), *Haemophilus* spp. (95%), *Streptococcus* spp. (91%) and Pasteurella spp. (77%), similar to bacterial cultivation. Of particular note is the high incidence (70%) of *Mycoplasma spp.* in this healthy population with no statistically significant differences between pregnant and non-pregnant animals. The sequencing approach provides deeper insights into the vaginal microbiome of the healthy proestrus bitch, but further analysis is necessary to define potential candidate pathogens regarding infertility among the vaginal microbiota.

Impact of the environment on metabolic parameters of newborn piglets and their vitality

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Larger litters are associated with a greater number of low birth weight piglets, which have a reduced vitality and die at a disproportionally high rate. The aim of the study was to show the influence of the housing system and a birth induction on metabolic parameters related to piglet vitality. Therefore, sows were housed in farrowing crates (1 m^2) or farrowing pens (6 m^2). Half of the sows in each farrowing system were induced with 0.175 mg cloprostenol and 70 µg carbetocin. The other sows farrowed spontaneously. In total, 96 German Landrace piglets of three birthweight classes (800–1100 g, > 1100–1500 g, > 1500 g) were studied during the neonatal adaptation period and blood samples were taken at 0.5–6 hours, day (d) 1 and d 4 postpartum. In piglets, key factors of energy utilisation (e.g. blood glucose, triglycerides) were quantified. Results show that hypotrophic piglets had the lowest and hypertrophic piglets the highest blood glucose levels (4.1 ± 2.8 mmol/l vs 6.0 ± 2.8 mmol/l) at 0.5–6h of life ($p < 0.001$). Neither sex nor birth induction, but housing of the sow in pens, were found to effect negatively the glucose-levels at different ages ($p < 0.05$). Hypotrophic piglets had a lower body temperature than hypertrophic piglets within the first six hours after birth (36.3 ± 1.8 °C vs 37.7 ± 0.7 °C, $p < 0.001$). Piglets from induced birth had a lower body temperature than those from natural birth (36.9 ± 1.6 °C vs 37.5 ± 1.0 °C, $p < 0.05$). Overall, hypotrophic porcine neonates may be more predisposed to a hypoglycaemia-hypothermia complex influenced by housing system and birth induction.

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Bacterial detection in antibiotic-free boar semen portions under routine storage conditions

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In semen portions used for Artificial Insemination (AI), responsible use of antibiotics (AB) is required to minimize resistances and thus to contribute to the One Health approach. The aim of this study was to examine the presence of bacteria in routinely produced boar semen doses in order to identify targets for antimicrobial concepts. For this purpose, 233 ejaculates from 16 European AI stations were extended in antibiotic-free Androstar® Plus medium (Minitüb, GmbH Tiefenbach). Samples in routinely used Beltsville Thawing Solution (BTS) extender with gentamicin served as control. Bacterial culture on sheep blood agar after 144 h semen storage at 17 °C revealed bacterial counts > 10^3 CFU/ml in 25.8 % of the AB-free samples. Among these, 14 different bacteria species were found in 57 semen samples, of which 86.4 % belonged to the Enterobacterales, mostly *Proteus species* (n = 13 samples), *Providencia species* (n = 12 samples), and *Serratia marcescens* (n =19 samples). Sperm agglutination was increased, and motility was reduced compared to controls ($p < 0.05$) only in samples with > 10^7 CFU/ml *Serratia marcescens* (n = 19) or *Klebsiella oxytoca* (n = 3). In 90.1% of the control samples, no bacteria were detected (< 10 CFU/ml) indicating the high efficiency of gentamicin. In conclusion, *S. marcescens* and *K. oxytoca* are bacterial species of high concern for sperm longevity at 17 °C long-term semen storage. They should be the primary target for any antimicrobial strategy in porcine AI.

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Arnold-Chiari malformation and associated anomalies in a stillborn dicephalic Angus calf

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A two-year-old, primiparous Angus heifer was presented with dystocia due to a full-term, deceased, and malformed calf in posterior presentation with apparent arthrogryposis and ascites that excluded vaginal delivery. Cesarean section revealed a dicephalic female calf with spina bifida and umbilical hernia, which was submitted for pathological investigation. Main findings were a dicephalus with two necks and double formation of the thoracic organs (two tracheae, two left and two right lungs, two hearts, and two esophagi ending in one reticulorumen). Furthermore, spina bifida in the lumbar region and arthrogryposis of both hind legs were present. In both heads, a dorso-ventral compression of the skull, platybasia, and enlargement of the foramen magnum was apparent and confirmed by x-ray examination. Furthermore, a prolapse of the caudal cerebral hemispheres underneath a hypoplastic cerebellar tentorium and an invagination of the cerebellar vermis through the foramen magnum was found in both skulls, indicating Arnold-Chiari malformations (ACM) in both heads. Since malformations of the central nervous system (CNS) in cattle can be caused by intrauterine infections with teratogenic viruses, like bovine viral diarrhea (BVD) virus, an exclusion of viral infections is always recommended in calves with CNS malformation. However, in the described case, a virological investigation did not confirm a teratogenic infectious etiology, such as Schmallenberg, blue tongue, bovine herpes or BVD virus. Although a predisposition for ACM is reported especially in the Angus breed, its sporadic incidence has little economic impact on livestock producers.

Development of a four-color flow cytometric assay for the assessment of plasma membrane remodeling and cholesterol efflux during bovine sperm capacitation

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Our experiments evaluated the incorporation of two probes, that target the phospholipidic scrambling and cholesterol depletion in the plasma membrane, in a four-color flow cytometric assay for the assessment of bovine sperm capacitation. The Violet Ratiometric Membrane Asymmetry Probe F2N12S and BODIPY-cholesterol were combined with reporters of plasma membrane integrity (propidium iodide) and mitochondrial membrane potential [cyanine dye DiIC1(5)] to quantify the degree of phospholipid scrambling (expressed by the ratio of orange/green F2N12S fluorescence) and cholesterol efflux (expressed by the shift of BODIPY-cholesterol signal) in sperm diluted with a capacitating (Tyrode's solution supplemented with HCO^3; CAP) vs control medium (CON). Single cryopreserved sperm samples produced by four bulls were split in CAP and CON aliquots and flow cytometrically assessed within 30 min post-thaw (0 h) and after 2-hour incubation at 37 °C (2 h). Already at 0 h, CAP sperm showed a loss of plasma membrane integrity and mitochondrial function against CON sperm by 32.49% and 40.12%, respectively. A right shift of the BODIPY-cholesterol fluorescence was detected in the CAP vs the CON sperm (up to 99.41%, 134.15% and 82.25% for the total, viable and dead sperm population, respectively). An increase of the F2N12S orange/green ratio at 0 h and 2 h was mainly observed for sperm with damaged plasma membrane and/or compromised mitochondrial function. This is the first report of the above-described staining panel as a method to assess plasma membrane changes during capacitation in bull sperm.

Grants: This study was funded by Swissgenetics, Switzerland.

Zinc – essential molecule for mitochondrial function and trigger of acrosomal destabilization in viable porcine sperm

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Sperm cells are precisely orchestrated by various molecules and ions. Our aim was to evaluate excess and insufficiency of free zinc ion effect on sperm cells' physiology. To this end, spermatozoa stored for 24 h at 15 °C in Beltsville Thawing Solution (n = 3 boars) were separated from the extender by density gradient centrifugation (20% Percoll) and subjected to 15 min incubation (38°C) in a Zn^{2+}-free HEPES-buffered medium (control). Concentration series with $ZnCl_2$

(10–1000 µM) or TPEN, an intracellular Zn^{2+} chelator (0.1–1000 µM), were used to either elevate or lower free intracellular Zn^{2+}-levels. The impact on sperm viability, acrosome integrity and mitochondrial function was assessed. One millimolar extracellular Zn^{2+} increased intracellular Zn^{2+}-levels 1.8 ± 0.6-fold compared to the control samples (n.s.). Viability remained at a high level (control: 89.1 ± 3.7 %; 1 mM Zn^{2+}: 89.3 ± 5.9 %). At the same time, the viable, PNA-positive sperm population rose from 4.7 ± 4.2 % to 64.4 ± 15.3 % ($p < 0.05$). This destabilizing effect was specific for ZnCl2 and could not be induced by either $CaCl_2$ or $MgCl_2$. Chelating free intracellular Zn^{2+} levels (1 µM TPEN) reduced in 2 of 3 animals the fluorescence intensity of JC-1 aggregates by > 90% compared to the controls. Although sperm were viable at 1 µM TPEN (90.7 ± 1.3%) the percentage sperm with high mitochondrial membrane potential was virtually zero for two of the three boars. Our preliminary data highlight the importance of regulating extra- and intracellular free Zn^{2+}-levels for acrosomal stability and mitochondrial function.

A one health issue: Hyperestrogenism and hyperandrogenism in dogs, cats and children due to secondary exposure

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Both children and pets that have close physical contact with users of topical hormone replacement therapy can develop clinical symptoms of exogenous hyperestrogenism or hyperandrogenism. Clinically, secondary hyperestrogenism in dogs presents predominantly with disorders of the oestrus cycle and pyometra, feminisation in males (pendulous prepuce, reduced penis and testicle size), teat hyperplasia and dermatological signs. Cats also display disorders of the oestrus and teat hyperplasia but predominantly show behavioural signs such as vocalisation, hyperactivity and urine marking. In children breast budding and breast masses in prepubertal females, precocious puberty, gynaecomastia and breast masses in prepubertal males following unintentional secondary exposure to estradiol spray/gel were reported. The product information of these products contain uniform information throughout Europe that children should not come into contact with the part of the body on which the medicinal product have been sprayed or applied (EMA 2022). Exogenous hyperandrogenism may be underdiagnosed in veterinary medicine. Reports due to endogenous hyperandrogenism as well as toxicological tests indicate that the symptoms in dogs and cats could be mainly behavioural signs, but also urogenital, dermatological, and hepatic. Dogs and cats may show virilisation. A close dialogue between the medical and veterinary professions in line with the One Health concept is needed to further improve drug safety of topical hormone preparations.

Endometrial and cervical cancer impair tubal morphology and function

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It has been hypothesised that the human fallopian tube may be the organ of origin for high-grade serous ovarian cancer. However, little is known about the role of the tube in endometrial and cervical cancer. To this end, the aim of this study was to investigate the effects of cervical and endometrial cancer on oviductal microarchitecture and ciliary beating (CBF) using light microscopy, scanning electron microscopy and quantitative digital live cell imaging. Tubal samples (0.5 cm) from patients with endometrial cancer (EC, n = 14), cervical cancer (CC, n = 8) were examined and compared to control patients (n = 10) after routine ovariohysterectomy. As shown by scanning electron microscopy, the inner tubal surface of CC and EC patients showed fusion of the tubal folds and areas of necrotic cells with loss of cilia. Quantitative analysis showed that the thickness of the primary folds was significantly increased in CC and EC patients. In the oviductal epithelium of patients with EC and CC, the number of ciliated cells was significantly decreased, whereas the number of protruding cells and basal cells was significantly increased. CBF was significantly decreased in CC patients. EC patients with a favourable prognosis (grade I adenocarcinoma) had a significantly lower CBF compared to those with a poor prognosis (high-grade serous carcinoma). Taken together, our studies show that tubal structure and function are profoundly affected by endometrial and cervical cancer. Detailed knowledge of these effects provides the basis for establishing new cancer staging criteria, thus providing valuable tools for refining prognosis in these patients.

Variation of semen quality of rams and bucks during the year

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In small ruminants seasonal and aseasonal breeds exist. In aseasonal breeds mating is performed throughout the year whereas in seasonal breeds mating is mainly in autumn and winter. Independently of seasonality, various factors can have negative effects on the semen quality (fever, heat, stress, diseases). Records from the AI-center D-KBSZ-003-EWG between 08/2017-08/2023 were evaluated. Semen was weekly collected and analyzed for volume, progressive motility, density and total sperm count. Data from seasonal breeds (East Frisian Milk Sheep (EFMS), Alpine Goat (AG)) and aseasonal breeds (Lacaune sheep, Boer goat) were compared. Each year was divided into spring (Mar.–May.), summer (Jun.–Aug.), autumn (Sept.–Nov.) and winter (Dec.–Feb.). Ejaculate volume of the AG was significantly higher in spring than in winter. In summer, on the other hand, the volume of the EFMS was higher than in the rest of the year. Regarding motility, there were no significant differences between breeds and seasons. Density and total sperm count were subjects to high animal-specific variation throughout the year and were highest across breeds in spring and summer. Unlike comparable studies, there were no significant differences in semen quality of seasonal and aseasonal breeds throughout the year. This can be mainly explained by the constant temperature in the AI-center even in summer. In addition, rams become shorn twice a year to avoid heat stress. Moreover, only males with a consistently good libido and excellent sperm quality are used for semen collection.

Development of an ovine dystocia simulator for obstetrical training

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The incidence of pre-weaning lamb mortality is estimated at 10–30% worldwide. More than 70% of lamb deaths occur within three days of parturition. Fetal malpresentation accounts for the majority of ovine dystocia cases, followed by fetopelvic disproportion and other fetal causes. Dystocia is a major contributor to stillbirth and perinatal mortality, leading to significant economic losses and partly posing a welfare problem. Training opportunities in obstetrics for farmers and veterinary students have frequently contributed to a significant improvement in lambing management. The use of dead lambs is common but potentially hazardous with regards to biosecurity and potential transmission of zoonotic agents such as *Coxiella burnetti*. Obstetrical simulators are a standard training aid in human medicine and have increasingly gained importance in veterinary training. Veterinary universities have progressively developed "skills labs" within recent years, aiming at providing a wide range of training opportunities using models and simulators. No commercially available ovine dystocia simulators are however available to date. To fill this gap, a lamb model was developed providing realistic flexibility of limb joints and the head, a realistic birth weight, and a characteristic body shape. Eye sockets allow lifelike manipulation of the head. The materials used for building the fetus, uterus, birth canal and pelvic bones allow the use of lubricant and are washable. This newly developed simulator thus allows repeated and safe training of diagnosing and managing fetal malpresentation in sheep.

Variability of oxygen consumption rate in cryopreserved bovine sperm

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A detailed characterization of cryopreserved sperm quality is crucial for evaluation of its

fertility. Parameters that are routinely used to assess sperm quality are motility, plasma membrane and acrosome integrity (PMAI) and DNA integrity but little information exists on metabolism of sperm. Therefore, our aim of this study was to establish a test for measurement of oxygen consumption rates (OCR), to investigate its variability in cryopreserved bovine sperm and to relate it to routinely measured parameters like rapid motility (RM), PMAI, and DNA fragmentation index (%DFI). Sperm ejaculates from 24 bulls (17 Brown Swiss, 4 Holstein, 2 Silian and 1 Simmental) were measured for PMAI and %DFI using flow cytometry. RM was measure using computer-assisted semen analysis. After thawing, sperm was selected using density gradient centrifugation. Sperm pellets were re-suspended in assay medium and seeded in culture plates. Basal oxygen consumption rates were measured using the Agilent Seahorse XFp analyzer for a duration of 1 hour, and given as average, standard deviation and coefficient of variation (CV), using Pearson's for correlation coefficients. Basal oxygen consumption rates averaged 14.29 ± 3.44 (CV 0.24) O_2 pmol/million sperm/min, while PMAI was 52.92% ± 10.09 (CV 0.19) and rapid motility and %DFI were 26.03% ± 11.89 (CV 0.45) and 3.99% ± 2.26 (CV 0.57), respectively. No significant correlations were found between OCR and PMAI (r = 0.27, p = 0.21), RM (r = 0.02, p = 0.94) and %DFI (r = –0.15, p = 0.48). Since there is a sizeable variability in OCR across samples with no correlation to other routinely measured sperm parameters, ongoing studies will aim to link OCR with sperm fertility.

Computer assisted analysis of canine vaginal cytology

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Vaginal cytology belongs to the routine gynecological examination procedures in the female dog. The stage of the estrus cycle, inflammation, neoplasia, and other conditions can be diagnosed using this technique in daily practice. It has been shown, however, that the determination of vaginal cell and the interpretation of the findings are subjective and heterogeneous. Therefore, new definitions of cells and a tutorial were developed to standardize determination of vaginal cells based on their size, appearance, size of nucleus, and sings of cornification. Based on this tutorial, a pilot project with the aim to assess vaginal cells using AI was conducted. In a first step, cell type specific algorithms (CTSA) were developed which identify the cells (parabasal cells, intermediate cells, superficial cells, squames), erythrocytes and neutrophils on digitized glass slides. The CTSAs decide whether a cell matches with a given cell type or not. With the help of the open- source bioimage analysis software QuPath™ 2900 cells per cell type were annotated. The vaginal cells were taken from 54 vaginal smears of 41 bitches in different stages of the estrus cycle. To train the algorithm, 2100 cells per cell type were used. The remaining 800 cells per cell type were used for validation. The accuracy of the CTSAs were between 82.8% and 99%. The not correctly identified cells were reviewed by humans and these results are being fed into the algorithms with the aim to improve the accuracy. Next step will be to combine the CTSAs into one single process. The future development of AI could potentially lead to more reliable results than the assessment by the human eye.

Eligibility of a rapid cooling test to identify boars with chilling sensitive spermatozoa

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Preservation of boar semen at 5 °C instead of the conventionally used temperature at 17 °C efficiently inhibits bacterial growth and yields high fertility. However, some boars might not be eligible for hypothermic semen preservation due to the enhanced chilling sensitivity of their spermatozoa. The aim was to identify chilling sensitive boars by a rapid semen cooling test. Semen volumes of 90 ml (n = 31 boars) diluted split-sample with Beltsville Thawing Solution (BTS) and Androstar Premium (APrem) were cooled to 5 °C after adaptation for 24 h at 17 °C and examined as rapid test. Control samples in APrem were slowly cooled to 5 °C [Paschoal et al. 2020]. At 72 h, rapid cooling in BTS resulted in 41 ± 23.4% motility with no correlation to control (p = 0.22). Rapid cooling in APrem showed 78.8 ± 13.1% motility and was positively correlated to the control (r = 0.69, p < 0.05). Sperm motility in control samples of three boars (9.7%) were below minimal values for the use in artificial insemination (< 65%), of which two were identified by the rapid test. These boars also had enhanced morphological abnormalities. One boar with high motility was wrongly identified as chilling sensitive by the rapid test. In conclusion, the rapid cooling test was not useful to identify chilling sensitive boars. Furthermore, most of boars are eligible for the hypothermic semen storage, especially when their semen is normospermic.

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Analysis of bedding materials used in boar artificial insemination centers

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The spermatogenesis process is very sensitive to external changes, therefore the aim of this study was to examine influences of different bedding materials (wood shavings, hemp, linen, spelt husks, and regional wood shavings) on sperm quality and quantity of 40 randomly selected breeding boars (8 boars per group, age: 2.35 ± 1.23 years). For this trial, 40 fresh and Beltsville Thaw Solution (BTS) – extended semen samples (160 samples in total, 4 consecutive ejaculates per boar, 32 samples per group) were collected weekly for the duration of four weeks. An extended range of spermatological methods (e.g., computer-assisted sperm analysis, flow cytometry) was used for semen analysis. Bedding temperature was assessed at two depths (5 and 15 cm) at the start and end of the bedding period. Generalized linear mixed models for each sperm characteristic were calculated. There were no significant differences between groups in any sperm characteristic (p > 0.05). However, for most spermatological parameters, we found significant differences between sampling weeks (p ≤ 0.001). We postulate an influence of bedding temperature on sperm characteristics, as temperature results were highly significant for both depths (p ≤ 0.001). Generally, temperatures were higher at 15 cm depth of the material and at the start of the bedding period. Linen and spelt husks exceeded maximum levels for pesticide residues (VO (EG) No. 396/2005). Based on the pesticide results, we strongly recommend a quality analysis of any new bedding material before use in artificial insemination centers.

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Exogenous FSH/LH does not affect in vitro embryo production in German Fleckvieh heifers

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The use of exogenous FSH is common in OPU-IVP programs to improve embryo production in cattle, but up to now there is no information about its effect on embryo production in dual-purpose German Fleckvieh cattle. Therefore, twelve German Fleckvieh heifers (age 484 ± 62 days) underwent two OPU-IVP cycles in a latin square design, with one cycle serving as a non-treated control cycle (CC) and the other cycle stimulated with exogenous FSH/LH (FLC). In all heifers and cycles follicular waves were synchronized using 0.5mg of cloprostenol on Day 0, followed by 10µg of buserelin on Day 2. Subsequently, half of the heifers were randomly assigned to receive FSH/LH (four injections of 75 IU each of FSHp and LHp, 12 hrs apart on Days 4 and 5) before the first OPU. The remaining heifers received FSHp/LHp before the second OPU. OPU was done on Day 7 after start of synchronization and seven weeks apart. Oocyte recovery rate (CC: 58.9% vs FLC: 59.1%; p = 0.98) as well as number of oocytes (CC: 13.2 vs FLC: 14.8; p = 0.71) were similar in CC- and FLC-cycles, resp. Although there was a trend (p = 0.07)

towards a higher number of excellent-quality oocytes in FLC (CC: 2.9 ± 0.6 vs FLC: 7.9 ± 2.4), cleavage rate (CC: 83.6 ± 3.8% vs FLC: 84.6 ± 4.2%, p = 0.80), blastocyst rate (CC: 40.3 ± 4.5% vs FLC: 42.9 ± 6.8%, p = 0.93), and the total count of transferable embryos (CC: 4.8 ± 1.1 vs. FLC: 4.1 ± 0.9, p = 0.67) were similar among groups. In conclusion, we did not find benefits on in-vitro embryo production by using exogenous FSH/LH in German Fleckvieh heifers.

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Lower gene expression of ZNF613 in the right uterine horn of genetically selected cows

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In bovine genetic association studies, the zinc finger protein 613 (ZNF613) gene on bos taurus autosome 18 (BTA18) has been found to be strongly associated with gestation length, calving ease and productive lifetime of cows. Aim of this study was to quantify gene expression of ZNF613 in endometrial tissue obtained from three locations (uterine corpus, right uterine horn and left horn) of cows (n = 36), that had been genetically selected for divergent BTA18 haplotypes. The samples were taken 39 ± 4 days after calving, when the animals were sacrificed 24 or 96 hours after intramammary challenge with *Escherichia coli* or *Staphylococcus aureus*, respectively. Gene expression of ZNF613 was determined via RT-qPCR and quantified via standard curve technique. Data were analyzed with a robust linear mixed effects model executed with R 4.0.4 including the individual animal as random effect. ZNF613 expression neither differed between the two haplotype groups, nor along gestations length or between pathogen challenge groups (p > 0.1). However, ZNF613 expression was significantly lower in the right uterine horn compared to the corpus and left horn (p = 0.021). The reportedly higher frequencies of right horn pregnancies might be related to this finding. Since the physiological function of ZNF613 in the bovine uterus is still unclear, further studies on this promising biomarker are required.

Metabolic stress in early pregnancy – insight from the ApoE$^{-/-}$ rabbit model

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Apolipoprotein E (ApoE) is a key molecule in lipid metabolism that mediates the uptake of plasma lipoproteins. Whether it plays a role in early pregnancy and embryonic preimplantation development is not yet known. In this work, the function of ApoE was investigated in female ApoE$^{-/-}$ rabbits in comparison to wild types rabbits at day 6 of pregnancy. ApoE$^{-/-}$ rabbits were fertile, with a lower number of blastocysts observed on day 6 of gestation, indicating an impaired reproductive phenotype. ApoE$^{-/-}$ rabbits showed a hypercholesterinaemia and hypertriglyceridemia accompanied by a systemic shift of liver metabolism towards the ß-oxidation of free fatty acids. In addition, a lower amount of free fatty acids was observed in the plasma and the expression of oxidative stress markers was increased in liver tissue, emphasizing an adversely affected metabolism. The hyperlipidaemic metabolic state influenced the reproductive tract organs of the ApoE$^{-/-}$ rabbits. In the ovary, the lipid metabolism shifted towards gaining energy mainly from fatty acids degradation and inducing oxidative stress in ovary tissue. In the endometrium, the expression of signal molecules important in cholesterol metabolism were affected accompanied by a strong decrease of mitochondrial transcription factor A (mtTFA). Taking together, an ApoE deficiency imbalances the lipid metabolism and stress defence in the ovary and endometrium with consequences for maternal fertility during preimplantation period.

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Comparison of imaging techniques for the diagnosis of choanal atresia in alpaca crias

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Choanal atresia is a malformation described in newborn alpacas. It is a unilateral or bilateral membranous, cartilaginous or osseous closure of the choanae, which obstructs airflow during breathing. The condition has been diagnosed primarily postmortem. In this case report, the possibility of diagnosing choanal atresia by contrast radiography, endoscopy and computed tomography (CT) is compared. Postmortem, the diagnosis was verified by necropsy as gold standard. The examinations were performed on three newborn alpacas. One of the alpacas had no abnormalities in the upper airway, one had unilateral complete choanal atresia, and one had a bilateral complete choanal atresia. Endoscopic examination was performed with a rigid endoscope through the nostrils in the sedated neonate. The examination was limited by the physiological narrowness of the nasal cavities. In the presence of choanal atresia, occlusion of the choanae could be visualized. Physiological choanae could not always be reliably visualized. During contrast radiography, contrast medium was applicated in the nostrils in the sedated neonate. In the presence of choanal atresia, the contrast medium remained in the nasal cavity; in the presence of physiological choanae, it was also distributed in the oral cavity. Contrast radiography could reliably show the presence of choanal atresia. CT examination was able to show the three-dimensional structure of the upper airway and thus the formation of the choanae very well. CT was the gold standard of intra vitam diagnosis. Contrast radiography also provides reliable diagnosis with less effort.

Effect of Mito-TEMPO on cryogenic viability and gene expression of bovine IVP derived blastocysts

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Addition of Mito-TEMPO increased developmental rates and reduced ROS levels of in vitro produced (IVP) embryos whereas cryoviability has not been investigated, yet. This study investigates the effect of Mito-TEMPO supplementation to maturation and culture media on freeze-viability and gene expression level of selected candidate genes of IVP derived embryos. Maturation of oocytes was performed in TCM199-medium supplemented with (treatment) or without (control) 1 µM Mito-TEMPO. In vitro fertilization was conducted by routine procedures. Presumptive zygotes were cultured for up to 8 days in SOFaa again with or without 1 µM Mito-TEMPO. Subsequently, Day 7 blastocysts were vitrified using „BO-VitriCool"-media emerging the Cryotop- vitrification system. Warming of these blastocysts (Bo-Vitri-Warm-media) was followed by post-warming culture for 72 hrs to determine viability, expansion and hatching rates. Blastocysts from both experimental groups were analyzed in terms of relative expression of a set of apoptosis- and oxidative stress-related candidate genes via qRT-PCR. As a result, supplementation of maturation and culture media with Mito-TEMPO significantly (p < 0.05) enhanced expansion and hatching rates after warming and significantly downregulated BAX, GPX1, GSTA4 and SOD2 transcripts in blastocysts whereas the expression of CAT was significantly upregulated. These results confirm our hypothesis that the antioxidant Mito-TEMPO reduces cryo-induced damage after vitrification. That finding goes along with differential expression of candidate gens playing a role as antioxidants as well as downregulation of pro-apoptotic genes.

Establishment of a method to study the growth dynamic of *Leptospira* in extended boar semen

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Leptospirosis is a zoonotic disease that may cause reproductive disorders in sow farms. With the advent of antibiotic-free semen preservation, the risk for *Leptospira* growth in semen used for artificial insemination is under debate. The aim was to establish a method for the analysis of *Leptospira* survival and growth in extended boar semen. A microdilution method was adapted for susceptibility testing of Leptospira, which allows testing the growth dynamic of different *Leptospira* serovars, strains and isolates in semen extenders with and without antibiotic additives. The viability of *Leptospira* was detected by quantitative analysis of their metabolic activity using a special reagent (alamarBlue®), and was compared with microscopy and long-term culture in agar. Due to the slow growth (doubling time for pathogenic leptopires adapted to in vitro culture is estimated 6–8 h) and resulting long incubation of leptospires compared to most other bacteria, the risk of contamination is quite high and the test system must be carefully controlled. Initial results showed that there is a threshold for survival and in-vitro growth of leptospires of about 10^7 leptospires per ml. Semen extenders without antibiotic additives already showed a growth-inhibiting effect during storage at 17 °C, but leptospires seem to be able to recover when re-exposed to a *Leptospira*-specific culture medium (EMJH medium, Becton Dickinson). Further studies are underway to validate the system for properly understanding the survival and growth dynamics of leptospires in extended boar semen.

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Can breed be a risk factor for feline dystocia? – A retrospective analysis

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Data on the frequency of dystocia in cats are often inaccurate and vary from rare to 20%. The data found are not always comparable because the frequency of dystocia was determined differently depending on the literature reference. Although thorough analyses have yet to confirm it, some experts assume that the incidence is breed-dependent. Therefore, this study focuses on investigating the relationship between breed characteristics and dystocia. Data from 166 cats with dystocia who visited our clinic between 2000 and 2021 were analyzed in terms of maternal breed characteristics. The breed distribution of cats with dystocia was as follows: Abyssinian (n: 3, 2%), Bengal (n: 1, 1%), Birman (n: 4, 3%), British Shorthair (n: 27, 16%), British Longhair (n: 2, 1%), Curly rex (n: 1, 1%), European Shorthair (n: 77, 46%), Carthusian (n: 8, 5%), Maine Coon (n: 9, 5%), Mongrel (n: 8, 5%), Norwegian Forest Cat (n: 4, 2%), Persian (n: 7, 4%), Russian Blue (n: 1, 1%), Savannah (n: 1, 1%), Siamese (n: 5, 3%), Siberian Cat (n: 1, 1%), not specified (n: 7, 4%). It could not be shown that individual breeds are particularly frequently affected. The British shorthair cat was one of the most common breeds suffering from dystocia (16%). The percentage of purebred cats was 54%. Those cats are affected as often as non-purebred cats. No conclusion regarding a breed predisposition for the development of dystocia can be drawn from the present results. This is certainly due to the breed composition of the clinic's referral population. More detailed studies on the topic will be needed in the future.

Effect of bovine sperm selection using microfluidics compared to density gradient centrifugation on sperm quality

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In this study, the effect of microfluidics (MF) compared to density gradient centrifugation (DGC) on bovine sperm quality was investigated. Eighteen cryopreserved semen samples produced from seven bulls were used. Each sample was divided into two aliquots post-thaw for preparation with either a MF (Fertile Plus™, KOEK EU®, Germany) or DGC (BoviPure™, Nidacon, Sweden). Sperm function was assessed prior to aliquoting (C) and after selection with either the MF or DGC. Rapid motility (RM%) was assessed with computer-assisted sperm analysis. The subpopulation of cells with high esterase activity (HEA%), intact plasma membrane and acrosome (PMAI%), low intracellular Ca^{2+} (LCA%), and high mitochondria membrane potential (HMMP%) was quantified simultaneously using flow cytometry. Cells with high DNA fragmentation index (%DFI%) were assessed with the flow cytometric Sperm Chromatin Structure Assay™. Sperm selected by MF showed higher values of RM% (73.3% ± 17.4% vs 29.5% ± 12.8%), HEA%-PMAI%-LCA%-HMMP%-sperm (50.8% ± 15.3% vs 29.1% ± 11.8%) and lower values of %DFI (1.7% ± 2.8% vs 7.5% ± 13.8%) compared to those selected by DGC ($p < 0.001$). The improvement after MF was more pronounced for samples with lower RM, PMAI, and HEA%-PMAI%-LCA%-HMMP%-sperm before sorting ($b < 0$, $p \leq 0.05$), but independent ($p > 0.05$) from the %DFI% of C-samples. Sperm traits did not differ ($p > 0.05$) between DGC and C. In conclusion, microfluidics substantially improves the functional status of bovine sperm compared to density gradient centrifugation.

Effect of low-dosage iron dextran injections on selected hematological blood parameters in neonatal calves

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Objectives To prevent anemia, neonatal calves are often supplemented with iron as a first day booster treatment. The effects of such treatments have been examined for high dosages of iron dextran but not for an often-used dosage in the field. Our study therefore examined the effects of low-dosage iron dextran injections in neonatal calves on hematocrit (Ht), red blood cell counts (RBC), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), serum iron (FE) and serum ferritin during the first 10 days of life.

Materials and Methods Blood samples from 40 neonatal Holstein-calves were examined. Calves were randomly allocated to a treatment group (T) and a control group (CON). Treatment consisted of an iron dextran injection (10 mg Fe^{3+}/kg i.m. once at day 1 of life). Blood samples were taken once daily until day 10.

Results Low-dose iron dextran injections enhance serum iron but have no effect on serum ferritin levels in neonatal calves. Serum ferritin and serum iron concentrations peak at day 2 (ferritin) and day 3 (iron) of life independently from supplementation. RBC, Hb and Ht are significantly correlated to each other without an effect of treatment (RBC vs Ht: $p < 0.0001$; ρ (T) = 0.88; ρ (CON) = 0.78) (RBC vs Hb: $p < 0.0001$; ρ (T) = 0.87; ρ (CON) = 0.98). MCV, MCH and MCHC showed similar timely changes between the groups. MCH and MCHC increased; MCV values declined.

Conclusions Low-dose iron dextran enhances the serum iron concentrations only transiently. Serum iron and serum ferritin increase within the first 10 days independently from iron supplementation.

Passive diffusion of fatty acids into granulosa cells may contribute to metabolic stress-induced ovarian follicle dysfunction

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Fatty acids (FA) are essential nutrients and signal transducers for cellular functions. It was suggested that the uptake of FAs should be closely regulated to ensure that normal cellular functions are not impeded by the uncontrolled uptake of FAs. It has been shown that CD36 and FATP1 mediate FA uptake into various mammalian cells, but the role of

these genes has not been studied in ovarian granulosa cells (GCs). In this study, we asked whether CD36 and FATP1 are involved in FA uptake in GCs and contribute to lipid droplet (LD) accumulation as excessive lipid accumulation in ovarian in GCs hampers steroido-genesis and viability. We treated cultured GCs with 200 µM of oleate (OA), palmitate (PA), and stearate (SA) and found that OA induced significantly higher LD accumulation compared to PA and SA. CD36 expression was proportional to LD content, as OA-treated cells showed higher CD36 expression than other treatments. On the contrary, FATP1 expression is decreased in OA treatment, suggesting FATP1 might not be involved in LD accumulation in GCs. The lipidomics analysis showed that FAs are accumulated as triglycerides and cholesterol esters, and the chemical composition of LD depends upon the FA treatment. We then performed CD36 knockdown to verify its role in FA accumulation. Results showed that neither LD accumulation nor triglyceride content was affected by the knockdown of CD36, indicating CD36 does not mediate LD accumulation in GCs. Based on these data, we propose that ovarian follicle dysfunction in metabolic stress conditions could be due to the uncontrolled passive diffusion of FAs into GCs.

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Pro-inflammatory interleukin 1β affects the function of decidualized dog uterine stromal (DUS) cells – first insights into immune-mediated effects in the canine placenta

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Maternal-derived decidual cells play a pivotal role in the success of pregnancies in dogs, as they exclusively express the nuclear progesterone (P4) receptor (PGR) in the canine placenta. The disturbance of PGR activity within these cells impairs their function, leading to pregnancy termination. Recent studies using an *in vitro* model of canine decidualization with dog uterine stromal (DUS) cells, revealed yet unexplored immunoactive features of decidual cells, including their expression of the IL1β receptor IL1R1. During pregnancy maintenance, immune signaling, including the expression of the pro-inflammatory IL1β, remains decreased in the canine placenta, unlike the pro-inflammatory events observed during pregnancy termination. On the other hand, uterine infections, a major cause of pregnancy loss in dogs, are associated with local inflammation including increased IL1β availability. However, immune-mediated intra-uterine mechanisms remain largely unexplored. Herein, the effects of pro-inflammatory signaling in decidual cells were explored by exposing decidualized DUS cells to recombinant IL1β. The transcriptional availability of factors associated with decidual function (i.e., decidualization markers) including I*GF1, PTGES* and *PTGS2/COX2* was decreased by different tested IL1β concentrations ($p < 0.001$). Moreover, IL1β also reduced the mRNA availability of the gap junction component *CX43* and extra-cellular matrix components *ECM1* and *TIMP2* ($p < 0.01$). These findings suggest that IL1β disrupts the function of decidual cells and, consequently, placental tissue homeostasis, potentially contributing to pregnancy loss (abortion) in bitches during infectious events.

Corpus luteum activity in dairy cows under heat stress

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Heat stress has a detrimental effect on the fertility of dairy cows. The objective of this study was to assess the effect of heat stress (HS) on the activity of the corpus luteum (CL). The CL development of 22 clinically healthy Holstein-Friesian cows was monitored on a commercial dairy farm in Argentina. After 50 days (d) postpartum, the cows were assigned to an ovulation synchronization programme followed by artificial insemination (AI). The CL were examined weekly by ultrasound (US) from the day of the first AI until 21 d after AI, and US images were analysed. The CL size (CLS) was the average diameter of the two greatest perpendicular CL cross-sections. The CL blood flow (CLBF) was assessed as percentage of CLBF area to CL area (objective score) and on a 3-point scale using power doppler images (subjective score). Climate data, ambient temperature and relative humidity, were recorded every 15 minutes on the farm. The time and amplitude of the temperature humidity index (THI) exceeding the threshold of 68 were calculated, with THI values ranging from 42 to 93. The CLS ranged from 10.2 to 47.7 mm, 9.1% (2/22) of the cows had small CLS (< 15 mm), and 13.6% (3/22) of the cows had no CL. The objective and subjective CLBF scores were positively correlated ($p < 0.05$). In the first week following AI, a negative correlation was observed between the subjective CLBF score and CLS ($r = –0.46$, $p < 0.05$) and HS ($r = –0.682$, $p < 0.05$). The correlation between the objective CLBF and HS tended to be significant in first week after AI ($r = –0.409$, $p < 0.1$). This preliminary data suggested that HS negatively affects CL activity in dairy cows.

Age-related changes of mitochondrial metabolism in adipose-derived stromal stem cells (ASC)

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Adipose tissue is a multicellular, metabolic and endocrine organ maintained throughout life by adipose-derived stromal stem cells (ASCs). In a previous study in ageing rabbits, we demonstrated a reduction of the ASCs plasticity in correlation with a higher chronological age of the ASC donor. The proliferation and differentiation of adult stem cells depend on cell metabolism and the associated mitochondrial functions. In order to investigate the role of changes in mitochondrial metabolism in stem cell ageing, the metabolic properties of six primary subcutaneous (inguinal subcutis, sASCs) and visceral (renal adipose capsule, vASCs) ASC lines from young (16 weeks) and reproductive pre-senescent (> 108 weeks, old) rabbits were compared using the Seahorse Mito Cell Stress Test (XF96, Agilent, mitochondrial respiration). The metabolic capacity of subcutaneous ASCs differed depending on age of the donor with a significant increase of maximal mitochondrial respiration and spare respiratory capacity in old sASCs. In addition, an increase of nonmitochondrial oxygen consumption in old sASCs is indicating higher cellular stress levels. Visceral ASCs showed no alterations in their mitochondrial parameters depending on age of the donor. Our data suggest that age-related disturbances in cell metabolism effect the stem cell quiescent state depending on localisation in the body and demonstrating the importance of the stem cell niche, for mesenchymal stem cell ageing.

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Vaginal leiomyoma in a goat expressing the progesterone receptor

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Goats experience a considerably longer lifespan in hobby husbandry compared to their time spent in agricultural use. Concomitantly, the risk of tumorous diseases in the genital tract increases with age. One of the most frequently described genital tumors in small ruminants is leiomyoma. Currently, our knowledge of the pathogenesis of this tumor in goats is limited. This includes the question whether these tumors express steroid hormone receptors. Here we describe the case of a vaginal tumor in a seven-year-old Angelo-Nubian goat. The goat was presented due to bloody vaginal discharge. The gynecologic examination revealed a singular pedunculated mass in the vagina measuring approximately 3 cm × 4 cm × 4 cm. After epidural anesthesia, the mass was removed electrothermally. The pathological examination identified a leiomyoma and the presence of nuclear progesterone receptors (PGR) was demonstrated immunohistologically. One year after the surgery the goat was in good general health. To the best of the authors‘ knowledge, this case report presents the first evidence of PGR presence in a vaginal tumor in a goat. This finding prompts the need for more extensive

research to explore the significance and functions of PGR in the development of genital tumors in goats. Drawing parallels from research in canines, where castration is recognized as a preventive measure against vaginal tumors, it raises the possibility that castration could similarly serve as a preventive strategy for genital tumors in goats. Further investigations in this area are warranted to establish potential preventive measures.

Nanoplastics impair bovine granulosa cell functions *in vitro*

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Plastic pollution poses a serious threat to humans and animals. However, there are still numerous unexplored aspects that need to be investigated to elucidate the pathophysiological consequences of exposure to nanoplastics within the mammalian system, particularly with respect to reproductive functions. This study aims to investigate the effects of different concentrations (5, 25, and 75 µg/mL) of nanoplastics (NPs) with a size of 100nm and made of polystyrene in an 8-day serum-free culture of bovine granulosa cells (GCs). The study focuses on assessing cell viability, steroidogenic activity and cytokine production. The first results showed a significant decrease in GCs viability (mean ± SD) as measured by the MTT assay, compared to the control (Ctrl). The viability was found to be only 25.25% ± 6.15%, 24.91% ± 7.46% and 27.64% ± 8.05% ($p < 0.0001$) for the concentrations of 5, 25 and 75 µg/ml of NPs, respectively. Instead, 17β-Estradiol (mean ± SD) secretion, measured using ELISA, was significantly enhanced by NP treatment. After 8 days of culture, we detected 2.0 ± 0.3, 14.2 ± 3.3 ($p < 0.05$), 17.6 ± 1.7 ($p < 0.01$), 63.8 ± 1.0 ng/ml ($p < 0.0001$) for Ctrl, 5, 25 and 75 µg/ml of NPs, respectively. Since, so far, little is known about the possible interaction of NPs with the available assays, we aim to explore different analytical approaches to obtain more reliable and robust results. Additionally, further studies, especially related to inflammatory and molecular status are needed.

Spermatogonial stem cell dynamics: Effects of slow-release GnRH agonist implants in canine testes

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Slow-release GnRH agonist implants (SRI) are commonly used in small animal medicine to temporarily downregulate testicular endocrine and germinative function. Treatment effects are fully reversible, but the influence of SRI on spermatogonial stem cells (SSC) and their niche has not been investigated yet. We studied SSC markers PGP9.5, FOXO1, and DMRT1 during downregulation and subsequent recovery. Healthy male dogs were treated with an azagly-nafarelin SRI for 5 months. The SRI was removed surgically (week, W, 0), and 3-5 dogs were castrated at W0 and 3, 6, 9, 12, and 24 weeks after SRI removal. Similarly to W0, 3 dogs each received a buserelin (PG) or deslorelin SRI (SG) and were castrated. Testes of 5 adult (CG) and 3 juvenile (JG) male dogs served as controls. Immunopositive spermatogonia (spg) in 20 round seminiferous tubules were counted at 200x magnification. Statistical analysis (GraphPad Prism) was performed comparing 1. downregulated testes (W0, PG, SG), CG and JG (Group, G_{down}) as well as 2. testes during recovery (W0, 3, 6, …, 24) and CG (G_{recov}). Numbers of PGP9.5, FOXO1 and DMRT1-positive spg differed significantly between groups in G_{down} (ANOVA; all: $p<0.001$) with lowest numbers in JG, followed by W0, PG and SG. In G_{recov}, group comparison revealed significant differences (ANOVA; PGP9.5/DMRT1: $p < 0.01$, FOXO1: $p < 0.05$) with lowest numbers in WO but counts in late recovery not differing from CG. In conclusion, treatment with a SRI reversibly affects the number of PGP9.5, FOXO1 and DMRT1 positive SSCs.

Grants: A. Vasetska receives a scholarship of the Philipp Schwarz Initiative, AvH Foundation.

Placental characteristics of different pig breeds and their relationships to litter size

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Pig breeds, modern lines or indigenous breeds, differ massively in litter sizes. In most breeds a notable increase of litter size was achieved during the last decades. The available uterine space and surface for each fetus as well as the capacity of the uterus becomes limited in large litters. This study focusses on the expression of the placenta in different pig breeds. Placentas of 18 litters of Hungarian Mangalica (HM), 19 litters of German Saddleback (GS), 24 litters of German Landrace (GL) and 14 litters of Yorkshire sows (Y) were weighted, the length measured and the placental efficiency per litter calculated. Only first and second litters were used, and piglet data collected. All breeds differ significantly in the number of total born piglets (HM 7.9 ± 1.9; GS 11.5 ± 3.2; GL 14.9 ± 2.9; Y 19.6 ± 2.5; Tukey: $p < 0.001$), life born piglets (HM 6.8 ± 2.3; GS 11.2 ± 3.1; GL 13.9 ± 2.8; Y 17.2 ± 2.6; Tukey: $p < 0.01$) and litter weight (HM 11.9 ± 2.7 Kg; GS 15.2 ± 3.9 Kg; GL 19.5 ± 4.2 Kg; Y 26.8 ± 3.2 Kg; Tukey: $p = 0.037$). But, while a clear negative correlation between litter size and piglet weight were seen ($r = –0.3$ to $r = –0.4$), the mean piglet weights (HM 1.5 ± 0.3 Kg; GS 1.4 ± 0.3 Kg; GL 1.3 ± 0.2 Kg; Y 1.4 ± 0.1 Kg) and mean placental weights (HM 234 ± 73 g; GS 223 ± 74 g; GL 261 ± 56 g; Y 220 ± 31 g) were comparable between breeds. Accordingly, the placenta efficiency (fetal weight/placental weight) were comparable between most breeds (HM 6.86 ± 1.5; GS 6.42 ± 1.8; Y 6.33 ± 0.9) but significantly lower in GL sows (5.23 ± 0.9; Dunn's: $p > 0.05$). Our findings suggest that the realization of high fertility in sows was mainly achieved by increasing uterine capacity/space, but further improvements could be done by selection for placental efficiency.

In vivo dynamics of polymorph nuclear neutrophils and pro-inflammatory factors in the bovine oviduct

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Dynamic changes within the oviductal microenvironment are the prerequisite for embryonic development and fertility. The objective of this study was to examine dynamics of polymorph nuclear neutrophils (PMN) and the mRNA expression of selected genes in the oviduct of living cows. From healthy heifers (n = 6) and cows (n = 7) mini-cytobrush (mCB) samples were taken from both oviducts during the follicular (FOL) and luteal phase (LUT) by transvaginal endoscopic sampling. The mCB were used for cytological examination to determine the percentage of PMN and for mRNA expression analysis. RT-qPCR was performed for selected pro-inflammatory factors and glycoproteins. The mean PMN percentage tended to be greater during LUT (5.2%) than in FOL (2.1%; $p = 0.062$). The Oviduct-Specific Glycoprotein-1 mRNA expression was 2-fold greater during FOL than in LUT, whereas the pro-inflammatory factors *IL1B, CXCL1, CXCL3* and *CXCL8* were downregulated during FOL. Strong positive correlations were observed between PTGS2 and CXCL8 and IL1B ($r = 0.6–0.9$), suggesting a cytokine regulated prostaglandin synthesis in the oviduct. Heat map and Principal component analysis illustrated grouping of the pro-inflammatory factors and PMN according to their known biological functions. Our results consolidate the idea that the oviductal microenvironment switches to an immune-tolerant state during FOL, which might be important for sperm survival and early embryonic development. This novel approach for repeated oviductal sampling opens promising perspectives for recording detailed pathophysiological processes in the bovine oviduct.

The mRNA expression of MUC4, MUC5B and MUC16 in bovine endometrial cells is estrous cycle-dependent with higher expression in periovulatory phase compared to luteal phase

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Mucins (MUC) are part of innate immunity in the female reproductive tract. Membrane associated MUC like MUC1, MUC4, MUC12, MUC15, and MUC16 mainly participate in cell signaling. Secreted MUC such as MUC5B and MUC6 contribute to barrier functions by forming the mucus gel covering many epithelia. This study investigated mRNA expression of the mucins in bovine endometrial epithelial cells (BEEC) at different stages of the estrous cycle. BEEC were collected from uteri harvested at the slaughterhouse at three regions (corpus, ipsi- and contralateral horns) and classified into one of the following four groups (n = 8 per group): post ovulation (day 1–5), early luteal (day 6–12), late luteal (day 13–18), and pre ovulation (day 19–21) phase. Total RNA was extracted and subjected to RT-qPCR. mRNA expression of MUC1, MUC6, MUC12, and MUC15, was not influenced by endometrial region or stage of estrous cycle. mRNA expression of MUC4, MUC5B and MUC16 was significantly higher ($p < 0.05$) in the periovulatory phase compared with the luteal phase. mRNA expression of MUC4 in all regions of endometrium was significantly higher in the post-ovulatory phase compared with early and late luteal phases. mRNA expression of MUC16 in ipsi- and contralateral horns was significantly higher in pre-ovulatory phase compared with late luteal phase. Results support the hypothesis that BEEC can play an important role in the immune function of uterine microenvironment by expressing MUC around ovulation to facilitate gametes transport and counteract possible invading pathogens.

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Influence of a combined treatment with two commensal lactobacillus strains on uterine health and fertility of dairy cows

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Bacterial contamination of the genital tract during parturition can result in inflammation of the uterus leading to subclinical or clinical endometritis. These diseases have a high prevalence in dairy cows and can lead to subfertility. The aim of this study was to evaluate the influence of an intrauterine application of *Lactobacillus buchneri* and *Lactobacillus ruminis* on uterine health, reproductive performance, and endometrial mRNA expression of selected pro-inflammatory factors. Dairy cows with clinical endometritis were investigated on day 24–31 postpartum (pp) and cytobrush samples were taken. Afterwards a suspension of *L. buchneri* DSM 32407 and *L. ruminis* (LAC) (n = 32) or Placebo (PLA) (n = 30) was applied intrauterine. A second sampling was performed after three weeks (d 45–51 pp). After extracting mRNA, the expression of pro-inflammatory factors was analyzed by RT-qPCR and the fertility data have been evaluated. The cows from the LAC and PLA groups were each inseminated for the first time on d 100 (± 15) pp. Median days to conception were numerically but not significant shorter in the PLA group than in the LAC group (PLA 126 ± 27 vs. LAC 135 ± 39 DIM). A reduced IL8 mRNA expression was observed in the LAC cows that were healthy on d 45-51 pp compared to the other groups on this day. Apart from this, significant differences in mRNA expression could be detected depending from health status, but not from the treatment. In conclusion, intrauterine treatment with a combination of *L. buchneri* and *L. ruminis* did not have beneficial effects on uterine health and fertility.

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A case of interrupted labor in a Van cat

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Physiological birth in domestic cats encompasses the period during which fetuses are expelled from the birth canal through uterine contractions, cervical dilatation, and abdominal contractions. This process varies in cats but generally takes about 8 hrs. The term „interrupted labor" refers to cases where the physiological birthing process it is not entirely completed, despite an apparent completition of parturition. In cats, birth typically terminates within a few hours (approximately 90%) during the second stage of labor. Labor has rarely been recorded to be completed within 24–48 hrs (around 0.3%). In our clinic, a Van cat was presented with incomplete labor 54 hrs after the first delivered kittens. To the best of our knowledge, such a case has not been previously documented. The 2-year-old Van cat was presented with complaints of difficult labor. In this cat, two live fetuses were born normally at 45-minute intervals, but labor ceased after the second kitten, as identified by the cat owner. After a lengthy wait, the cat was admitted to our clinic as an emergency at the 54th hour. The cat's full medical examination revealed that all health parameters were normal. Ultrasonographic and radiological examinations identified numerous live fetuses (200/minute heart rate) in the uterus. Consequently, an emergency cesarean section was performed in consideration of the elapsed time, leading to the successful delivery of six healthy kittens. In conclusion, this case demonstrates that Van cats may experience interrupted labor because of hyperfetosis (8 fetuses) and subsequent uterine inertia. More research is required to confirm this hypothesis.

Evaluation of estrogen receptor alpha (ERα), neuropilin 1 (NRP1) and e-cadherin expression in normal, hyperplastic, and neoplastic endometrium

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The roles of estrogen receptor alpha (ERα), neuropilin 1 (NRP1) and e-cadherin in the progression from benign to malignant endometrium is not fully elucidated. Paraffin blocks of 97 specimens with the diagnoses of proliferative endometrium (n = 23); endometrial hyperplasia without atypia (n = 16) or with atypia (n = 13); and endometrioid type endometrial cancer of low (n = 20) or high grade (n = 25) were immunostained with ERα, NRP1 and e-cadherin. All specimens of endometrial hyperplasia without atypia showed a more intense staining for ERα compared to high-grade endometrial cancer ($p < 0.05$). In all specimens, proliferative endometrium stained better than endometrial hyperplasia with atypia for NRP1 ($p < 0.05$). A stronger staining with NRP1 was evident in both low- and high-grade endometrial cancer compared to proliferative endometrium ($p < 0.001$) and endometrial hyperplasia with and without atypia ($p < 0.001$ and $p < 0.05$, respectively). Staining with e-cadherin was stronger in proliferative endometrium, endometrial hyperplasia with atypia and low-grade endometrial cancer compared to high grade endometrial cancer ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively). Gradual increment in NRP1 expressions in malignant transformation of pre-malignant endometrium, accompanied by lower ERα and E-cadherin expressions in high grade cancerous endometrium might indicate that estrogen induced gradual NRP1 increase in early carcinogenesis may lead to the downregulation of E-cadherin expression in high grade malignant endometrial lesions.

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The Giessen testis biopsy repository: A histopathologic-clinical analysis

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Infertility affects about 10% of couples in industrial countries with about 50% being caused by male factor infertility. In 10% of

infertile men, semen analysis reveals azoospermia, which can be stratified in obstructive and non-obstructive, the latter demonstrating impaired spermatogenesis. Detailed histological evaluation of testicular biopsies is essential to delineate underlying pathologies and patients' prognosis regarding testicular sperm extraction (TESE). Here, biopsies of a Giessen cohort of azoospermic men (n = 545; aged 18–58y.) are evaluated using hematoxylin and eosin stains. Semi-quantitative score count analysis (% of tubules containing elongated spermatids, value 0–10) is performed, and presence of immune cell infiltrates, Leydig cell appearance etc. recorded. Via immunohistochemical analysis, we will investigate changes in biopsies' cellular composition in selected patients. Preliminary results show that a Sertoli cell-only phenotype is the most prevalent histopathological category (33.1%; score 0), followed by severe tubular damage (19.5%; score 0.1–1.0), hypospermatogenesis (19.5%; score 1.1–7.9), and spermatogenic arrest (8.6%; score 0). Intact spermatogenesis was found in 23.8% of cases (score 7–10). Despite considerable intra- and inter-organ heterogeneity, histological categorization significantly correlates with clinical parameters such as testicular volume and serum FSH. Deep phenotyping of our patient cohort linking histopathology to the clinical database will help to improve andrological diagnostic work-up and patient stratification in terms of male reproductive and general health as well as fertility prognosis.

Single cell sequencing reveals transcriptional response to TGFB1 and MFGE8 in equine endometrial fibroblasts

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In older horses, fibrotic degenerations of the endometrium are a major cause of subfertility. Fibroblasts, a heterogeneous cell type being descended from different precursor cells play a central role in disease development. Transforming Growth Factor Beta 1 (TGFB1) is a prominent pro-fibrotic factor in this context. In arteriosclerosis Milk Fat Globule Epidermal Growth factor 8 (MFGE8) seems to be a key player in the pathogenesis of fibrotic changes. Single Cell Sequencing allows for investigation of the entire transcriptome and comparison of treatment effects on different fibroblast subpopulations. We have used this technology to study the heterogeneity of in vitro cultivated endometrial fibroblasts from four different mares and the effects of treatment with TGFB1 and MFGE8 on the transcriptome. We found a clustering by TGFB1 treatment, reflecting the tremendous effect of this cytokine on fibroblast gene expression. This could not be seen for treatment with MFGE8, as transcriptional changes were more subtle. Ingenuity pathway analysis finds strong association for TGFB1 treatment of all clusters towards idiopathic pulmonary and hepatic fibrosis as well as wound healing signalling pathway. One cluster in the MFGE8 treated group displayed upregulation of PTEN signalling. PTEN plays a critical role in inhibiting cell proliferation and acts as a tumour suppressor and has previously been associated with the action of MFGE8.

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