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ABSTRACT

Common ethics issues of the application of fertility preservation technologies in different user groups

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Our project aims to present the first results of the ethical research within the BMBF-funded Interdisciplinary Junior Researchers Center for Fertility Protection (FePro-Ulm). The specific research task was to identify and analyse from an ethical perspective the issues of fertility preservation common for various user groups: children and adolescents, men with cancer, women with cancer, women with endometriosis, women wishing social egg freezing, and transgender people. Through a systematic literature search, 125 articles were identified and further analysed and discussed from the perspective of the four principles of bioethics. The identified issues related to the principle of autonomy were: enhanced reproductive autonomy, decisional pressure, and posthumous usage of stored reproductive material. The issues linked to the principle of beneficence involved the possibility of achieving genetic parenthood and the prevention of patients' regret. The issues related to the principle of non-maleficence comprised of the risks of misinformation, delayed therapy for the main disease, and nurturing false hope for future reproduction without a guarantee of success. The issues connected to the principle of justice included resource allocation questions, unequal access to fertility preservation technologies, and discrimination concerns. The added value of our research lies in building up a comprehensive picture of the ethics issues of fertility preservation that goes beyond oncofertility. The identified common ethics issues can further serve as key points of ethical relevance in the development of fertility preservation guidelines and protocols.

Clinical and hormonal characterisation of pseudopregnancy in camels

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Pseudopregnancy occurs when pregnancy symptoms appear without the presence of an embryo or fetus. Reasons, characteristics, and behaviours differ across species. Camel pseudopregnancy has yet to be described. The purpose of this study was to describe the phenomenon of pseudopregnancy in dromedary camels. The genital tracts of 58 female camels exhibiting pseudopregnancy were examined, and breeding histories were obtained. Serum levels of estradiol-17β (E2), progesterone (P4), and prolactin (PRL) were measured. Refusal to mate, and a stiffened posture with the head held high and tail curled upwards when approached by a male were signs of pseudopregnancy. Normal pregnancy-related mammary or abdominal changes were absent. Risks associated with pseudopregnancy were age and previous history of reproductive disorders. Only ~25% of the pseudopregnant camels had elevated serum P4 levels. Serum E2 levels did not differ significantly among pseudopregnant, pregnant, and cyclic camels. Serum PRL levels were significantly higher in pregnant camels than in pseudopregnant and cyclic camels. Finally, pseudopregnancy camels exhibit external signs of pregnancy, but are distinguished by the absence of udder edema, milk production, or elevated P4 or PRL levels. It has also been linked to a high incidence of other reproductive disorders, particularly in older camels.

Fertility indices of dromedary camels in Saudi Arabia

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Camels are important to the Saudi community. It is commonly reported that reproductive performance is relatively low. Little information is available on the reproductive performance, factors influencing it, and causes of infertility in dromedary camels in Saudi Arabia. This article addresses the most prominent fertility indices of dromedary camels in Saudi Arabia. Data were collected from 115 camel farms containing 7122 heads from different regions in the Kingdom of Saudi Arabia. The overall pregnancy rate (PR) was the dependent variable. The main effects in the model were: herd size, herder/camels ratio (H/C), manager experience (ME), male/females ratio (M/F), housing system (HS), length of the breeding season, age at first mating, and time of mating after parturition. Most herds mate female at the age of 4y (67%), less at 5y (26%) and 3y (7%). PR did not differ across ages. Female reproductive life extends for 20y during which she gives 9 calves. Most herds use male camels by

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5y (60%), less by 4y (26.1%) and 6y (13.9%). Male age did not influence PR. Few herds replace male every 5y (10.43%), while the majority (89.57%) kept males for life. Length of the breeding season (average 6.17 ± 0.19 months, range 3–9 months) did not influence PR. Pregnancy duration did not differ between parities. PR was approximately equal in camels' which mated early postpartum (≤ 3 months) or later (> 3 months). Calving interval was shorter for camels mated early postpartum. The more years of manger experience (> 10 year) the better PR. The fewer H/C ratio (1:< 25), the higher PR. Other studied parameters did influence the PR. Finally, to improve camel reproductive performance in Saudi Arabia, reproductive management, manger performance, H/C ratio, and the use of stud males should all be optimised. It is possible to reduce both mating age and calving interval.

Single-cell transcriptomics reveals distinct cellular and molecular changes associated with the regression of bovine corpus luteum

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The corpus luteum plays an essential role in regulating the reproductive cycle, fertility, and the maintenance of pregnancy. It undergoes dynamic molecular and structural changes during its lifespan in accordance with the luteotropic and luteolytic signals. This study uses single-cell RNA sequencing to investigate the cellular and molecular changes in the bovine CL at the mature and regressive stages. Mature CL samples ($n = 3$) were obtained on day 11 post-ovulation from heat-synchronised, ultrasound-tracked animals via transvaginal surgery. Regressive CL samples ($n = 3$) were harvested 8 h following an intramuscular PGF2α analogue injection also on day 11. CL tissues were dissociated using collagenase, and the single cells were purified using Ficoll gradient centrifugation. The mature and regressive samples were pooled in equal proportions for each stage, and single-cell libraries were created using 10X Genomics technology and sequenced on the Novaseq X Plus platform. The sequencing reads were analysed using Cell Ranger and R software packages. Integration of mature and regressing CL cell clusters was performed using the Seurat tool kit. Post-quality control, 14,919 cells from mature CL and 13,131 cells from regressive CL were analysed. The mature CL composition included 43% steroidogenic, 19% endothelial, 15% immune, and 23% fibroblast cells. In contrast, the regressing CL displayed a shift to 35% steroidogenic, 14% endothelial, 32% immune, and 18% fibroblast cells. The data uncover novel markers for luteal cell types and provide insights into the molecular and cellular transitions that drive luteal regression.

Changes in the incidence of anestrus and its clinical forms during two last decades in dairy cows in north-eastern region of Poland

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The aim of the study was to evaluate changes in the incidence of anestrous conditions in several dairy cow herds over the past 20 years in north-eastern Poland. In data set 1, 1215 crossbred cows (Polish Black and White x Holstein Friesian) calving 2003–2004 from 8 herds were used. The average milk yield was about 5000 kg per year. In data set 2, 942 Holstein Friesian cows calving 2023–2024 from 5 herds were used. The average milk yield was about 9000 kg milk per year. Anestrous cows were examined twice by ultrasonography in a 7–10 day interval between 50 and 60 days after parturition. In data set 1, anestrus was observed in an average in 33.1% of cows The main clinical form of anestrus was silent heat (44.0%), followed by ovarian afunction (43.3%), ovarian cysts (9.2%), prolonged luteal phase (2.1%) and persistent follicles (1.4%). In data set 2, anestrus was observed in 43.5% of cows. Silent heat was diagnosed in 41.0%, persistent ovarian follicles in 34.9%, ovarian cysts in 18.0%, ovarian afunction in 4.9% and prolonged luteal phase in 1.2% of anoestrous cows. There was a tendency towards higher incidence of anestrus ($p < 0.058$) in data set 2, compared to data set 1. The difference between the data sets in the ovarian afunction and persistent ovarian follicles was statistically significant ($p < 0.05$). The study showed that over the past two decades there has been an increase in the incidence of anestrus in dairy cows and changes in the proportion of its clinical forms, in particular in ovarian afunction and persistence of follicular structures.

Detrimental effects of 100 nm polystyrene particles could be observed in bovine granulosa cells accumulating particles in vitro

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In recent years, the hazardous potential of micro- and nanoplastics has been elucidated in several studies. While the majority of marine plastic contamination originates from land-based sources, the terrestrial environment has only recently come in to focus. The current knowledge on the effects of plastics on farm animals, particularly ruminants, is still limited. The objective of this study is to analyse the effects of the polymer polystyrene (PS) on our serum-free granulosa cell (GC) model in order to assess potential effects on reproductive physiology. Therefore, GCs were treated for 8 days (37°C, 5% CO_2) with different concentrations (5, 25 and 75 μg/mL) of 100 nm fluorescent PS particles. Previously, we demonstrated that the treatment had no significant effect on hormone secretion or gene expression. However, our observations indicated that PS was capable of accumulating within GCs, although not in a uniform manner throughout the cell culture. Thus, GCs

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were subjected to fluorescence-activated cell sorting (FACS) to separate PS-positive and PS-negative GCs. The gene expression of the sorted cells was then analysed by quantitative RT-PCR. The GC-specific markers *CYP19A1* and *FSHR* were markedly reduced in PS-positive GCs in comparison to PS-negative GCs. The expression of *RGS2* was similarly affected, with a lower abundance observed in PS-negative GCs. It seems reasonable to assume that the effects of PS on cell physiology are obscured by the GCs that do not incorporate plastics. Nevertheless, these results indicate that PS may have detrimental effects on GC physiology when accumulating within the cells.

Regional differences of (induced) rat vas deferens contractility and its implications for sperm propulsion

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Mature spermatozoa, stored in the most distal part of the epididymis (dEpi), are trans-ported to the urethra during emission. This process is often described as "by noradrena-line-induced powerful contractions of the vas deferens (VasD)", but not detailed further and not fully understood. In our already published results we could show equally strong emission-like contractions induced by either noradrenaline or oxytocin in dEpi.
For the first time, we have investigated the VasD in its entirety in toto and in longitudinal pieces of 3 mm each to find and define regional differences in contractility and substance responses using live-imaging combined with a novel analysis method + organ bath studies. From morphology and responsiveness, we grouped the results from the rat VasD into distinct comprehensive regions: 1st loop, pars epididymica (PE), pars libera and pars prostatica. In addition, we investigated regional histological differences.
In all VasD pieces, contractions were observed predominantly longitudinally while effects of the circular layers were nearly absent. Only in PE the response was similarly strong to dEpi while the rest of VasD, despite thicker and more intertwined smooth muscle layers, showed little responsiveness. Especially in toto data indicated that the strong contractions of dEpi and PE drive pump-like the expulsion of spermatozoa. In these two "emission-relevant" tissues (dEpi and PE) oxytocin induced very strong contractions, equally strong to noradrenaline in dEpi.
This confirms a role for oxytocin during ejaculation and opens up a new alternative therapeutic tool for ejaculatory disorders, including paraplegic patients.

Effect of timing of milk sampling on the results of the bacteriological and cytological milk examination in Holstein Frisian cows

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Mastitis is usually caused by bacteria which can be detected using a bacteriological examination of the milk. The gold standard is the examination of a sterile milk sample retrieved before milking. However, under certain management conditions (e.g., AMS milking) sampling before milking is challenging, and therefore samples are often collected after milking. Our aim was to assess the effect of timing of sampling (before or after milking) on the results of the bacteriological and cytological milk examination. Cows were sampled which had in the last monthly milk control a somatic cell count (SCC) in the milk of > 300,000 cells/mL ($n = 110$) and milk samples of four quarters were taken before and after milking. A cytological and a standard bacteriological (culture plates) examination was performed. In 17.3% of all samples bacteriological growth was detectable. Samples retrieved before milking had significantly more often a positive bacteriological result (60.1%) than samples retrieved after milking (39.9%). The somatic cell count was significantly lower in samples collected before milking (356.137 cells/mL) than after milking (661.806 cells/mL), and in samples with bacteriological growth this was even more evident. From this it can be concluded that bacteriological analysis is samples retrieved before milking bacteriological growth is more likely to be detected than after milking.

Relationships between metabolism of cryopreserved equine sperm determined by the seahorse analyser and their characteristics measured by flow cytometry and computer assisted analysis of motility

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The aims of the study were to investigate intra- and interindividual variabilities of sperm metabolism in cryopreserved equine sperm and relationships of metabolic parameters with other sperm characteristics. Three cryopreserved ejaculates from each of 21 warmblood stallions were examined. The quality of the semen samples was characterised using different assays during a period of 120 min after thawing every 15 min. The sperm metabolism was quantified using the Seahorse analyser by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Total motility of sperm was assessed by CASA. Using a flow cytometric multicolor assay, the percentage of sperm with an intact plasma membrane and negative acrosomal status, a high esterase activity, a high mitochondrial membrane potential, and a low intracellular Ca2+ level was determined. The SCSA was performed to determine the percentage of sperm with a high DNA fragmentation. There were high variabilities in OCR- and ECAR values within and between stallions (within: CVs: OCR: 22.83–93.03; ECAR: 29.46–90.16; between: CVs: 65.33; ECAR: 11.39). The variations of OCR- and ECAR values could only described by up to 30% (R2-values) by flow cytometric as well as CASA parameters were. In summary there are high intra- and interindividual variabilities in oxygen consumption as well as extracellular acidification in cryopreserved equine sperm. As the determined metabolic parameters cannot be predicted by conventionally determined sperm parameters the Seahorse Analyser provides new information about sperm quality.

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Factors affecting endometrial thickness and relationships between endometrial thickness and fertility in dairy cows

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In this study we examine the impacts of a clinical endometritis, parity, time after parturition and breed on endometrial thickness (ET) and effects of ET during diestrus on fertility in dairy cows. B-mode sonography was carried out on 200 cows to determine ET; 21% in the first, 46% in the second or third and 33% in the fourth or higher lactation. The cows belonged to four different breeds (Holstein Friesian: 45.5%; Brown Swiss: 30%; Red Holstein: 17%; Swiss Fleckvieh: 7.5%). Three puerperal controls (PC1: 4–5 weeks pp., PC2: 4–5 weeks pp., PC3: 8–9 weeks pp) were performed in 100 cows without (OE) and 100 with (ME) clinical endometritis at PC1. ME-cows showed higher ET values than OE-cows ($p < 0.001$). ET values were independent ($p > 0.05$) from time after parturition (PC1, PC2, PC3) if other parameters were not taken into account. Primiparous cows had lower ET values ($p < 0.05$) than cows with ≥4 lactations. Cows in the second and third lactation had by tendency ($p = 0.05$) higher ET values than primiparous animals. Red Holstein OE-cows had higher ET values ($p < 0.05$) than Brown Swiss OE-cows. No effect ($p > 0.05$) of breed on ET was noticed in ME-cows. Primiparous ME-cows with ET values of < 0.94 cm at PC3 showed with a sensitivity of 75% and a specifity of 94.4% a service period < 150 days. If OE-cows in the second or third lactation had an ET of < 0.89 cm at PC3, the sensitivity was 80% and the specificity 80.6% for the prediction of a service period of < 200 days. In conclusion, the endometrial thickness can be used to predict fertility if the factors presence of an endo-metritis, time after parturition, parity and breed are taken into account.

High concentrations of interferon-tau (IFN_τ) influence the expression of epithelial cell markers in bovine caruncular epithelial cells (BCEC) in vitro

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Pregnancy losses in dairy cattle can occur in every stage of gestation for a variety of reasons, but happen mostly during early embryo development prior to implantation and negatively influence the reproductive efficiency in dairy farms. The trophoblast-derived cytokine interferon-tau (IFN_τ) is specific to ruminants and acts not only as the pregnancy recognition signal, but also affects the trophoblast and uterine epithelium. We hypothesize, that IFN_τ alters the expression of epithelial cell components to prepare the endometrium for a successful implantation. As in our former study 10 ng/mL IFN_τ had no significant effect on epithelial cell markers in bovine caruncular epithelial cells (BCEC), BCEC were now stimulated with 100 and 1.000 ng/mL IFN_τ and compared to two control groups—serum-reduced medium and PBS. Via quantitative Real-Time PCR the mRNA expression of Ezrin (*EZR*), Cytokeratin 18 (*CK18*), E-Cadherin (*CDH1*), Occludin (*OCLN*) and Tight Junction Protein-1 (*TJP-1*) was examined. Additionally, the protein localisation (EZR, CK18 and CDH1) was investigated by immunofluorescence. Both IFN_τ concentrations significantly upregulated *EZR* ($p < 0.001$), *CDH1* ($p < 0.05$), *OCLN* ($p < 0.05$, $p < 0.001$) and *TJP-1* ($p < 0.05$, $p < 0.001$) compared to gene expression of the control groups in a dose-dependent manner. On the other hand, no differences could be observed concerning the protein localisation after IFN_τ-application. In conclusion, IFN_τ upregulates epithelial cell markers within BCEC only when applied in sufficient concentrations. Further investigations are planned regarding protein expression via Western Blotting.

Comparison of the steroidogenic activity of spheroids derived by luteinisation of different antral follicle cell types and mixtures of the domestic cat

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In this study, we compared the steroidogenic activity of spheroids obtained by luteinisation of different cell types/mixtures originating from antral follicles of domestic cats to identify a suitable luteal cell model with high progesterone (P4) production. In the first experiment, granulosa cells (GC), theca cells (TC) and a cell mixture from large (~3 mm) antral follicles (LAFC), were used. In the second experiment, cells from small (~1 mm) follicles (SAFC) and LAFC were compared. For each group, cells isolated from at least 3 animals were pooled and cultured in 3 technical replicates for 14 days in ultra-low attachment plates. Media were collected on days 2/3, 7 and 14 for P4 measurement by ELISA. RNA was isolated from cells before (day 0) and at the end of the culturing period (day 14). Quantitative PCR was used to measure the gene expression of various steroidogenic factors, collagen type I alpha 1 chain and vascular endothelial growth factor A. Although gene expressions for steroidogenic acute regulatory protein, side chain cleavage enzyme and 3-beta-hydroxysteroid dehydro-genase were in the same range in GC, TC and LAFC on day 14, the P4 production of LAFC was increased more than 40-fold in comparison to luteinized GC or TC alone. Furthermore, luteinized LAFC produced over 20 times more P4 than luteinized SAFC on day 14. We conclude that not only using an antral follicle cell mixture, rather than GC or TC alone, is better for obtaining a luteal cell culture model with high steroidogenic activity, but also that the size of the antral follicles used plays a role.
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How to train artificial intelligence systems for morphological assessment of spermatozoa in routine bull semen analysis: preliminary results

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Bull sperm morphology is assessed visually using bright field microscopy, which includes the evaluation of at least 200 sperms according to The Society of Theriogenology for Bull Breeding Soundness Evaluation (BBSE). The process is time-consuming and requires trained personnel to ensure consistent results. At present, the artificial insemination industry increasingly relies on genomic selection for young bulls, thus the need for a more standardised method of assessing semen quality is growing, especially for sperm morphology that influences semen freezing viability, survival and fertilisation capability. To address this, we are developing an Artificial Intelligence (AI) algorithm for automated classification of microscope-acquired images of spermatozoa. We are using neural networks, specifically YOLO networks (YOLOv8), which are based on convolutional neural networks (CNNs) able to learn and extract relevant features from complex visual data through segmentation of images. This allowed us to classify spermatozoa morphology and vitality (normal spermatozoa, primary and secondary abnormalities). We used a training dataset consisting of thousands of images, which have been evaluated by expert operators, labelled and annotated with bounding boxes to enable algorithm to learn (training phase). Results showed a performance up to 80% in accuracy, even if not for all the classes, and 85% in precision for the correct classification of spermatozoa morphology performed by the algorithm. In the future, the algorithm could be improved to achieve optimal performance for implementation into the field practice, thus permitting the standardisation of the analysis.

Evaluation of three protocols for ovulation synchronisation and timed insemination in arabian mares

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The present study aimed to evaluate 3 protocols for ovulation synchronisation and timed insemination in Arabian mares. Thirty nulliparous, reproductively sound and cyclic Arabian mares aged 4–7 years, weighing 350–450 kgs and averaged 3 body condition score were assigned for the study. The mares were examined for two successive cycles to ensure their behavioural and reproductive cyclicity before the designed treatment. Studied mares were assigned into three groups; group I (GPG, $n=10$) received 40 µg GnRH agonist (D0), 250 µg $PGF_2\alpha$ analogue (D7) and 40 µg GnRH agonist (D12); group II (G-P-PG-H (D19), $n=10$) treated with oral progesterone for 14 days, 250 µg PGF2α analogue on Day 9 and 2500 hCG on Day 19 and group III (PG-PG-H (D19), $n=10$) received 14 days interval-double injections of 250 µg $PGF_2\alpha$ analogue and 2500 hCG (D19). All animals were artificially inseminated 24 h after the end of each protocol. Animal were examined and bled daily to monitor the ovarian changes and estimate E2 and P4 levels. Compared to G-P-PG-H- and PG-PG-H-treated mares, GPG group mares had longer estrus duration, GnRH-ovulation and AI-ovulation intervals with larger DF. Conception rate was significantly lower (10%) for GPG compared to G-P-PG-H (50%) and PG-PG-H (50%) groups. It can be concluded that the long interval between insemination and ovulation among other factors affects the efficiency of ovulation synchronisation-timed insemination protocols in Arabian mares.

Doppler ultrasonography could be an important tool to detect the viability of the fetus in female dromedary affected by postcervical uterine torsion

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The detection of the fetal viability in camel is often difficult due to the passiveness of the fetal response especially during uterine torsion. Therefore, the aim of the present study was to evaluate the use of doppler ultrasonography of the vaginal artery to assess the viability of the fetus in postcervical uterine torsion compared with normal pregnancy. The waveform pattern of the vaginal artery was characterised in 18 pluriparous dromedaries with right, post cervical, high degree (over 90^0) uterine torsion (UT). As a control group, 17 pluriparous full term dromedaries were used (CONT). According to the viability of the fetus, the UT group was further categorised into two groups; UTL (uterine torsion with live fetus, $n=8$) and UTD (uterine torsion with dead fetus, $n=10$). All animals were examined to determine the site, degree and side of the torsion in the affected animals. Torsion was right in all examined camels. Only animals with high degree postcervical uterine torsion were included in the present study. The waveform of the vaginal artery was examined by the same examiner. Results indicated that pulsatility index (PI), resistive index (RI) and average blood flow velocities were greater ($p=0.02$) in UTD group compared with UTL and CONT groups indicating that doppler could be important for detecting the viability of the fetus when it is not accessible by palpation or B-mode ultrasonography. There is no significant difference between the waveform of the vaginal artery of UTL compared to CONT groups.

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Relevance of diseases and imaging of the thymus in canine neonates

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In contrast to human medicine, thymus diseases in neonates receive little attention in veterinary medicine. While ultrasound examinations of the thymus are a standard imaging procedure in human paediatrics, there is a lack of corresponding examinations in veterinary medicine. The objective of the study was to assess and describe the ultrasonographic appearance and localisation of the thymus in mature canine neonates. Therefore ultrasonography (Canon Aplio a CUS-AA000; Canon Medical Systems Europe B.V., Zoetermeer, Netherlands; 4.5–17 MHz linear transducer) was performed in ten mature puppies that deceased due to hypoxia during dystocia. To confirm the ultrasonographic findings autopsy was carried out afterwards. Then, ultrasonography was performed in one-day-old live puppies while being restrained in right lateral recumbency. The thymus was localised in the ventral part of the cranial mediastinum, next to the cranial margin of the heart (Margo ventricularis dexter). The ultrasonographic structure was rough and relatively homogeneous with scattered hyperechogenic foci. As a conclusion sonographic evaluation of the thymus in canine neonates is possible, though it is restricted by surrounding bone and lung and by strong defensive movements of unsedated patients. The findings obtained in this study should serve as a basis for identifying pathologic changes in the thymus and its sonographic appearance.

Comparison of RNA- versus DNA-based 16S rRNA V3-4 amplicon sequencing for the analysis of the low biomass bacterial microbiome in the equine uterus

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The uterine microbiome is recognised as a key player in endometrial receptivity. However, characterising a low biomass microbiome in uterine samples is challenging due to a high risk of contamination and a high background of host nucleic acids. This study aimed to establish a highly sensitive 16S rRNA V3-V4 amplicon PCR protocol for microbiome analysis starting from DNA or RNA isolated from cytobrush samples. RNA-based amplicon sequencing was compared to DNA-based 16S analysis because the higher copy number of rRNAs (=number of ribosomes) per bacterial cell vs. rRNA gene copy numbers (1–15 copies) should provide a much higher sensitivity. In addition, RNA-based 16S analysis mainly detects alive bacteria whereas DNA-based analysis also detects dead bacteria and free DNA. DNA and RNA were extracted from uterine cytobrush samples collected from 14 mares in estrus. The V3-V4 region of the 16S rRNA gene was amplified and sequenced (Illumina NextSeq 2000) resulting in 1–2 million amplicon reads per sample which were analysed using QIIME 2. The RNA-based analysis identified significantly more amplicon sequence variants (ASVs) and associated taxonomic units, indicating higher sensitivity. Although the overall microbiome composition was similar between the paired DNA and RNA samples, significant differences in alpha (Simpson, Chao1) and beta diversity were identified. Differential abundance analysis also revealed significant differences at all taxonomic levels. Overall, the findings indicate that combining RNA and DNA-based approaches provides a more complete representation of the uterine microbiome in the mare.

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Influence of glycemia on farrowing characteristics in a free farrowing system

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The farrowing process requires a high level of energy from the sow. The aim of this study was to evaluate the impact of blood glucose on farrowing kinetics.

In 147 sows, feeding time, peripheral glycemia of sows (measured in the ear vein) at the onset and at the end of parturition were measured.

A mean farrowing duration of 422 min ±130 min with 16.1 ± 3.6 total born piglets was observed. Glycemia at the begin of farrowing was 4.44 mmol/L ± 0.63 and rose to the end of farrowing to 4.72 mmol/L ± 0.79. In the linear model, the time between the last meal and onset of farrowing does significantly affect the farrowing duration ($p = 0.007$). Sows with their last meal before farrowing in the morning had lower glycemia levels (4.33 mmol/L) than those with their last meal in the evening (4.51 mmol/L) ($p = 0.009$) but tended to have shorter farrowings (397 min vs. 439 min) ($p = 0.053$).

At 49.7% of the farrowings, obstetrical interventions (manual extraction of piglets and administration of oxytocin) was performed. The farrowing process with assistance lasted 59 min longer than without ($p \leq 0.001$) and tended to have 0.5 more stillborn piglets ($p = 0.054$), but there was no difference in blood glucose levels between sows. Older sows (> 4 litters) had lower blood glucose levels at the start of farrowing, received significantly more farrowing assistance (62% vs. 37%), had almost twice the number of stillborn piglets (1.42 vs. 0.78) than younger sows. Glycaemia had no significant effect on farrowing duration and therefore this parameter cannot be used to predict sows with dystocia.

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Evaluation of macroscopic parameters and their influence on the sexual cycle of the urogenital tract in slaughtered sows

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Evaluation of the urogenital tract in slaughtered sows is a valuable diagnostic tool. Therefore, the aim of the study was to evaluate macroscopic parameters and their relationship to the sexual cycle of the urogenital tract in slaughtered sows.
The following parameters were assessed in 105 sows within 40 h after slaughter: Weight of the empty urinary bladder and the reproductive tract (from the ostium urethrae externum to the ovary). Furthermore, length of vagina, cervix and the uterine horns were measured. The sexual cycle was evaluated on the surface of the ovary and the mucosa of the uterus and the urinary bladder was assessed macroscopically for signs of inflammation (accumulation of fluid, severe redness and/or pus).
The average weight of the uterus was 1376.0 ± 623.3 g and the median was 1311.0 g (355.0–3213.0 g). The mean weight of the bladder was 245.3 ± 100.8 g and the median was 226.0 g (87.0–847.0 g). An inflammation in 43.8% of all uteri was detected and in 30.5% of all bladders. In the linear multiple regression model, a significant influence of oestrus ($p = 0.019$), dioestrus ($p \leq 0.01$), and the signs of inflammation of the uterine mucosa ($p \leq 0.01$) on the uterus weight was detected. Furthermore, a significant influence of the dioestrus ($p \leq 0.01$), the signs of inflammation of uterine mucosa ($p \leq 0.01$), as well as the bladder mucosa ($p \leq 0.01$) on the bladder weight was detected.
The study provides current data on the urogenital tract of slaughtered sows. Considering the sexual cycle, the weight of the uterus and the urinary bladder can be used as an indicator of inflammatory processes in the genital tract.

Calving management of farm veterinarians compared to private veterinarians

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While it is known that veterinarians can differ substantially in their knowledge of calving management (Mee et al., 2023), the underlying reasons for this are poorly documented (Voß et al., 2021). Hence, a 30-question calving management survey was administered by online questionnaire (Google Forms) to 490 veterinarians (339 private clinicians and 91 veterinarians employed by the farmer) working in Türkiye. In total, 430 veterinarians responded (88% response rate). The majority (96.3%) were male with 3.7% female and the duration of professional experience was 12.7 ± 8.7 years. More farm veterinarians (72.5%) did not intervene prophylactically at calving compared to clinicians (47.5%) ($p = 0.001$). More farm veterinarians (65.9%) used a calving jack sometimes than clinicians (46.0%), ($p = 0.008$). More farm veterinarians (34.4%) did not suspend the calf to remove birth fluids compared to clinicians (17.1%), ($p = 0.001$). More farm veterinarians (87.9%) did not administer analgesic/anti-inflammatory medications after eutocia than clinicians (80.2%), ($p < 0.04$). More farm veterinarians (75.8%) did not administer antibiotics to cows after eutocia, compared to clinicians (57.9%), ($p < 0.002$); also after dystocia (22.0% vs. 11.6%), ($p < 0.02$). It was concluded that farm veterinarians were less proactive to intervene during calving but when they did so were more likely to sometimes use a calving jack but were then less likely to administer analgesia, anti-inflammatories and antibiotics than clinicians. Given these findings, similar studies are warranted in other countries to determine the external validity of these results and to explore why these differences exist.

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Computed tomographic dynamics of the abomasum in newborn calves taking milk and feed intake into account

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This study aimed to follow the postnatal changes in abomasum topography in calves using computed tomography, under the influence of milk and feed intake. For this purpose, computed tomography scans (PQ 2.000, Picker International, Cleveland in Ohio, USA) were performed on 15 clinically healthy Holstein Friesian calves. These scans were taken on the 1st, 7th, 14th, and 21st day of life. On the calves' first day of life, three scans were taken 6 hours apart. The first examination of the calves took place immediately after birth. A significant increase in both the height and width of the abomasum was measured in the transverse scan between the fasting intake (1-hour postnatum) and after the administration of colostrum. The width of the organ expanded by 98%. Based on the total stomach volume, the abomasum occupies about 42% of the volume immediately after birth. Twelve hours postnatum and after ingestion of colostrum, the volume of the abomasum increases to 1.6 L. The abomasal volume reaches its maximum one-week postnatum and decreases with the beginning of the first fibre intake. At the same time, the volume of the reticulum increases steadily in its total volume and percentage among the total stomach volume. In conclusion, the computer tomography offers the possibility of repeatedly examining the same calf in a life-preserving manner over a defined period and thus recording the individual course of development. It therefore provides the basis for further investigations, e.g. in connection with the question of drenching.

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Cholesterol, proximate and fatty acid contents of anatolian buffalo milk at various lactation stages

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The aim of this study was to determine proximate and fatty acid composition of Anatolian buffalo milk at various lactation stages. Milk samples were collected from Anatolian buffaloes in three different lactation periods (early lactation, mid-lactation, and late lactation) in a local farm in Osmaniye in Türkiye in order to determine cholesterol content, proximate and fatty acid compositions. The results showed that significant effect of lactation period on milk composition was observed. The highest fat and cholesterol contents were found in the early lactation stage with values of $6.88 \pm 0.08\%$ and $38.52 \pm 0.38\%$, respectively. The protein contents in the Annatolia buffalo milk samples during early, mid, and late lactation stages were $3.51 \pm 0.07\%$, $4.47 \pm 0.09\%$, and $4.27 \pm 0.03\%$, respectively. The lactose profiles of the Anatolia buffaloes milk samples ranged from 51.62 ± 0.08 to 57.90 ± 0.48 g/L. The results also showed that saturated fatty acids (SFA) contents of all milk samples were similar during all lactation stages. While monounsaturated fatty acids (MUFA) content was observed to be high in milk samples taken in the early lactation period, MUFA content in milk samples taken in both late and mid lactation periods decreased. Polyunsaturated fatty acids (PUFA) were 4.97% in mid- and late lactation and 4.57% in early lactation. It can be concluded that lactation stages have an impact on cholesterol, proximate and fatty acid composition of Anatolian buffalo milk. Our findings suggest that buffalo milk has high nutritional value in all periods, especially PUFA and MUFA, and its health benefits may be better in the middle and late stages of lactation.

The effect of body condition score on oxidative stress and milk quality in healthy anatolian buffaloes

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The aim of the present study was to investigate whether body condition score (BCS) affects oxidative stress and milk quality in healthy Anatolian buffalos. The study comprised four groups as Group 1 ($n = 12$; BCS values ≤ 2.5), Group 2 ($n = 15$; BCS 2.5–3), Group 3 ($n = 17$; BCS 3–3.5) and Group 4 ($n = 13$; BCS ≥ 3.5). Milk samples were collected under aseptic conditions from all groups. California mastitis test (CMT) test and microbiological analyzes were performed on milk samples. If the CMT results were negative and there was no pathogen isolation, milk samples were considered healthy and included in the study. The milk samples were analysed for Somatic Cell Count (SCC), total antioxidant status (TAS) and total oxidant status (TOS). Milk serum oxidative stress index (OSI) was also calculated. TOS, OSI and SCC values differed significantly between the groups ($p < 0.05$). TOS level was higher in Group 1 compared to other groups ($p < 0.01$; $p < 0.05$). OSI level decreased in the Group 3 compared to the Group 1 ($p < 0.01$). While there was no difference in terms of SCC between all groups, it was higher in the Group 1 than the Group 2 and Group 3 ($p < 0.05$). In conclusion, decreased and increased BCS value significantly affected oxidative stress parameters and SCC value. It is suggested that oxidative stress occurred as a result of accelerated fat mobilisation and thus milk quality was negatively affected.

The influence of AFP III on the cryopreservation of canine sperm

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Cryopreservation impacts sperm motility, integrity, and fertilisation potential. This study assessed antifreeze protein III (AFP III) on frozen–thawed semen from eight mature male dogs. Ejaculates were diluted with a Tris-fructose-egg yolk extender with 0, 0.75, 1.0, and 2.0 µg/mL AFP III to 50×10^6 sperm/mL, transferred into 0.25 mL straws, cooled at 4°C for 2 h, and frozen in nitrogen vapour for 10 min. Post-thaw samples were then analysed for different parameters. Motion parameters showed no significant differences ($p > 0.05$), but 0.75 and 1.0 µg/mL groups had better movement than 2.0 µg/mL. Membrane integrity differed significantly between control and treated groups ($p < 0.05$). Higher values were found in the 0.75 and 1.0 µg/mL AFP III groups compared to control and 2.0 µg/mL. Parameters of phosphatidylserine translocation, lipid peroxidation, mitochondrial membrane potential, and H2O2 levels didn't differ significantly ($p > 0.05$), but the 0.75 and 1.0 µg/mL groups performed better. Thus, incorporating AFP III, especially type III, can enhance sperm protection during cryopreservation.
Acknowledgments: We extend our sincere appreciation to Simone Heerth and Katrin Koslowski for their exceptional contributions and collaboration in the andrology laboratory.

Impact of MitoQ on canine sperm cryopreservation

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Cryopreservation is crucial in reproductive biotechnology, but frozen/thawed semen has a limited lifespan due to integrity loss. This study explored MitoQ's effects on sperm from eight mature

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dogs. Ejaculates were divided and diluted with a Tris-fructose-egg yolk extender containing 0, 100, 200, and 400 nM/mL MitoQ to a final concentration of 50×10^6 sperm/mL, then transferred into 0.25 mL straws, cooled at 4°C for 2 h, and frozen in nitrogen vapour for 10 min. Post-thaw samples were assessed for various parameters. CASA results showed no significant differences in motion parameters from the control group ($p > 0.05$), though the 200 nM MitoQ group had slightly better motion characteristics. There were no significant differences in viability, membrane, or acrosome integrity between control and treatment groups ($p > 0.05$), but the 200 nM group showed a tendency for better results. Lipid peroxidation differed significantly between the 200 nM group and others ($p < 0.05$). The 200 nM group also had significantly fewer dead sperm (ANX+/PI+) ($p < 0.05$). Finally, the 200 nM group outperformed others in active mitochondria and intracellular ROS-H2O2. In conclusion, the current study concludes that MitoQ benefits certain parameters of frozen–thawed canine sperm cells.
Acknowledgments: We thank Simone Heerth and Katrin Koslowski for their exceptional work and collaboration in semen collection and analysis at the andrology laboratory.

Characterisation of multilamellar structures in the seminiferous tubule of wild-type mice and mice with a transgenic sertoli cell specific connexin43 knockout (SCCx43KO)

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The deletion of the gap-junction protein connexin43 (Cx43) in murine Sertoli cells (SC) significantly affects their morphological and functional integrity and causes metabolic disturbances. In adult mice, Cx43 deficiency results in an arrest of spermatogenesis at the level of spermatogonia and/or a Sertoli cell-only syndrome. Cx43-loss in SC can further be associated with histological changes of the seminiferous epithelium, including disrupted microtubule organisation, vacuolization, and intratubular SC clusters/agglomerates. Using transmission electron microscopy and serial block-face scanning electron microscopy, we were interested in detecting additional alterations at the ultrastructural level. First experiments demonstrated multilamellar structures within the cytoplasm of SC or between adjacent SC. Thus, the aim of the present study was to characterise the observed multilamellar structures found in SC and to compare them (1) between SCCx43KO and wild-type mice as well as to compare their appearance (2) between the seminiferous epithelium and intratubular SC clusters. Multilamellar structures that are already known from other organs and cell types can be differentiated in multilamellar bodies, annulate lamellae, annular gap junctions and/or other lamellar bodies. Furthermore, in the testis, formations of the basal ectoplasmic specialisation of SC and autophagosomes may also form structures with a multilamellar appearance. First results lead to the supposition that the appearance of multilamellar structures might be linked to the basal ectoplasmic specialisations and could be associated with augmented or disturbed autophagy in adult SC of mutant mice.

Extended focus imaging of in vitro produced bovine embryos—preliminary data

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The objective of the present study was to compare images of bovine embryos produced via light microscopy or extended focus imaging (EFI), being multi-dimensional. At day 7 of in vitro culture 35 embryos (morulae, blastocysts, expanded and hatching blastocysts) were placed in TCM-199 and two pictures of every single embryo were taken: one directly with a light microscope (Olympus IX73, Eppendorf, Germany; control group) and one using "Instant extended focal imaging" (EFI group), a manual acquisition process of the imaging software used (cellSens Dimension, version 1.18). Recording was started with a focus at the lowest point of the embryo, then focusing manually through the whole embryo, while the software took as many pictures as possible. These were combined to a multi-dimensional image afterwards. Pictures of both groups were presented in random order to five well experienced practitioners for staging and grading according to the IETS-Manual (5th edition; study part A). In part B, examples of the five embryonic stages mentioned above were shown with both groups in direct comparison. Results were analysed anonymously, average values of stage and grade were calculated for every embryo. In part A, half of the embryos were staged and graded the same, independent of the group (control vs. EFI). The others differed in stage or quality grade, but in most cases difference was less than one point. Part B revealed a preference for the EFI group, as being easier to assess. EFI seems to be a comparable technique for evaluation of bovine embryos. Further surveys with more participants are needed to draw general conclusions.

Canine decidual cells communicate through Extracellular Vesicles (EVs) containing factors involved in trophoblast invasion

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Successful canine pregnancy requires maternal uterine stromal cells to differentiate into placental decidual cells. Located near maternal endothelial cells and embedded within the trophoblast layers, they are the only placental cells expressing the nuclear progesterone (P4) receptor (PGR). Disruption of P4/PGR signalling induces parturition/abortion, linked with luteolytic PGF2α output. The immortalised dog uterine stromal (DUS) cell line enables in vitro study of decidualization. Being of epithelioid character, surrounded by interstitial space encompassing COL IV and other matrix proteins, decidual cells communicate directly and indirectly with their environment. Here, the involvement of decidua-derived extracellular vesicles (EVs)

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in the cell-to-cell communication was hypothesized. EVs were isolated from both control and in vitro-decidualized DUS cells. The functional approach tested the effects of aglepristone. EVs were isolated with ultracentrifugation and size exclusion chromatography, and characterised by immunoblotting, transmission electron microscopy and nanoparticle tracking analysis. Proteomics revealed the presence of factors associated with decidualization and trophoblast invasion, e.g., CX43, THSB1. The antigestagen diminished their EV levels as well as ADAM9/10. Decorin and PTGR1 (prostaglandin reductase) levels were decreased by decidualization. The uptake of EVs by decidual cells was validated using a recombinantly expressed CD9-mEmerald reporter. The study confirmed EV release and internalisation by decidual cells, and indicated their roles in cell communication, decidualization, and control of trophoblast invasion.

Video surveillance and artificial intelligence in ovine parturition management

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Dystocia is an important factor contributing to lamb and ewe mortality. Early intervention is crucial to reduce losses and avoid unnecessary suffering. However, continuous monitoring is time-consuming and labor-intensive. This study therefore aims to explore the use of computer vision and artificial intelligence (AI) algorithms in monitoring sheep flocks at lambing time, with the aim of detecting lambing behaviour and developing a parturition alert system to enable timely intervention.
At first, the video data collected on four co-operating farms was annotated using bounding boxes to detect sheep and lambs, and later for key points on prominent body parts such as head and limbs, which can be reliably recognised. These key points were used to develop a 3D (three-dimensional) sheep skeleton. Using the software Blender, the skeleton model was further developed, and motion sequences were animated to provide the program with information on the behaviours from any perspective.
In addition, the recorded births were analysed regarding the frequency of parturition-related behaviours and events during the entire recorded birth period.
The average percentage share of behaviours and events shown in the total duration of births observed was highest for contractions: 20.1%, circling: 10.2%, raising the head: 9.1%, extending the hind legs to the side: 6% and parts of lamb visible: 5.7%. These results form the basis for refined AI algorithms. Good progress has already been made in recognising simple behaviours and tracking the individual animals. Work on refining the models for recognising complex movement patterns is ongoing.

Single cell RNA sequencing from testes of conditional sertoli-cell specific Connexin 43 Knockout mice

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Spermatogenesis, which describes the formation and maturation of sperm, is a highly regulated process requiring intensive regulation by somatic Sertoli cells to synchronise germ cell proliferation, migration and differentiation. Connexin 43 (Cx43), a crucial gap junction protein in Sertoli cells, plays a significant role in maintaining the blood-testis barrier (BTB), and its loss in Sertoli cells results in altered spermatogenesis and infertility. This study investigated the role of Cx43 in testicular development and function using single-cell RNA sequencing in adult Sertoli-cell specific Cx43 knockout mice compared to their wild-type littermates. In the first step, the study analysed the gene expression profiles of KO and WT animals and focused on how the transcriptional profiles of testicular cells are altered under pathological conditions of Cx43 deficiency in order to gain better insights into the molecular mechanisms of male infertility. By profiling gene expression in individual testicular cell populations, this approach allows for the identification of changes in cellular heterogeneity and the impact of Cx43 deficiency on Sertoli cells, germ cells, peritubular cells and Leydig cells, and BTB-related components. The goal is to map transcriptional changes associated with Cx43 deficiency, providing key insights into the genes role in both normal testicular development and altered spermatogenesis. These findings will finally contribute to a broader understanding of the molecular mechanisms governing male fertility, the specific role(s) of Cx43 and the maintenance of the testicular environment.

Placental extracellular vesicles as messengers in revealing transplacental transfer of maternal cells

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During pregnancy maternal (immune) cells are vertically transferred to the fetus. These cells, known as maternal microchimeric cells (MMc), can reduce the offsprings' susceptibility to diseases later in life. However, there is limited understanding of how MMc overcome the placental barrier. This study aims to unearth the mechanisms of transplacental MMc trafficking by analysing placenta-specific extracellular vesicles (EVs) in normally

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progressing pregnancies and pregnancies affected by infections. EVs from third-trimester serum samples of healthy and SARS-CoV-2-infected pregnant women were isolated via cushion ultracentrifugation. EV isolation was validated and subsequently placental EVs were enriched and their proteome was analysed by imaging flow cytometry and mass spectrometry. The EVs' proteome was then correlated with the MMc frequencies detected in cord blood. Lastly, C57BL/6J (CD45.2, H-2Db) and Balb/c CD45.1 (CD45.1, H-2Dd) mice are being mated allowing the identification of MMc in the fetus with subsequent validation of the identified proteins driving MMc trafficking. MMc transfer is significantly decreased following maternal SARS-CoV-2 infection. The placenta comprises an altered EV messaging upon infection with increased EV secretion and an altered protein cargo. This includes the significant downregulation of HMGA2 (High mobility group AT-hook 2), a transcriptional regulator and PSME1 (proteasome activator complex subunit 1), a component of the immunoproteasome. The regulation of MMc transfer is closely linked to placental EV messaging. These findings provide novel insights into human and murine placental transfer regulation.

Lesson learnt—A disturbed pregnancy with good outcome and frustrating insights

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After abortion caused by multiresistant *Pseudomonas (P.) aeruginosa*, a 4-year-old female Great Swiss Mountain Dog was presented for ovulation determination in the next heat. As the strain was no longer detectable by microbiological vaginal testing, the bitch was mated and pregnancy confirmed. In a subsequent control, however, pregnancy in the left uterine horn was disturbed due to *P. aeruginosa* infection with empyema, determined by microbiological punctate analysis. As the right horn contained vital foetuses and the bitch was in good general condition, unilateral hysterectomy was performed on day (D) 45 of gestation. After resistance testing, tazobactam/piperacillin (50 mg/kg i.v.) was administered four times daily, following the drug reclassification cascade. Bitch and foetuses recovered despite mild leucocytosis. Progesterone concentrations were below 2 ng/mL at one occasion, but recovered without treatment. On D60 of pregnancy, four puppies—two viable without apparent deformities and two dead ones—were delivered via planned caesarean section. *P. aeruginosa* was detected throughout the uterus. Nevertheless, mother and both vital puppies developed well. This case questions vaginal microbiological testing, as it could not detect the persistent *P. aeruginosa* strain in the uterus, and therefore did not prevent abortion twice. In addition, administration of a reserve antibiotic in high dosage did not prevent puppy death. Furthermore, single low progesterone levels not necessarily indicate luteal insufficiency.

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Treatment with a deslorelin slow-release implant influences the expression of androgen receptor and prostaglandin-pathway in the canine epididymis

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The effects of deslorelin slow-release implants (DSRI) on the canine epididymis are, in contrast to testicular function, poorly analysed. Prostaglandins (PG) play a role in epididymal function and have been postulated to be regulated by androgens. To gain deeper insights into the impact of DSRI treatment and the role of PGs on canine epididymis, 6 male dogs were treated with a DSRI and hemicastrated after 5 months, concurrent with implant removal (TG). Five months later, the other side was removed (RG). Testes of 3 untreated dogs served as controls (CG). Gene expressions of the AR and the PG pathway (PTGS2, HPGD, hPGDs, lPGDS, DP, PTGES, EP2, EP4) were studied individually for each epididymal segment. Statistical analysis was done comparing groups (TG vs. RG, TG vs. CG, RG vs. CG) and epididymal segments (head/body/tail) using GraphPadPrism. Comparison between TG and RG revealed a significant influence of group and segment on AR and HPGD expression (AR: $p < 0.05$, HPGD: $p < 0.001$), with higher expressions in the body (AR, HPGD) and tail (HPGD) in RG. The group significantly impacted PTGES and EP2 ($p < 0.05$) and the segment lPGDS ($p < 0.05$). Regarding TG vs. CG, EP2 and EP4 were influenced by group and segment ($p < 0.05$), lPGDS by segment ($p < 0.01$). Comparing RG and CG, the segment influenced PTGES ($p < 0.05$), EP2 and 4 (each $p < 0.01$), lPGDS ($p < 0.01$), HPGD and AR ($p < 0.001$). Although DSRI treatment alters the epididymal expression of the AR and some PG pathway genes during downregulation, induced effects are reversible proving restoration of epididymal function subsequent to treatment.

Differences in the triacylglycerol composition of epididymal fat tissue between mammalian species

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Although there is evidence that the epididymal fat tissue is essential for spermatogenesis (Hansel, 2010, Endocrinology, 151, 12, 5565–5567), its molecular function has not been elucidated. As triacylglycerides (TAG) are the main lipid components of white adipose tissue, we analysed the TAG composition of epididymal fat in 55 adult males from 29 mammalian species. Samples were collected over ten years and stored frozen until cell disruption

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and lipid extraction. TAG were determined by mass spectrometry (MALDI-TOF). Since the oxidation of TAG was unexpectedly low, it is possible to store the samples in a frozen state for a longer period of time. In adult Carnivora ($n = 40$) the predominant TAG of the epididymal fat tissue consist of 52 carbon atoms (C), followed by 50 and 54 C with mainly two and three double bonds (db). Felidae ($n = 33$, 16 species) and Caniformia ($n = 7$, 7 species) have a similar TAG composition of the epididymal fat, whereas the TAG composition of adult Rodentia ($n = 15$, 6 species) shows a greater variability. Rodent epididymal fat also predominantly consists of TAG with 52 C, but contains more TAG with 54 C than 50 C. In addition, the examined rodent species possess mainly more unsaturated TAG with three and four db. This is the first comparative study to describe the TAG composition of epididymal fat tissue in distinct mammalian families. Hypotheses regarding the reproductive role of epididymal fat in an evolutionary context and in relation to dietary fatty acid intake need to be investigated in more detail.

Optimising the voluntary waiting period of dairy cows: Effect of gynaecological health combined with milk yield on days open

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The ideal length of the voluntary waiting period (VWP) is a complex topic that is essential for optimising productivity in dairy herds. The present study investigated a classification of dairy cows ($n = 245$) based on gynaecological health and milk yield regarding median days open (DO) for three VWPs. Gynaecological exams were conducted on day 32 ± 3 *post partum* (*pp*) using rectal ultrasonography and a speculum. Cows were either free for insemination on day 42 *pp* (VWP1; $n = 102$) or postponed until the exam was repeated on day 74 ± 3 *pp*. After the second exam, cows were either free to inseminate on day 84 *pp* (VWP2; $n = 87$) or 126 *pp* (VWP3; $n = 56$). Milk yield for the exam days was classified as "high" with a mean of > 32 kg (1st lactation), > 40 kg (2nd lactation) and > 45 kg ($\geq$ 3rd lactation) per day, calculated over the previous seven days. Cows were categorised into: no gynaecological abnormalities and low milk yield (GN-L), gynaecological abnormalities (endometritis, pyometra or cysts) and low milk yield (GA-L) and gynaecological abnormalities and high mil yield (GA-H). Cows in the GN-L group had significantly shorter median DO when assigned to VWP1 compared to VWP2 (85 vs. 141; $p = 0.012$). However, no significant differences in median DO were observed between VWP1 to VWP2 in GA-L and GA-H. Interestingly, cows of all three groups had significantly shorter median DO ($p \leq 0.05$) when assigned to VWP2 instead of VWP3: GN-L (117 vs. 182.5 days), GA-L (119.5 vs. 167 days) and GA-H (122 vs. 171 days). These findings suggest that a gynaecological exam on day 74 ± 3 *pp* does not benefit VWP selection with respect to days open. *Funded by LfULG, Germany.*

Targeted stabilisation of hypoxia-inducible factor (HIF) 1α during in vitro maturation of bovine cumulus-oocyte complexes increases IVP outcomes

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HIF1α plays a critical role as a transcription factor in regulating cellular responses to reduced oxygen (O_2) tension, essential during ovarian follicular development, oocyte maturation, and ovulation. Previously, we identified HIF1α as a key regulator impacting in vitro maturation/production (IVM/IVP) outcomes, associated with the modulation of factors involved in the maturation of cumulus-oocyte complexes (COCs). Here, we assessed the effects of targeted HIF1α stabilisation in bovine COCs during IVM on IVP output. HIF1α stabilisation was achieved by increasing dosages of the prolyl hydroxylase (PHD) inhibitor Roxadustat, which prevents HIF1α degradation.
Functional outputs included assessing meiotic resumption, cleavage and blastocyst rates, and evaluating transcriptional and translational effects on selected maturation markers. Our main findings revealed significantly increased blastocyst rates in the presence of lower dosages of the PHD inhibitor. Contrary to expectations, increased blastocyst rates did not correlate positively with maturation markers' expression (e.g., HAS2, TNFAIP6, or TMSB4). Another important finding indicates a negative self-regulatory loop between stabilised HIF1α protein availability and its decreasing transcriptional levels, which appears essential for maintaining its controlled provision. An upward trend in steroidogenic activity was observed in response to increasing Roxadustat. Finally, we report the positive effects of targeted HIF1α stabilisation on IVP outcomes in the bovine model, though the underlying molecular mechanisms require further investigations.

Targeted stabilisation of hypoxia-inducible Factor (HIF) 1α during in vitro maturation of bovine cumulus-oocyte complexes increases IVP outcomes

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Generation of bovine placental allantochorionic cell lines via SV40 *large* TAg transfection or lentiviral libraries with multiple, non-SV40-related transgenes

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Coxiella burnetii is the causative agent of human Q-fever and an obligate intracellular bacterium. Ruminants are asymptomatic carriers and the primary source (placenta, milk) of human *C. burnetii* infections. In order to generate permanent bovine mesenchymal cell lines to study the interaction of *C. burnetii* with relevant host cells, primary bovine allantochorionic (Alc) tissue was enzymatically digested. The isolated cells were transfected with a plasmid expressing the SV40 *large* TAg or lentiviral libraries containing multiple, non-SV40-related transgenes. Genomic SV40 *large* TAg-integration was confirmed by PCR and protein expression by immunofluorescence. Y-chromosomal presence was confirmed by PCR (SRY, microsatellite). Primary Alc cultures displayed both mesenchymal and also epitheloid cell phenotypes. 3D spheroids were generated by *hanging drops*. Within 3 days, the SV40 *large* TAg transfected cells formed spheroids. Cultures (2D, 3D) of transfected cells were ultrastructurally characterised which indicated that the SV40-transfected cells are co-cultures of mesenchyme and epithelium (identified by microvilli formation).

Five single cell clones were grown from lentivirally transduced Alc cells into monoclonal cell lines. Four cytokeratin-negative (mesenchymal morphology, no colony formation) and one cytokeratin-positive clone (epitheloid morphology, colony formation) were selected for continuous culture. After further characterisation of the permanent Alc cell lines generated to confirm their mesenchymal character, these cell lines can be used to study the interaction of *C. burnetii* and other zoonotic abortificient agents with bovine placental cells.

Does the polled intersexuality syndrome (PIS) also affect genetically male goats?

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Bovidae are distinguished by their prominent horns. Dehorning has been common in practical husbandry, but is controversial for reasons of animal welfare and, depending on the species and country, not (or no longer) permitted by law. Different genetic factors cause hornlessness (or polledness) in cattle, sheep, and goats and are therefore used for breeding ruminants without horns. In female goats, homozygous genetic polledness can cause the polledness intersexuality syndrome (PIS), leading to infertility that is also assumed in genetically hornless male goats. So far, this has never been systematically investigated. One reason may be that in the past, no routine determination of genetic sex and horn status was possible in goats. We evaluated sperm production and histological morphology in a study group of heterozygous (Pp $n=15$) and homozygous (PP $n=4$) polled male goats in comparison with horned individuals (pp $n=3$) using ejaculate and testicular tissue. All animals (breed "Weiße Deutsche Edelziege") were tested for sex (XY) and horn status by PCR. They were trained for sperm donation with an artificial vagina at the age of about 1.5 years within the breeding season. No significant differences between Pp, PP, and pp. male goats regarding their semen parameters were observed. Testicular biopsies taken directly after routine slaughter, fixed in Bouin's solution, embedded in paraffin (FFPE), cut and stained with haematoxylin and eosin. These displayed regular stage-specific tubular architecture independent of the genetic horn status and no hints to a reduced sperm production in comparison to horned male goats.

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Diagnosis of *Neospora caninum* in a low prevalence bovine foetal population using three diagnostic techniques

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The objective of this study was to determine the animal-level prevalence of *N. caninum* in bovine foetuses submitted to an Irish veterinary laboratory (RVL, Cork) (Sept. 2022—Feb. 2023). Thoracic fluid/blood, a midbrain swab and fixed brain tissue were collected. Histopathological diagnoses were made by research officers. Foetal fluid/serum ELISA testing was carried out (IDEXX *Neospora caninum* Antibody Test Kit). A PCR assay was carried out on the brain swabs (LSI VetMAX *Neospora caninum* Detection Kit, ThermoFisher). PCR results were available for all 363 foetuses; 17 (4.7%) were *N. caninum*-PCR-positive and 3 were inconclusive. Antibody ELISA results were available for 326 foetuses; 4 (1.2%) were positive and 1 was inconclusive. Histopathology results were available for 90 foetuses; 5 (5.6%) had lesions consistent with *N. caninum* and 1 was inconclusive. Twenty-one foetuses (5.8%) were positive by at least one of the three tests. Of the 17 PCR-positive foetuses, 14 had available antibody ELISA results and 4 had available histopathology results. Of these, 1 was antibody-positive and 3 were histopathology-positive. One of the 4 antibody-positive foetuses was PCR-positive. Histopathology results were available for 2 antibody-positive foetuses, only 1 of which was positive. Three of the five histopathology-postive foetuses were PCR-positive and one was antibody-positive. No foetus was positive in all three diagnostic tests. Agreement between the different diagnostic options for *N. caninum* was poor. The 5.8% overall positivity rate was lower than an apparent prevalence of between 9 and 23% reported internationally (Mee, 2023).

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The expression of osteopontin in bovine endometrial gland cells (BEGC) is regulated by oestrogen in vitro

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The peri-implantation period is a critical time for the successful establishment of pregnancy. During this time, the extracellular matrix protein osteopontin (OPN) is of great importance. OPN is secreted by endometrial glands and required for adhesion and signal transduction at the uterine-placental surface. In sheep, endometrial OPN secretion is mediated by progesterone (P4) through down-regulation of its own receptor (PR). In former studies we could show that stimulation with P4 and combinations of P4 with oestrogen (E2) and/or interferon tau had no effect on the mRNA expression of *OPN* in bovine endometrial gland cells (BEGC) in vitro. Interestingly, the combination of P4 and E2 led to an increase in *PR* expression. Therefore, BEGC were now stimulated with E2 alone and the mRNA expression of *PR* and *OPN* was analysed by quantitative real-time PCR. Cells incubated in serum-reduced medium (SR) served as controls. Additionally, OPN protein expression was analysed by immunofluorescence (IF). Stimulation with E2 led to a significant increase in the *PR* ($p < 0.01$) and *OPN* ($p < 0.001$) mRNA expression. IF staining showed a diffuse cytoplasmic distribution, but also strong signals at the cell borders in both the E2 stimulation and SR control group. OPN regulation seems to be different in cattle compared to sheep. Our results show that E2 but not P4 led to an increase in the *OPN* gene expression. However, OPN function might be similar as the localization of OPN protein at the cell borders may reflect OPN secretion by BEGC. Quantitative investigations at protein level using Western Blot are planned.

Role of rare T cell subtypes in human testicular germ cell tumours

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Testis cancer is one of the most common malignancies in young men, with testicular germ cell tumours (TGCT) being most frequent. Most TGCTs arise from de- or wrongly differentiated gonocytes that develop to pre-invasive *germ cell neoplasia* in situ (GCNIS) and may—or may not—form invasive tumours such as seminoma (SE) and embryonic carcinoma (EC) later on. The factors promoting this development and also metastatic spread are still unclear, but increasing evidence suggests an essential involvement of the immune system in this pathogenesis. Our previous studies showed a significant contribution of T cells to the immune environment of seminomas, e.g. the presence of regulatory T cells (T_{reg}) and follicular helper T cells (T_{fh}) utilising various patient cohort, materials, and experimental approaches. By this, we were able to show highest numbers of T_{reg} and T_{fh} cells in tumour centers of SE samples compared to other locations in the diseased testis as well as to the contralateral side without tumour hinting at a biological role of these cells in tumour biology. In ongoing experiments, we are analysing the presence and function of type 17 T cells using immunohistochemistry and flow cytometry. Additionally, we will analyse TGCT patient peripheral blood monocytes (PBMC) alone and within a

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tumour cell-somatic cell culture models (TCam2-FS1) to unveil the functional connection between tumour, somatic and immune cells. By this, we aim at deciphering the tumour micro(immune)environment to pave the way for future immune-modulatory therapies.

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Vascularisation in human testicular germ cell tumours—High endothelial venules and their significance for tumour development

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Testicular germ cell tumours (TGCTs) are most prevalent among young men (14 to 44yo). They arise from pre-invasive germ cell neoplasia in situ (GCNIS) and can proceed to invasive seminoma (SE) and non-seminoma (NSE). Disease-free survival rates are usually high, but metastases can lead to poor prognosis. High endothelial venules (HEVs) and immune cell infiltration have been described as prognostic factors in various cancer entities and we aim to investigate these in TGCT patients. For this, we performed histological evaluation (HE) and immunohistochemistry (IHC) to analyse immune cells and HEVs in testis biopsies from azoospermic men with and without neoplasia. Testis biopsies (each $n=10$) with intact spermatogenesis (NSP), non-neoplastic inflammation, GCNIS, SE, and NSE were stained with haematoxylin/eosin and for macrophages (CD68+), B cells (CD20cy+), dendritic cells (CD11c+), and T cells (CD3+, CD25+, FOXP3+). For HEV detection we will use MECA-79, HECA-452, PECAM-1, and SMA (smooth muscle actin). HE showed massive immune cell infiltration and a high number of blood vessels in SE/NSE compared to NSP, with formation of follicular-like structures in some SE patients. IHC revealed an abundance of T cells and macrophages in tumour centres compared to tumour borders and control samples. In further experiments, we will analyse HEVs by IHC and correlate results to clinical data. In the future, IHC characteristics of immune cells/HEVs in TGCTs could serve as diagnostic/predictive markers and be placed in the clinical context of tumour development and further progression.

Use of morphometry to estimate dairy bovine foetal age at abortion and stillbirth

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In the absence of AI or natural service dating it can be difficult to estimate bovine fetal gestational age (GA), (Mee, 2021). Hence, the objective of this study was to use multiple foetal morphometrics to predict GA. Measurements were collected at necropsy from 1295 single abortion/perinatal mortalities from Irish dairy cows which had recorded case histories including service date. The following foetal morphometrics were measured: DD—digital diameter, CRL (crown rump length) was measured in three ways:- Pollc—curved from the base of the tail to the crown, CRL eye—curved from the base of the tail to the eye, Str CRL—straight from the base of the tail to the crown, girth and body weight. Data from Je and Non-Je calves were analysed; Je = Jersey or Jex sire or dam ($n=394$); non-Je = other dairy breeds or crosses ($n=450$). The final dataset (844 records) was divided into train (80%) and test (20%) sets. Recorded GA varied between 128 and 316d. Morphometric variables were highly autocorrelated (0.86–0.98), hence models with one morphometric variable and breed category were built. While breed category was statistically significant, it had little impact on the output values. sCRL (0.63), girth (0.61) and DD (0.61) and had the highest R^2 in train and test datasets combined with lowest mean absolute error (MAE), (mean difference in predicted days from GA) (10.3–10.8). Body weight had the poorest associations with GA (R^2 0.49, MAE 11.8). Straight CRL had the highest and body weight models had the lowest predictive ability. Bovine gestation length, from the fourth month of pregnancy onwards, could be predicted with ~ ±10 days model error in dairy calves.

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Are selected markers of cell growth arrest present in bovine placenta?

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Bovine placenta, like other placentas, is a temporary organ with clearly defined life cycle. Despite sharing the same purpose, the bovine placenta is unique in its histological structure compared to other mammalian species, like the human placenta. This difference significantly impacts fetal-maternal interaction, immunological response and the mechanism of parturition as well as raises the question how and whether the cells signal the end of their life cycle in the developing placenta. Such signalling molecules may include proteins p-38, phosphorylated p-38, p-21 and p-53. Biological material were placentomes collected at slaughterhouse from HF pregnant cows (2nd, 4th, 5th month; $n=4$ per each month) and at parturition during caesarean section ($n=4$). Placentomes were manually separated into maternal and foetal parts and homogenised. Supernatants were subjected to western blotting and ELISA determination of proteins p-38, phosphorylated p-38, p-21 and p-53. The presence of all examined proteins was confirmed in bovine placenta but with different densities and localisation within examined placental parts. Phosphorylated p-38 was detected only in the foetal part. The concentrations of p-38 and phosphorylated p-38 increased

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between the 2nd and 5th month of pregnancy. P-38 expressed the tendency to decrease in the foetal part but no changes were observed in the maternal part at parturition. Studying markers of cell growth arrest accompanied by already studied apoptosis markers may provide new insights into the preparation of the bovine placenta for delivery, particularly in the context of hormonal regulation and inflammatory signals.
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Studies on the topographical anatomy of the spermatic cord of the raccoon (*Procyon lotor*)

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The raccoon (*Procyon lotor*) is a mammal native to North America and is listed as an invasive alien species of union concern. In recent years there has been a significant increase in the population in Germany. In addition to various management strategies, controlling reproduction in accordance with animal welfare regulations appears to be the most important issue. One method used is Trap-Neuter-Return (TNR). It has been shown in tom cats that sterilisation is successful in the long term, as testosterone-induced territorial behaviour is maintained. Therefore, the aim of this study was to examine the anatomical structures and proportions of the spermatic cord in order to establish a basis for the surgical approach to sterilisation of male raccoons. For this purpose, the inguinal and peri-scrotal area of five adult male raccoons was dissected. An incision in the inguinal area was made to access the processus vaginalis including the funiculus spermaticus. The vaginal process is covered by a 15–20 mm thick subcutaneous fat layer and was visualised after blunt preparation. It appears as a very thin membrane, which cannot be fixed and opened easily. The Ductus deferens and parallel running vessel have a diameter of approximately 2 mm and the mesoductus deferens is approximately 2–3 mm wide. Intraoperative dissection would be correspondingly delicate and the risk of ligating or injuring neighbouring blood vessel, e.g. *A. femoralis* and A. pudenda externa, is high. Therefore, it does not appear to be a suitable technique for reproductive control in raccoons. Epididymectomy seems to be a more simple and quick implemented alternative.

Selective dry cow treatment—Simple and safe with "MastiSelekt"

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Selective drying off of dairy cows is intended to reduce the use of antibiotics while maintaining udder health. To select cows for drying off without antibiotic therapy, a data- and IT-based decision tool has been developed as part of the "MastiSelekt" initiative, which enables automated assessment of the mastitis risk of cows. The aim of the project was 1) to develop an algorithm, 2) to enable automated data exchange between different data sources and 3) to show that selective drying off is possible while maintaining udder health. Data on a total of 15 criteria based on milk performance, health data as well as bacteriological milk tests are automatically transmitted and a score of mastitis risk is determined. The algorithm was tested on five project farms over a period of 13 months and udder health data from the subsequent lactation were recorded. For 906 animals the algorithm issued a recommendation for antibiotic treatment due to a high score of mastitis risk. For animals with a low score of mastitis risk, either a recommendation with ($n = 569$) or without antibiotics ($n = 572$) after random selection was issued and those low mastitis risk animals were analysed using multivariate mixed linear models. There were significant differences in somatic cell count in the first, second and third milk performance test of the subsequent lactation, pathogen detection by bacteriological examination on the first and fifth day in milk, but no significant differences in the occurrence of clinical mastitis within the first 7-, 14- or 30-days postpartum. In conclusion, selective drying off with an automated decision tool is possible while maintaining the udder health of the herd.

Transrectal ultrasound in dairy cows: Do hyperechogenic endometrial areas affect reproductive performance and cytological findings?

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In dairy cows, reproductive performance is highly affected by uterine health. Two of the diagnostic methods are transrectal ultrasound and endometrial cytology. With ultrasound, pathological purulent filling located in the uterine lumen presents itself mostly isoechogenic, accumulated and often fluctuant. Another clinical finding is the presence of irregularly distributed, hyperechogenic and non-fluctuant endometrial areas. The aim of this study was to compare cows with hyperechogenic areas (HA; $n = 53$) to cows with no clinical findings (nCF; $n = 78$) regarding the uterine ultrasound in view of endometrial cytology and fertility. Therefore, cows were examined using rectal ultrasound

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on day 32 ± 3 *post partum* (*pp*). In addition, an endometrial swab was taken to evaluate the percentage of polymorphonuclear neutrophils (PMN). Median days to first insemination did not differ comparing nCF to HA (80 vs. 79; $p = 0.535$), but the first service conception rate (FSCR) was 51.3% for nCF and only 26.4% for HA ($p = 0.005$). However, median days open failed to reach a significant difference for nCF vs. HA (101 vs. 122; $p = 0.158$). Cytology revealed significantly higher percentages of PMN in the HA group compared to the nCF group ($p = 0.008$). Furthermore, a classification of HA into three grades and a comparison among themselves did not reveal any significant differences regarding fertility and endometrial cytology. The results indicate that the diagnosis of hyperechogenic endometrial areas on day 32 ± 3 *pp* can be associated with an activated endometrial immune system and a decrease of the FSCR, but a classification into grades does not seem to be useful.
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Verification of a fertility classification system for predicting the fertility of thoroughbred mares

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This present study aimed to classify the reproductive status of thoroughbred mares after many years of regular field fertility examinations. For this purpose, a fertility classification system (Identification and classification of animals with and without reproductive problems) was used, which is used in thoroughbred horse breeding to predict the fertility of a mare. The study was carried out during fertility examinations of thoroughbred mares during the autumn months of 1997–2003 in the Hesse region (Germany). The data were analysed retrospectively. A total of 1118 mares with 3072 examination cards were included in the dataset. After submission of the results of the gynaecological examination the thoroughbred mares were classified according to their fertility prospects as follows: GroupI: Pregnant mares and broodmares without clinical or bacteriological concerns, Group II: Mares without clinical or bacteriological concerns that have remained not pregnant for one year. Group III: Mares without clinical or bacteriological concerns that have been not pregnant for more than one year, as well as mares in Group IV after healing (animals that become pregnant after treatment). Group IV: Mares showing clinical signs of disease or bacteriological concerns. Within the scope of this classification the number of animals in Groups I, II, III, and IV, were 2090, 600, 369, and 4 respectively. The age of the mares had a statistically significant influence ($p < 0.0001$) on the classifications of the mares. The pregnancy rates in the following year differed between the classification groups but were not statistically significantly different ($p = 0.065$).

Laparoscopic cryptorchidectomy in a holstein friesian bull—A Case Report

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A 2-year-old Holstein Friesian bull was presented at the Clinic for Cattle with "testicle present on one side only". The owners, an animal welfare organisation, were interested in the animal's survival. The clinical examination revealed a good general condition. During andrological examination, the left testicle was palpable in the scrotum. The right testicle was palpable transrectally and visualised by transcutaneous and transrectal ultrasonographic examination in the abdominal cavity. The animal was castrated on the left side via scrotal orchiectomy. After 3 days, a laparoscopic cryptorchidectomy of the right testicle was performed at the Clinic for Horses. The bull was sedated and locally anaesthetised for the procedure. Laparoscopy was conducted on the standing animal via three surgical sites at the right flank. A spray block and infiltration of the mesorchium with a local anaesthetic was performed for the removal. The blood supply was ligated with LigaSure and the testicle was removed. To extract the testicle, the organ was fixed with a traumatic laparoscopic grasping forceps and pulled out after widening the surgical site of the endoscope. The procedure was accompanied by treatment with antibiotics and NSAID's. Within 2 days after the procedure, subcutaneous emphysema developed at the surgical site, which was absorbed without treatment. The stitches were removed after 12 days and the animal was transferred home. Overall, laparoscopic castration is a less invasive method for the animal and should be preferred to laparotomy if appropriate equipment is available.

Primary uterine inertia (PUI) in dogs is associated with impaired placental availability of factors involved in the parturition cascade

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The initiation of the parturition cascade in dogs involves the decrease of progesterone (P4) signalling in the placenta, mediated by its receptor PGR, expressed by decidual cells only. This results

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in increased PGF2α production by the trophoblast, promoting luteolysis, and stimulates myometrial activity. The local role of glucocorticoids in initiating parturition, linked to increased placental cortisol availability and glucocorticoid receptor (*GR*/*NR3C1*) expression, possibly affecting P4-PGR signalling, was suggested. PUI, one of the main causes of dystocia in dogs, is characterised by insufficient uterine contractility. Recent works focused on myometrial function, but the pathophysiology of PUI remains unclear. To test if the dysregulation of the parturition cascade could contribute to the development of PUI, the placental availability of factors related to the parturition cascade was assessed in dogs with PUI ($n=4$) and at prepartum luteolysis (LUT, $n=4$). Compared with LUT, prostaglandin-related factors *PTGS2*, *PTGES*, and *HPGD* showed no significant transcriptional changes in PUI ($p>0.05$). Concomitantly, the PGF2α synthase *PTGFS* was lower in PUI ($p<0.0001$), whereas *PGT* was more abundant ($p<0.0001$). Importantly, the expression of *GR*/*NR3C1* was reduced in PUI ($p<0.05$). Strikingly, both the mRNA and protein levels of PGR were increased in PUI ($p<0.001$), although no significant changes in the number of decidual cells were observed ($p>0.05$). These findings suggest that PUI could be associated with disturbances of the parturition cascade, possibly due to inadequate decrease of P4-PGR signalling and its interaction with *GR*/*NR3C1*.

How the epididymis helps the uterus-development of a promising biosensor model for drug testing

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Published results of our group demonstrate that the very last part of the rat epididymis (segment 19, S19) showed no spontaneous contractions but responded with strong contractions to the hormone oxytocin and not only to the sympathetic neurotransmitter noradrenaline. The main use of oxytocin is the prevention and treatment of postpartum haemorrhage by stimulating contractions of the uterus. Using live-imaging in combination with a new method of analysis, we aimed to clarify whether S19 (in comparison to uterine tissue) is a superior drug-testing biosensor targeting the oxytocin pathway.

Uterine tissue shows spontaneous contractions and is subject to hormonally induced changes but 1 nM oxytocin induces strong contractions. In contrast, S19 shows no spontaneous contractions but also a sensitive and more consistent and easily reproducible dose-dependent contraction strength (10–500 nM) that could already be correctly recognised during recording. Due to this sensitivity, smallest impurities of free oxytocin can be discerned when testing inactive newly developed oxytocin-conjugates. S19 allows the generation of up to 16 tissue aliquots, which reduces the number of animals needed for multiple drug testing set-ups. In addition, using live-imaging and our method of analysis, a 10-min recording time per experiment is already sufficient, making this biosensor model resource- and time-efficient.

In conclusion, S19 of the rat epididymis is a superior biosensor model for quickly assessing the bioactivity of new drugs.

Case report: A rare case of uterine torsion in a bitch in the first half of pregnancy

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Uterine torsion in bitches is typically observed either in the last trimester of pregnancy or as a cause of dystocia during parturition. It has also been described in non-pregnant bitches, mostly associated with tumours or trauma. In this case, a 2.5-year old Nova Scotia Duck Tolling Retriever bitch was presented on day 29 of pregnancy due to repeated vomiting, a severely disturbed general condition and bloody vaginal discharge. The bitch was presented in an excellent general condition to the primary vet one day prior and the pregnancy was confirmed sonographically by the primary vet the day prior to presentation. Clinical examination revealed a reduced general condition with a rapid worsening and slightly pale mucous membranes, a tense abdominal wall and bloody vaginal discharge. During ultrasound, free peritoneal fluid was identified as well as bilateral thickening of the walls of the uterine horns. Fetal heart beats could not be confirmed sonographically. Focal perfusion was only identifiable in the left uterine horn. Blood analysis revealed severe thrombocytopenia. Laparotomy confirmed a uterine torsion at the uterine body and approximately 300 mL free intraabdominal haemorrhagic fluid. A routine ovariohysterectomy was performed. The bitch received intensive post-operative stabilisation including several blood transfusions. Anti-thrombocyte-antibodies were identified and considered causative for thrombocytopenia. The bitch was discharged 11 days post-operatively in good general condition. This case highlights the importance of considering the rare possibility of uterine torsion in bitches presenting with metropathy in the first half of pregnancy.

The dummerstorf high-fertility mouse line 1—A worldwide unique model for increased female reproductive performance

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Over the past few decades, various mouse models have significantly advanced our understanding of folliculogenesis. Among these, the Dummerstorf high-fertility mouse line 1 (FL1) stands

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out as a unique selection experiment aimed at enhancing female reproductive performance. FL1 mice can nearly double the number of ovulated oocytes compared to unselected control lines, and they exhibit various endocrine and molecular alterations that may support improved follicular development (Ludwig et al., 2022).

To delve into the cellular mechanisms driving this high fertility phenotype, we isolated granulosa cells from antral follicles for mRNA sequencing. Following transcriptome analysis, we assessed the levels of hormones and growth factors linked to follicular maturation. While we noted a decrease in FSH and IGF1 levels during estrus in FL1 mice, no significant differences were found in insulin, prolactin, or oxytocin when compared to the control line. LH levels were decreased in FL1 females.

Our findings suggest that FL1 mice benefit from enhanced interactions among various intracellular signalling pathways in granulosa cells. This results in reduced follicular atresia, attributable to improved survival rates of granulosa cells, along with more effective intracellular signalling, glucose metabolism, and signal transduction. As a result, FL1 mice demonstrate significant advantages in reproductive performance. Understanding these mechanisms and their interplay could be crucial for advancing our knowledge of fertility in humans.

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Transcriptomics of outgrowing cells from bovine embryos in feeder cell culture

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Bovine embryonic stem cells (bESC) comprise a huge potential to reduce animal experiments when used in research. These cells can be differentiated into different cell types and subsequently serve as in vitro models by mimicking embryogenesis. The aim was to replicate the cultivation of intact, in vitro produced bovine day 7.5 blastocysts on murine embryonic fibroblasts (MEF), to induce proliferation of bESC and suppress the growth of trophoblast cells (TE) contemporaneously. Stem cell medium by Bogliotti et al. (2018) containing WNT-inhibitor IWR1 and Fibroblast Growth Factor 2 was used to maintain pluripotency. Activin A was supplemented to induce definitive entoderm differentiation. The effect of two different MEF cells (CF1-MEF and PMEF-CF) was tested. Outgrowing cells were collected after 5 days in culture and the transcriptomic profile was analysed. Pathway analysis revealed that culture approaches with only IWR1 supplement keeps the outgrowing cells the most similar to the non-cultivated day 7.5 blastocysts. RNA sequencing gene count detects high amounts of *IFNT2* (trophoblast marker) in all outgrowths. However, principal component analysis revealed that outgrowing cells cluster in groups according to the different MEF. Additionally, there were 1010 exclusive genes, expressed only in non-cultivated embryos, whereas the outgrowths revealed averagely only 111 exclusively expressed genes. The preformulated stem cell medium did not sufficiently restrict the TE growth. Accordingly, stem cell medium composition needs to be improved in terms of maintenance of pluripotency standards.

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Serratia species affects the quality of preserved boar semen based on the total bacterial count, but not the duration of storage

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Serratia species (*Serratia* spp.) are among the bacteria of greatest concern in stored boar semen due to their rapid growth, high sperm toxicity, and multi-drug resistance. The goals of the study were to compare the growth rates of different *Serratia* spp. strains in extended semen portions during long-term storage at 17°C and to determine whether the total bacterial content or the length of exposure affects semen quality. To measure the growth rate, we spiked portions of boar semen ($n = 3$) with four different *Serratia* spp. strains isolated from different semen samples. After 24 h, 48 h, and 72 h, the bacterial content was counted on blood agar (CFU/mL). All four strains exhibited similar growth of six log levels up to 72 h of storage. To assess the sperm-damaging effect, 60 ejaculates, spiked with different amounts (10^1–10^4 CFU/mL) of different *Serratia* spp. strains, were analysed for sperm motility, agglutination score and bacterial content up to 72 h of storage. The agglutination score ($r = 0.48$; $p < 0.001$) and total motility ($r = -0.62$; $p < 0.001$) correlated with the bacterial content but not with the length of semen storage. In 17 samples, low semen quality was evident after 48 h and was associated with bacterial counts $> 10^7$ CFU/mL. Conversely, 12 samples maintained normal semen quality even after 72 h of exposure to *Serratia* spp., all of them with a bacterial count of $< 10^7$ CFU/mL. In conclusion, the contamination degree with *Serratia* spp. is decisive for the loss of sperm quality in preserved boar semen, but not the length of exposure.

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Mastectomy in a seven-year-old white alpine sheep with inappropriate lactation syndrome

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Inappropriate lactation syndrome (Lactatio falsa), the inception of secretion in the mammary gland without a previous pregnancy, is a well-known problem in sheep and goats. A seven-year-old female, non-postpartum White Alpine sheep was presented with severe, bilateral but asymmetric (left>right side) enlargement

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and induration of the lactating udder. Ultrasonography revealed parenchyma of the mammary gland with multiple, well-defined abscesses (diameter, 0.5 to 7 cm). The abdominal wall was intact and there was no indication of muscle rupture. Bacteriology of the puncture fluid of one abscess revealed coryne- and obligate anerobic bacteria. Surgical removal of the udder was performed under general anaesthesia with the sheep in dorsal recumbency. Additionally, distal paravertebral anaesthesia and a pudendal nerve block were used. The incision at the basis of the mammary gland was ellipsoidal. Special attention was paid on the ligature of the big blood vessels, i.e. the superficial caudal epigastric veins and the external pudendal arteries and veins. Furthermore, dissection of the glandular tissue was avoided to prevent contamination of the surgical area. Post-operative care included antibiotic treatment, pain management, and daily wound cleaning until full recovery of the sheep. In summary, mastectomy is a suitable therapy for the inappropriate lactation syndrome that cannot be treated conservatively. However, a surgical removal of the udder requires a profound knowledge of the anatomy of the mammary gland and the supplying blood vessels, and is possibly restricted to small ruminants that are kept as companion animals, due to the cost restriction in livestock husbandry.

Photodynamic inactivation reduces bacterial load in boar semen doses

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The Photodynamic Inactivation (PDI) of bacteria is a well-established alternative to antibiotics in human medicine and is also applied for food decontamination. The PDI process is mediated by a biocompatible photosensitizer (PS) and low-intensity light. The excited PS transfers energy to molecular oxygen, resulting in the generation of singlet oxygen ($^1O_2$), which causes irreversible damage of bacterial cell walls. The aim of this interdisciplinary study was to test the PDI concept for antimicrobial treatment in preserved boar semen. The main challenge was to maintain sperm fertility while inactivating seminal bacteria. The illumination setup consisted of a tower with a ten-channel current controller at the bottom and an LED light source on top, on which the semen sample bags were placed. Singlet oxygen generation and the localization of the photosensitizer (PS) outside the sperm were confirmed through phosphorescence spectroscopy and fluorescence microscopy. The samples consisted of extended semen ($n = 8$ boars) containing the photosensitizer 5,10 ,15,20-tetrakis(1-methyl-pyridinium-4-yl) porphyrin (TMPyP). Dose–response studies with white and blue LED light revealed no light toxicity on sperm viability, motility, mitochondrial activity and DNA fragmentation after illumination for 90 s with an intensity of up to 4.5 mW/cm^2 ($p > 0.05$). During storage for 144 h at 17°C, the bacterial load in the control semen increased to ~10^4 CFU/mL, whereas bacterial counts in the PDI-samples were below the detection limit (< 10 CFU/mL). In conclusion, the photodynamic treatment of semen doses is a novel approach for antibiotic-free semen preservation.

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Sperm functional status: A multiparametric assessment of the fertilising potential of bovine sperm

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Sperm viability is routinely assessed for the quality control of cryopreserved bovine sperm but is not always conclusive about their fertilising potential. In this study, we investigated the fertility predictive value of bull sperm viability in combination with DNA integrity or the functional status of viable sperm post-thaw. For this purpose, we examined two sets of 791 (set A) and 733 cryopreserved batches (set B) with non-return rate (NRR) records after ≥100 first services, respectively. Sperm viability was flow cytometrically assessed for set A and B as the percentage of plasma membrane- and acrosome-intact sperm (PMAI) after staining with propidium iodide and peanut agglutinin, respectively. The percentage of sperm with high DNA fragmentation index (%DFI) was determined for samples of set A with the Sperm Chromatin Structure Assay. The percentage of sperm with high esterase activity, intact plasma membrane and acrosome, low intracellular Ca^{2+} content and functional mitochondria ($C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$) was quantified in set B with a five-colour flow cytometric panel that included calcein violet, propidium iodide, the phycoerythrin-conjugated peanut agglutin, Fluo4-AM and the cyanine stain $DiIC_1(5)$, respectively. Using linear mixed effects models and conditional inference trees, we examined the predictive value of PMAI combined with either %DFI or $C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ to predict the batch-specific NRR. Batches with %DFI ≤6.86% were more likely to have a NRR >60%, whereas %DFI values <6.86% were more likely associated with 55%–60% or lower NRR ($p < 0.01$). Combining PMAI with $C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ did not reliably predict the NRR of individual batches. Concluding, the incorporation of DNA integrity assessment can considerably improve sperm fertility prognostics.

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Inter- and intra-individual variability of motility and DNA fragmentation of boar sperm

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In this study, we analysed the inter- and intra-boar variability of sperm progressive motility and DNA integrity in the ejaculates from 92 boars of ten different breeds. In addition, we evaluated the effects of boar age, breed, season and method of semen collection on both sperm characteristics. Ejaculates were examined once per month for 13 consecutive months. Progressive motility (PMOT) and the percentage of sperm with high DNA fragmentation index (%DFI) were assessed on the day of semen collection using computer-assisted sperm analysis and the Sperm Chromatin Structure Assay, respectively. Both sperm traits showed high inter- and intra-individual variability (72.88% ± 31.42% PMOT and 7.91% ± 5.30% %DFI with 49.35% and 11.25% mean coefficient of variation, respectively), with no age-related but significant breed-related differences. As shown by mixed-effects linear models, autumn samples showed reduced PMOT and winter ejaculates had elevated %DFI values ($p < 0.05$ in both cases). Conditions of high temperature-humidity index led to high %DFI ($p = 0.01$). Ejaculates collected by hand or semi-automatically did not differ in PMOT and %DFI values. However, the factors examined only explained 14.6% and 10% of the overall PMOT and %DFI variability, respectively. In summary, sperm progressive motility and DNA integrity largely vary between and within boars. They are partly influenced by breed, season and microclimatic conditions on the day of semen collection, but not by boar age or the method of semen collection.

Developing an AI-driven system for boar sperm morphology assessment

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The morphological assessment of spermatozoa belongs to a well-established routine for evaluating semen quality. But, morphology assessment is still a manual task. This is counterintuitive, as objectivity and time management of semen quality assessment are of high importance. Therefore, we trained a computer vision model based on Yolov8s that can evaluate single spermatozoa. The model was trained with 145 pictures downscaled to $1200^2 px^2$, annotated with polygonal masks, and labelled by an extended version of the German Livestock Association standard for boar semen evaluation. We used 80% of the pictures for training and 20% for validation. Mean average precision at an intersection over union threshold of 0.5 (map50) was 18.1% for all classes, while ranging from 0% to 47% within the eight out of 46 subgroups present in the validation set. These values show the need for a modular system including an initial sperm separation step, followed by a hierarchical structure of classification models. For this initial separation step, we reduced the number of groups to only "Sperm" and "Waste". This class reduction improved the map50 (all groups: 61.5%, Sperm 76.6%, Waste 46.3%; Yolov8m; $1280^2 px^2$). Visual inspection of the predictions shows good sperm separation of the model, while medium-good map50 results are based on unclear boundaries in the separation of "Sperm" and "Waste". With this primary model we can now process larger numbers of pictures to generate sufficient training data for every single morphological abnormality and might lead to a fully automated sperm morphology assessment system in near future.

Current international trends in dairy cow fertility—From decline to recovery?

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It is generally accepted that dairy herd reproductive performance began to decline in the latter decades (1960–90s) of the 20th century across countries, production systems (confinement, hybrid, pasture-only), dairy breeds and milk production levels (Mee, 2024). This decline has been attributed to changes in dairy cow management, nutrition, genetics and level of milk production. The nadir of dairy cow fertility occurred in approximately 2005. Since then dairy herd reproductive performance has not only plateaued but has improved internationally (though variable across countries and across fertility metrics, e.g. conception rate, calving interval, days open, daughter pregnancy rate). Why? The quote *'success has many fathers, but failure is an orphan'* accurately summarises the competing hypotheses as to why dairy cow fertility declined and why it recovered. Three competing narratives of success exist: better veterinary reproductive and herd health management, better nutritional management, particularly transition cow management, and better genetic selection, specifically multi-trait, indices. Rather than a winning narrative, it is likely all three, plus other unknown variables, contributed substantially to the current improvements in dairy cow fertility. Throughout these periods of fertility decline and recovery, milk production/cow has continued to increase, albeit more slowly of late. This is critical, as milk yield/cow, a major driver of agricultural economics, was perceived as one of the primary causes of declining cow fertility. However, concurrent genetic selection for better fertility and increased milk production has removed this barrier for both traits.

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Bovine endometrial explants express different temporal courses of IL1B and IL1RA mRNA expression after challenge with heat inactivated *Lactobacillus buchneri* compared to *Escherichia coli* and *Bacillus pumilus*

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Intrauterine presence of pathogenic bacteria can cause severe inflammation and impacts fertility of affected cows. Adequate inflammatory host response towards invading bacteria is crucial. But bacteria can also provide protective traits in the bovine uterus. The objective was to compare temporal courses of the mRNA expression of proinflammatory interleukin (IL)1B and its receptor antagonist (IL1RA) in endometrial tissue after challenge with heat inactivated bacterial components of *Escherichia coli (EC)*, *Bacillus pumilus (BP)* and *Lactobacillus buchneri (LB)*. Explants ($n = 780$) were taken from 26 healthy uteri at the abattoir and challenged for 3, 14 and 24 h with bacterial components (10^7 cfu/mL). IL1RA and IL1B mRNA expression was measured via RT-qPCR. Mixed-effects models were used for statistical analysis. Challenge with EC, BP and LB led to higher IL1B mRNA expression compared to the control after 3, 14 and 24 h ($p < 0.001$), but 3 h after challenge, IL1B was lower after LB challenge compared to EC or BP ($p < 0.001$). Compared to the control, mRNA expression of IL1RA was higher 3 h after challenge with EC ($p < 0.001$) and BP ($p < 0.01$), but not after 14 and 24 h ($p > 0.1$), whereas challenge with LB led to higher IL1RA only after 14 h ($p < 0.001$), and 24 h ($p < 0.01$), but not after 3 h ($p > 0.1$). Thus, IL1RA was significantly lower after LB challenge compared to EC or BP at 3 h ($p < 0.01$) and higher at 14 and 24 h ($p < 0.1$). In conclusion, challenge with pathogenic EC and facultative pathogenic BC led to different time courses of the inflammatory host response compared to the apathogenic and potentially protective LB. Underlying mechanisms and benefits have to be elucidated.

Punctual differences in differential milk cell count, milk parameters and vaginal temperature between cows with diverging haplotypes after intramammary *Staphylococcus aureus* challenge

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Mastitis in dairy cows affects animal welfare and causes high economic losses. Genetic selection can be used to select cows with reduced mastitis susceptibility and analysis of Differential Milk Cell Count (DMCC) via flow cytometry may improve early mastitis diagnosis. Full quarter milk samples from a highly standardised in vivo infection model with 24 cows selected for divergent bovine taurus chromosome 18 haplotypes (Q vs. q) and challenged with *S. aureus*, were analysed every for 96 h after inoculation. Vaginal temperature (VT) was recorded every three minutes. The aim was to compare DMCC (polymorphonuclear neutrophils (PMN), vital, non-vital, lymphoid, large cells), milk constituents (fat %, protein %, lactose %) and pH as well as VT between favourable (Q) and unfavourable (q) haplotype cows using Bayesian models, with *p*-values based on the density at the Maximum A Posteriori. Somatic cell count in milk of challenged quarters was higher compared to unchallenged quarters (24 h—96 h, $p < 0.001$). Regarding DMCC, q cows displayed higher numbers of PMN, large cells and vital ($p < 0.05$) at 24 h, and higher numbers of non-vital at 36 h ($p < 0.001$) post challenge. VT dynamics (q > Q at interval 12–24 h, $p < 0.05$; q < Q at interval 48–60 h, $p < 0.05$) and milk constituents (protein %, q < Q at 60 h, $p < 0.05$; fat %, q < Q at 84 h, $p < 0.05$) differed significantly between the two haplotypes. In conclusion, Q and q cows exhibited selective differences in DMCC, milk constituents, and VT in a highly standardised in vivo infection model with *S. aureus*. The significance of these changes in relation to mastitis susceptibility remains to be elucidated.

Aging of adipose stem cells is mediated by AMPK/SIRT1 signalling pathway

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The capacity for self-renewal and cellular plasticity is diminished in adipose-derived stem cells (ASCs) during the aging process. We hypothesize that metabolic regulators interact with the ASC niche via the AMP-activated protein kinase (AMPK)/sirtuin (SIRT) 1 signalling pathway, thereby influencing the aging of ASCs. The objective was to analyse the aging of ASC in correlation with their capacity for adipogenic differentiation and expression of the SIRT1 and AMPK subunits. The effects of chronological aging and metabolic aging were modelled using donors from different age groups and an apolipoprotein (APO)E−/− rabbit. The subcutaneous adipose tissue of human donors (aged 18 to 88 years) and the New Zealand White rabbit served as sources for ASCs. The adipogenic differentiation of the ASCs was induced in a two-dimensional cell culture system. For all cell lines, the adipogenic differentiation capacity was determined using Nile Red and Oil-O-Red staining techniques. Quantitative polymerase chain reaction (qPCR) and Western blot analysis were employed to quantify the expression of the AMPK and SIRT1 genes at the mRNA and protein levels, respectively. In the undifferentiated ASCs, the gene expression of AMPK subunit PRKAG2 was reduced in older donors and APOE−/− rabbits, with no differences for the other subunits of AMPK. The expression of SIRT1 was found to be lower in APOE−/− ASCs. In conclusion, we observed a specific subunit shift of the AMPK/SIRT1 signalling pathway during aging, indicating that age-related factors influence this metabolic pathway in ASCs.

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Cryopreservation of epididymal domestic cat sperm: influence of sperm count in a commercial and a self-made extender

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We originally developed a simple and field-suited method for the cryopreservation of feline epididymal sperm based on a self-produced extender (TestG) and a final glycerol concentration of 4.7% (v/v) (Klaus, 2016, Reprod Dom Anim, 51, 195–203). Here, we tested the applicability of a commercial glycerol-containing ready-to-use extender for human sperm (Origio Cryo Sperm). It is normally applied 1:1 (v/v) to a sperm sample of 10 million sperm/mL. Since epididymal sperm collection in domestic cats (used as a model for sperm from wild felid species) often results in higher concentrations, the Origio-extender was applied at a higher ratio of 2:1 (v/v) to sperm samples between 30 and 200 million sperm/mL. A generalised linear model showed a significant influence ($p \leq 0.05$, $n = 9$) of extender and sperm count/vial (ranging from 3 to 20 million sperm) on post-thawing quality. The percentage of motile sperm was higher in the TestG than in the Origio-extender at 10 and 60 min after thawing, but the difference was less pronounced at high sperm counts/vial. The velocity (VCL) of progressive sperm was reduced after thawing but did not differ between the extenders. The percentage of thawed sperm with active mitochondria also did not correlate with the motility parameters, did not differ between the extenders, but was higher in samples with a high sperm count/vial. Origio Cryo Sperm is therefore an alternative commercial extender for the cryopreservation of feline sperm that does not require the addition of fresh egg yolk. However, we suggest diluting the sample as originally prescribed 1:1 (v/v) with the extender, if the initial sperm concentration is below 180 million sperm/mL.

Modulation of uterine responses in dogs: The divergent effects of semen and embryo signals

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Despite the absence of an acute luteolytic mechanism in non-pregnant dogs, precise embryo-maternal communication is needed to set the stage for a successful pregnancy. Uterine responses during early pregnancy include modifications of decidualization markers, the extracellular matrix, and immune factors. This has been shown in studies comparing early pregnant with non-pregnant uteri exposed to semen. Here, we hypothesized that embryo signalling differs from the signals initiated by semen alone. Three groups of healthy dogs we included (d. 10–12 of pregnancy/dioestrus): non-mated, mated and pregnant, and mated but non-pregnant (semen-exposed). Semi-qPCR and immunolabelling validated the nuclear receptors, immunomodulatory factors, and growth factors. The main findings indicate differences in P4 receptor PGR staining across the surface epithelium, superficial and deep glands, with some variations suggesting effects of semen that are potentiated by the embryo presence, while ERα staining implied embryo-specific signalling. Semen-effects included higher transcription of *CD163* and *CD206* and decreased of *CD8*, indicating anti-inflammatory immunomodulation (favouring M2 macrophages). Conversely, embryo-effects included increased *MHCII* and *NCR1* (an NK cell marker). Our study underscores the importance of differentiating seminal plasma- and embryo-driven signals, with implications for enhancing assisted reproductive technologies. Components of semen may play an essential role in preparing the uterus for implantation, while embryonal signals might aid in identifying early pregnant markers.

Long-term storage of liquid-preserved bovine semen: Effects of new cooling protocols and holding temperatures on sperm quality

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Liquid preservation (LP) of bull semen, characterised by a lower sperm cell concentration per straw compared to frozen semen, offers a highly resource-efficient way for utilising elite genetic material. However, the optimal preserving method including cooling rate and holding temperature is not fully defined. This study aimed to develop a new LP method to store bull semen for up to 7 days (long-term). Therefore, nine ejaculates from fertile Holstein-Friesian bulls ($n = 9$) were collected, processed and diluted to 40 x 10^6 sperm cells/mL in Caprogen at a German AI center. Next, semen straws (0.25 mL) were allocated to three cooling protocols: A (control) cooling at 0.2°C/min to 16°C, B cooling at 0.3°C/min to 5°C and C cooling at 0.1°C/min to 16°C followed by a holding time (6 h) and further cooling at 0.1°C/min to 5°C. Samples were evaluated on day 1, 4 and 7 of storage for total sperm motility after 30 and 120 min. (TSM30/120) of incubation at 38°C, plasma membrane and acrosome integrity (PMAI) and morphologically intact sperm cells (MIS). By day 7, analyses indicated that storage at 16°C (A) resulted in significantly lower ($p \leq 0.05$) semen quality compared to 5°C (B and C). Specifically, TSM30 was significantly higher in C (80.0 ± 3.5%) than in A (69.4 ± 5.8%). Moreover, TSM120 (A: 51.1 ± 13.4%, B: 69.4 ± 3.0%, C: 69.4 ± 6.8%) and PMAI (A: 43.2 ± 14.5%, B: 56.7 ± 11.9%, C: 60.2 ± 9.5%) were significantly higher in B and C compared to A. No significant differences in MIS were observed among protocols. In conclusion, our results suggest that Caprogen preserves higher sperm quality in LS at 5°C than 16°C for up to 7 days. *Supported by FBF eV Germany.*

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Autophagy's role in mouse preimplantation embryo development

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Nutrient homeostasis is essential for reproductive efficiency across species. Autophagy, a cellular process that recycles nutrients and cellular components, plays a significant role in regulating nutrient metabolism. This pathway allows cells to adapt under conditions of nutritional stress, such as nutrient scarcity or excess. Autophagy has been linked to numerous processes in mammalian reproduction, yet its specific functions in the early stages of embryo development remain unclear, especially given its sometimes contradictory roles in cellular processes. To analyse autophagy's role, the autophagy inhibitor bafilomycin A1 (Baf) was applied in hybrid mouse zygotes. Embryo development was tracked up to five days using the Primovision time-lapse imaging system. Autophagy levels were measured by tracking LC3 markers, with an emphasis on accumulation after Baf treatment. In untreated control embryos, normal development to the blastocyst stage occurred within five days. However, embryos treated with Baf showed degeneration starting on day 3. After a three-hour Baf exposure followed by a wash and culture in Baf-free medium, embryos continued to develop to the blastocyst stage, suggesting no long-term toxic effect from short-term Baf exposure. Autophagy levels were assessed via LC3 markers. In control embryos, LC3 signals were diffusely spread throughout the cytosol, whereas in Baf-treated embryos, LC3 dots accumulated at the oocyte periphery, indicating increased autophagy activity. These results highlight the essential role of autophagy in supporting oocyte fertilisation and early embryo development in mice.

The biomechanical properties of the pregnant vs. non-pregnant ovine uterus

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Uterine rupture during a trial of labor after caesarean delivery constitutes a severe emergency. In this context, obtaining samples for fundamental research appears impractical and challenging to standardise. Biomechanical tensile tests of ovine uterine tissue samples offer an alternative, standardised surrogate marker for simulating the tearing of the uterine wall ex vivo. Full-thickness samples of the uterine wall were collected from pregnant ewes during repeat caesarean deliveries, as well as from non-pregnant ewes after slaughter, which was conducted as part of the food chain. Under uniaxial tensile load, the tissue parameters "stiffness," "yield point," "maximum force," and "rupture point" were recorded. "Stress," "strain," and the energy expended were documented for each parameter. Overall, the samples from the gravid uterus exhibited lower stiffness. A greater force was required to tear these samples compared to the non-pregnant uterine tissue. The objective endpoint of "maximum force" holds the greatest clinical significance. We conclude that biomechanical testing of ovine uterine wall samples ex vivo yields reproducible results and allows for standardised simulation of uterine rupture. The quantitative findings can be correlated with histological and molecular biological tissue examinations. This approach enables the comparison of the impact of different intraoperative techniques aimed at optimising uterine wound healing on tissue strength, ultimately with the goal of preventing future uterine ruptures.

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The ovine uterus as a translational model for the study of caesarean delivery scars

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Fundamental research with the collection of extensive tissue samples from the scarred lower uterine segment of pregnant women after previous caesarean delivery (CD) is only possible to a very limited extent. We evaluated the advantages of standardised uterine scar tissue sampling in an ovine repeat CD model. Formalin-fixed paraffin-embedded sections of uterine scar tissue were obtained from repeat CD in 16 humans. Comparably, 40 sheep underwent repeat CD with hysterectomy and standardised sampling of uterine scar tissue. Sections were stained with Gomori trichrome and evaluated microscopically. A three-layered uterine wall with endometrium, myometrium and perimetrium was visible in specimens of both species. Evaluation of uterine scars was more informative in ewes, as the standardised protocol allowed the targeted sampling of uterine scar tissue with samples containing the uterine scar surrounded by unscarred myometrium in contrast to human samples with marginal parts of the uterine scar. Histomorphometrical collagen quantification showed similar results in both species. In conclusion, uterine scars in humans and sheep show similar histological scarring patterns. The ovine repeat CD model allows more systematic sampling, which makes it promising to evaluate uterine scarring in sheep. This translational model can be used to evaluate intraoperative approaches to optimise uterine wound healing with the aim of reducing the risk of uterine rupture in humans.
The study was funded by the Deutsche Forschungsgemeinschaft Research Grants Program (grant numbers: SCHW 1946/2-1,PA 4357/2-2,BR 2925/11-1,BR 2925/11–2).

Antibiotic-free storage of boar semen results in high sperm quality and fertility

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Antimicrobial resistance is an emerging threat in the artificial insemination (AI) of pigs, caused by the consistent use of antibiotics in extended semen. The storage of semen at 5°C, instead of the commonly used 17°C, was recently introduced as an antibiotic-free alternative. Launched in 2023, amended EU regulations now allow the omission of antibiotics in semen extenders. The aim of this study was to test fertility under German field conditions, without the use of antibiotics, for the first time. Semen from ten different boars at an AI center was divided and diluted in Androstar Premium semen extender (Minitüb GmbH, Tiefenbach) without antibiotics, or with 0.125 g/l gentamicin (control). The semen doses were shipped to the sow farm and cooled down the following day by the farmer: Antibiotic-free semen portions were cooled to 5°C, whereas control samples were stored at 17°C up to four days. Sperm motility, assessed after 144 h by computer-assisted semen analysis, was 79.6% ± 5.4 (5°C) and 82.5% ± 5.5 in the control ($p = 0.068$). Sperm membrane integrity, evaluated after 144 h with flow cytometry, was 87.3% ± 3.2 (5°C) and 87.8% ± 2.8 (control) ($p = 0.933$). Insemination of 270 sows resulted in high pregnancy rates (day 56) of 96.2% (5°C) compared to 97.8% (control) ($p = 0.496$). Preliminary data do not reveal a difference in litter sizes between the two groups. In conclusion, the novel 5°C semen storage concept provides a sustainable alternative in pig reproduction by allowing the omission of antibiotics in the extender.
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Granulosa cell tumours in two holstein friesian cows—A case report

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Granulosa cell tumours are the most common tumorous changes in ovaries of large animals. They are usually benign, unilateral and do not tend to metastasize or recur. In 2023, two Holstein Friesians with circumferential growths on one ovary each, diagnosed by the local veterinarians, were presented to the clinic for cattle. The first case describes a 2.5-year-old cow that showed no estrus after calving three months ago. In the clinic, a homogeneous isoechogenic mass with vessels and caverns of 3 cm in diameter was visualised on ultrasound. The second case describes a 1.5-year-old heifer that was intended to be flushed for embryo collection. The ultrasound findings in the clinic were very similar to those of the first case. Both animals underwent

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a laparatomic ovariectomy. The histological examination confirmed the clinical suspicion of granulosa cell tumours. The cow's tumour weighed about 3 kg, the heifer's tumour about 600 g. Both animals were discharged as healthy. After a while, the local veterinarian found metastases in case one and the cow was euthanized. After two months, the local veterinarian in the heifer diagnosed an overgrowth of the pelvic and abdominal cavity. Laparatomic ovariectomy usually is the low-risk treatment of choice. In these cases, the size of the tumours and the formation of metastases must certainly be considered as exceptional.

Optimising boar semen temperature management prior to cryopreservation

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Cryopreservation of boar semen often relies on shipping prediluted semen overnight to a lab where the freezing takes place. The aim was to determine which shipping temperatures are suited to maintain the quality of the semen. In addition, it was tested whether the shipping temperature was associated with semen quality after thawing. Semen ($n = 6$ boars) was diluted in Androhep Plus. Subsamples were cooled to different temperatures (25°C, 20°C, 15°C, 10°C and 5°C) and held for 24 h before being cryopreserved. A control sample was frozen at the day of semen collection. Samples were analysed freshly diluted, after 24 h of cooling, and after thawing, using computer-assisted sperm analysis and flow cytometry. Total motility remained high when chilled semen was held at 15°C, 20°C, or 25°C, respectively, but declined when the semen was held at 10°C or 5°C ($p < 0.05$). After thawing, total motility was highest for semen previously held at 25°C (34.1 ± 12.6%, and lowest for samples held at 5°C (11.0 ± 6.5%, $p < 0.05$). The population of viable, acrosome intact sperm with low intracellular calcium concentration and low plasma membrane fluidity steadily decreased the lower the cooling temperature got (fresh: 83.5 ± 9.0%, 5°C: 59.7 ± 13.3%; $p < 0.05$). However, values were still at a high level after thawing (25°C: 59.2 ± 11.2%; 5°C: 53.7 ± 11.2%; $p > 0.05$) thereby suggesting a lack of motility re-activation as a main deficit. In conclusion, boar semen for cryopreservation should be shipped at 15°C to 25°C. Refinement of the extenders, dilution ratios, and the cooling process, may further improve pre- and post-thaw boar semen quality.

Antimicrobial use in the breeding management of the Bitch: A cross-sectional study of german veterinarians

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Microbiological examinations of the vaginal tract are frequently performed in canine breeding management. Despite the growing threat of antimicrobial resistance, inappropriate use of antibiotics prior to mating is still carried out due to fear of unsuccessful breeding and uncertainty among many veterinarians regarding the interpretation of bacterial culture findings from the vagina. This study investigated the microbiological sampling and antimicrobial treatment habits of German small animal veterinarians using an online questionnaire via the platform LimeSurvey. It included 40 questions with single or multiple-choice options to ensure standardised responses for comparable analysis. The objective was to provide a comprehensive overview of the current situation in order to identify necessary research and recommendations in this field. A total of 211 participants answered the survey completely. Of these, 77.7% have dog breeders under their care, and 29.4% take vaginal swab samples regularly (at least once a month). Only 37.9% of respondents stated that breeders never demand a prescription of antibiotics before mating, while even 10.0% reported that more than half or nearly every breeder requests this. The primary rationale is prophylaxis (69.5%). In veterinary practice, clinical signs were the most cited indication for the use of antimicrobials prior to breeding (76.8%). However, 13.7% of the participants regularly use antibiotics in cases of positive bacterial findings. Moreover, 42.2% indicated feeling either unsure or very unsure about the interpretation of bacteriological examination results. Our current findings highlight the need for further studies and education.

Bovine congenital defects diagnosed in the Irish national veterinary laboratory service (2020–2024)

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A congenital defect is a structural/functional anomaly present at birth. A collaborative study was conducted between Regional Veterinary Laboratories (RVLs) of the Irish Department of Agriculture, Food and the Marine, the Irish Cattle Breeding Federation (ICBF) and Teagasc on the most common congenital defects seen in Irish dairy and beef cattle over five years; 2020–2024. Data on bovine congenital defects were extracted from the 'hereditary and congenital anomalies' field of the LIMS database in the 6 RVLs for January 2020 to June 2024. Epidemiological data associated with these cases were extracted from the national ICBF database. In total, 202 cattle with congenital defects were recorded in LIMS, of which 180 had associated data in ICBF. Three-times more cattle with a congenital defect were beef than dairy (3:1). Of the six RVLs, the one located in an area of high suckler beef herd density had the highest congenital defect submission rate (Sligo RVL; 36%). The 3 most commonly affected body systems were the cardiovascular (63%), gastrointestinal (22%) and the musculoskeletal systems (9%). Key features of cardiac defects were (a) they occurred predominantly in beef cattle (17:3 dairy), (b) affected cattle lived from 0 to 1374 days and (c) the most common were septal defects (79%

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of all defects). Key features of gastrointestinal defects were (a) atresia was the most commonly diagnosed defect, (b) the majority of atresia calves were male (63%), (Keane et al., 2023) and (c) atretic calves lived, on average, for 3 days. Schistosomus reflexus and dwarfism were the two most commonly diagnosed musculoskeletal defects (Mee et al., 2024).

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The endocrine profile of the mare at the time of oocyte collection can affect the outcome of OPU-ICSI

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There is significant variation in the results of ovum pick-up (OPU) followed by intracytoplasmic sperm injection (ICSI) among individual mares, and the reasons for this variation remain largely unknown. This study analysed the possible effects of the endocrine profile of the mare at the time of OPU on subsequent results, with a particular focus on growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) as oocyte-secreted factors. Blood samples were collected from 59 mares at the time of OPU and GDF9, BMP15, GDF9/BMP15 complex, AMH, progesterone (P4), as well as free and conjugated estrogens (E2) were determined by either ELISA or RIA. Mares were retrospectively classified into the low ($n=25$) or high ($n=34$) outcome group based on the number of blastocysts obtained (<2 or ≥ 2 embryos). The outcome of OPU-ICSI was not affected by the mare's age or body condition score, nor by the number of aspirated follicles, the number of recovered oocytes, or the recovery rate ($p>0.05$ in all cases). The circulating peripheral levels of all analysed factors were similar among the mares with low and high outcome ($p>0.05$ in all cases). Interestingly, random forest decision trees showed that mares with P4 $\leq$ 5.99 ng/mL and simultaneously E2 $\leq$ 11.0 pg/mL ($n=28$) had a $>90\%$ likelihood to yield at least 2 embryos per session. Taken together, these preliminary findings suggest that an above average number of blastocysts following OPU-ICSI can be obtained by selecting the timepoint for oocyte collection based on the endocrine profile of the mare, even in the absence of a specific marker for success rate.

Improvement of quality and fertility of cryopreserved bull semen through an innovative selection system

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The introduction of genomic selection in cattle has led to the practice of collecting and preserving semen from very young bulls, whose semen quality often does not yet meet the minimum requirements. This study analysed the effects of a selection system which separates viable from dead spermatozoa based on membrane traits. Semen collected from Holstein-Friesian bulls ($n=20$) aged 30 ± 6 months was analysed for motility with a CASA system and the percentage of sperm with high esterase activity (Cpos), intact plasma membrane (PIneg), unstained acrosome (PNAneg), low intracellular Ca2+ (Fneg) and high mitochondrial membrane potential (Mpos) was quantified using a flow cytometric panel. Selection and time (24 h equilibration before cryopreservation, 2 h incubation after thawing) effects on sperm characteristics were analysed by a two-way ANOVA. Selection significantly improved all sperm parameters both before freezing and after thawing ($p<0.01$ in all cases), while time affected viability and CposPInegPNAnegFnegMpos ($p<0.05$ in both cases). Using the Kruskal-Wallis test, higher total and progressive motility, as well as viability ($p<0.05$ in all cases) were observed immediately after thawing, while the analyses performed 2 h afterwards revealed an improvement in total motility, viability, PInegFneg as well as CposPInegPNAnegFnegMpos sperm ($p<0.05$ in all cases). These results suggest that sperm selection using the herein analysed selection system improves bull semen quality and might also improve fertility, as demonstrated by the preliminary IVF trials performed on slaughterhouse-derived oocytes.

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Hydrops of fetal membranes and conjoined twins in an ouessant ewe

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Hydrops of the fetal membranes is a rare condition in sheep, often associated with pathological placentome changes or caused by fetal malformations. In this case, an Ouessant ewe (1.9 years, 22 kg) at day 123 of gestation was presented at the clinic showing reduced activity, anorexia and bilateral abdominal distension. Clinical examination revealed tachycardia, dyspnea and dehydration. A closed cervix was found by manual vaginal examination. Through transabdominal ultrasound, a highly increased amount of fluid in the uterus was visualised, occupying the larger part of the abdomen. Due to the ewe's severely reduced general condition, caesarean section was performed. Pre-operative care included fluid therapy, antibiotics, pain management and tetanus serum. To counteract hypovolemic shock, continuous drip infusion was administered rapidly after uterine incision. Living premature conjoined twins (male, 1.2 kg) were delivered and euthanized. After surgery, the ewe (15 kg) was treated with fluids, antibiotics, NSAIDs, and carbetocin. The ewe was discharged 4 days after surgery, showing increasing food intake and good general condition. The fetal malformation was classified as thoraco-omphalopagus conjoined twins.

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The weight of uterine fluids, placenta and fetal membranes was calculated to 5.8 kg. Hydrops of fetal membranes in combination with fetal malformation is a very rare condition in sheep. Thorough clinical and ultrasonographic examination should be carried out if hydrops is suspected and induction of labor or caesarean section should be considered.

Reproduction of free-ranging european wildcats (*Felis silvestris*) in Germany: Samples of animals found dead are valuable sources of information

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The European wildcat *(Felis silvestris)* inhabits parts of Europe, Anatolia and the Caucasus region and is currently listed as "Least Concern" by the IUCN Red List. However, many populations are threatened by habitat degradation and fragmentation as well as hybridization with domestic cats *(Felis catus)*. There is a lack of information on the reproduction of free-ranging European wildcats, although this is key knowledge for the conservation of the species. We therefore investigated the potential of using frozen carcasses of animals found dead for collecting data on the reproductive status of female European wildcats. Samples of 99 female individuals (8 pregnant) were examined. The carcasses (mostly roadkill) were collected in Germany between 1996 and 2024 and stored at −20°C. The samples were in different stages of decomposition depending on the time span between the death of the animal and refrigeration. Genotyping using a microfluidic 96 SNP chip designed for hybrid detection was performed to distinguish wildcats from domestic cats and hybrids (99 adults, 25 foetuses). Size of the last litter was estimated by counting placental scars. In pregnant females, the sex distribution of the foetuses was determined through detection of the SRY gene in placental tissue. All samples were suitable for genotyping. Nine were classified as hybrids (4 adults, 5 foetuses) and four as domestic cats. Scar counting was successful in 47 wildcat uterus samples (litter size 3.06 ± 1.07). Sex ratio could be determined in 5 out of 8 pregnancies (1.6males/1female). We conclude that, despite their heterogeneous quality, samples from animals found dead are a valuable source for the collection of wildcat reproduction data.

Characterisation of primary mesenchymal stem/stromal cells (ASCs) isolated from subcutaneous adipose tissue of the APOE-knockout rabbit

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Primary ASCs of were studied to understand of the role of apolipoprotein E (APOE) in stem cell metabolism and differentiation. ASCs were isolated from subcutaneous adipose tissue of female New Zealand Withe (ZIKA hybrids) wild-type (WT) and APOE -knockout (KO) rabbits and cultured in vitro. ASC lines from different donors ($n = 22$) and at different passages (P) were characterised based on their morphology, proliferation kinetics and immunological phenotype. Both primary ASCs from WT and APOE-KO rabbits exhibited the mesenchymal stem cell morphology and expression of stem cell markers. While the multipotency markers were expressed, the pluripotency markers could not be detected in both groups. The ASC lines were positive for cluster of differentiation (CD)14, CD34, CD105 and negative for CD45, CD73 and CD90. CD44 expression was significantly higher in APOE-KO ASCs compared to WT controls at low passages (P4-7). The metabolic phenotype of the ASC lines was analysed using the Seahorse Cell Mito Stress Test (Agilent). APOE-KO rabbit ASCs showed significant differences in maximal respiration, reserve respiratory capacity, proton leak and coupling efficiency, demonstrating that mitochondrial efficiency of energy conversion is affected by APOE mutations in ASCs. The metabolic differences between APOE-KO and WT ASCs emphasise a functional role for APOE in stem cell metabolism and differentiation.
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Overview of the blood laboratory parameters determined in feline dystocia: A retrospective analysis

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The present study aimed to evaluate the laboratory analyses of maternal blood values in dystocic queens. This retrospective, observational study included 153 queens of various breeds and ages that presented to the clinic with dystocia. The following parameters were analysed from blood samples taken during clinical evaluations in these animals: Creatine (*n*: 33), urea (*n*: 33), leukocyte (*n*: 57), neutrophile granulocyte (*n*: 54), lymphocyte (*n*: 55), monocyte (*n*: 30), eosinophil granulocyte (*n*: 54), basophilic granulocyte (*n*: 54), erythrocyte (*n*: 56), haematocrit (*n*: 65), thrombocyte (47). Mean values of all blood laboratory parameters were found as follows: Leukocyte: 13.38 ± 8.56 G/L, neutrophile

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granulocyte: 74.17 ± 15.96%, lymphocyte: 16.53 ± 10.85%, monocyte: 7.03 ± 15.37%, eosinophil granulocyte: 2.82 ± 2.43%, basophilic granulocyte: 1.08 ± 2.43%, erythrocyte: 7.69 ± 2.14 T/L, haematocrit: 0.35 ± 0.09 L/L, thrombocyte: 257.2 ± 155.9 G/L, creatinine: 89.19 ± 33.32 µmol, urea: 12.45 ± 14.11 mmol. As a result; it should be emphasised that the laboratory diagnostic parameters for assessing renal function were almost exclusively within the reference range in the cats surveyed ($n = 33$). Only six cats were found to have severe anaemia (haematocrit < 18%). The patients suffered from uterine prolapse, uterine rupture, coagulopathy, severely impaired general condition, uterine torsion, and a relatively large fetus. The changes in the red blood count were only marginal.

Effects of Low-Dosage iron Dextran Injections on Selected Haematological Blood Parameters in Neonatal Calves

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To prevent anaemia, neonatal calves are often supplemented with iron as a first day booster. The effects of such treatments are known 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 haematocrit (Ht), red blood cell counts (RBC), haemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), serum iron (FE), serum ferritin and white blood cell counts (WBC) during the first 10 days of life. 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. 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. WBC counts were not influenced by iron supplementation. 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.

Characterisation of intratubular cell agglomerates in transgenic mice with a Sertoli cell-specific Connexin43 knockout (SCCx43KO)

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The expression of the gap junction protein Connexin43 (Cx43) in Sertoli cells (SC) is an absolute prerequisite for normal testicular development and spermatogenesis. Using a transgenic mouse model in which SC-specific Cx43 knockout mice ($SCCx43KO^{-/-}$-mice) were generated, it was shown that adult male mutants were infertile. Additionally, intratubular cell clusters/cell agglomerates were observed that seem to increase in size and number with aging. By using immunohistochemistry (IHC; e.g. Sox9, p27Kip1, pRB or Ki67), previous studies showed that these cells were predominantly identified as SC, although differing in morphology and phenotype from SC within the seminiferous epithelium. Clustered SC exhibit features of both adult and juvenile SC. The aim of this project is to shed more light into the fate of these agglomerates and cells with aging. For that purpose, it was investigated (1) whether clustered cells are still connected to the basement membrane of the seminiferous tubule using IHC (e.g., Laminin, Collagen IV, Claudin11) and serial block-face scanning electron microscopy, or (2) whether clustered cells can be detected within the epididymal duct. In addition, it was investigated (3) whether morphological signs for cellular death within the clusters can be observed with aging using IHC (e.g., Caspase3). IHC for Laminin and Collagen IV showed no evidence for connections to the basement membrane so far, and most clustered cells were found immunopositive for Claudin11. In a further investigation of epididymis samples, intraductal cell groups were detected that now need to be further characterised.

Oxygen depletion and HIF1α regulate steroidogenesis in granulosa cells by affecting significant players in cholesterol provision: A multi-omics approach

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This study investigates the role of hypoxia-inducible factor 1-alpha (HIF1α) in modulating steroidogenesis in granulosa cells under varying oxygen (O_2) levels, revealing key transcriptional and proteomic adaptations. As ovarian follicles function naturally in low O_2 environments, we explored how O_2 reductions (20%, 10%, and 1%) affect cAMP-stimulated steroidogenesis in murine granulosa cells (KK1). To assess the involvement of HIF1α, inhibitor, echinomycin, was used. Lowering O_2 altered the expression of genes involved in steroid metabolism, cAMP/PKA signalling, and responses to hypoxia. At 10% O_2 (2488 differentially expressed genes/DEGs) and 1% O_2 (7461 DEGs), notable pathways affected included lipid homeostasis, intracellular transport, and organelle organisation. These transcriptional shifts were mirrored at the proteomic level, where analysis of the 328 differentially expressed proteins (DEPs) under 1% O_2 underscored the importance of intracellular cholesterol transport and steroid metabolism, affecting key proteins such as ABCA1, SQLE, and LDLR. After blocking HIF1α activity, both transcriptomics and proteomics revealed disruptions in lipid and fatty acid metabolism and cholesterol transfer activity. This interplay between gene expression and protein activity under hypoxia highlights the regulatory role of HIF1α in maintaining cholesterol homeostasis required for steroid synthesis. Our findings provide understanding of the molecular adaptations in steroidogenic cells to altered O_2 availability, emphasise the critical role of HIF1α, and offer physiological insights needed for understanding pathological states, such as ovarian tumours.

Characterisation of mitochondrial miRNAs (mitomiRs) in bovine granulosa cells

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MicroRNAs (miRNAs) are a class of small noncoding RNAs, involved in posttranscriptional regulation of gene expression. Mitochondrial microRNAs (mitomiRs) are a subset of miRNA of either nuclear and mitochondria origin and localised within mitochondria. Emerging studies have identified that mitomiRs regulate gene expression processes in mitochondria as well as modulate cell health and function. This study characterises the mitomiR profile in steroidogenic granulosa cells (GCs) from cattle. GCs were cultured in FSH and IGF1-supplemented media for four days, and mitochondria were isolated and treated with ribonuclease A to digest any surface RNA molecules adhered to the mitochondria. Total RNA including miRNA was isolated from the mitochondria using the QIAGEN miRNeasy Micro kit. PCR analysis confirmed the presence of mitochondrial-specific RNAs (e.g., 12S rRNA, COX2) and absence of cytoplasmic mRNAs (e.g., CYP19A1, IGF1R and RPLP0), indicating high RNA purity. Western blot confirmed the absence of cytoplasmic proteins (e.g., beta actin) and the presence of mitochondrial proteins (e.g., COX IV, VDAC1). RNA sequencing analysis indicated that 192 high confident mitomiRs in GCs, which includes miR-21-5p, miR-23a, miR-181a, let-7a-5p, etc. Earlier studies have shown that these miRNAs have important cellular functions such as cell proliferation and glycolysis promotion, Gluconeogenesis, lipid metabolism and phosphorylation. Investigating the role of these mitomiRs under different pathophysiological conditions could deepen our understanding of ovarian function. These findings lay the groundwork for further studies on mitomiRs in reproductive biology.

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

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Heat stress (HS) affects dairy cow fertility and can lead to substantial economic losses. This study aimed to examine the effect of HS on the mRNA expression of selected genes in the corpus luteum (CL). The study was conducted on commercial farms in Slovakia and Argentina. Animals ($n = 86$) were included on the day of artificial insemination (AI) and examined on days 7, 14 and 21 post AI. The rectal temperature (RT) was measured weekly and CL tissue samples were collected on day 21 post AI by transvaginal biopsy. Pregnancy checks were done on day 42 post AI. Climate data were collected every 15 min using data loggers. HS exposure was assessed by calculating the area under the curve between the temperature humidity index (THI) threshold of 68 and the THI amplitude. The CL tissue was used for RT-qPCR for selected genes (*FGF1*, *IL1B*, *IL10*, *ISG15*, *MX2*, *OAS1*, *PTGS2*, *VEGF2*, *HSP70*, *HSP90B2*, *STAR*, *TNF*, and *VEGFC*). During the study period, 62% of the animals experienced HS, and a positive correlation was found between RT and THI values on days 7, 14 and 21 post AI ($p < 0.05$). The pregnancy rate was lower in HS cows (15.1%) than in non-HS cows (55.6%). The mRNA expression of *IL1B*, *ISG15*, *OAS1*, *VEGF2*, *HSP70*, and *VEGFC* significantly differed between pregnant and nonpregnant animals ($p < 0.05$). In pregnant animals, IL1B was lower and ISG15 was greater expressed in HS cows compared to no-HS cows ($p < 0.05$). The results illustrate that pregnancy status and HS significantly affect mRNA expression in the CL. Further work is required to understand the complex associations between HS, CL function and fertility.

The impact of non-esterified fatty acids on oxidative stress of bovine oocytes

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Non-esterified fatty acids (NEFA) are released during negative energy balance (NEB), a metabolic syndrome commonly seen in high-yielding dairy cows post-partum. To compensate for the energy deficit, adipose tissue is mobilised, releasing NEFA into the bloodstream. Regarding reproduction, NEFA exposure has been linked to decreased oocyte quality and impaired embryonic development. Sirtuins (SIRT), a family of nicotine adenine dinucleotide (NAD) dependent deacetylases, are essential for cellular energy regulation, mitochondrial function, stress responses, and meiosis. However, the link between high NEFA levels and SIRT expression in bovine oocytes is still not well understood. To explore this further, the aim of this study is to investigate the role of SIRT on oxidative stress control after exposure of bovine oocytes to different concentrations of NEFA during in vitro maturation (IVM). Nuclear maturation was assessed through polar body extrusion, while reactive oxygen species (ROS) levels were measured using H_2DCFDA and quantified through greyscale analysis (normalised data). Preliminary findings showed no significant differences in meiosis rates (Control negative = 23.3 ± 6.7; Control positive = 16.9 ± 7.0; Basal = 18.0 ± 5.8; High Combi = 15.7 ± 6.2, $p < 0.05$, $N = 1339$), but increased ROS levels in relation to higher NEFA concentrations (Control negative = 0.05 ± 0.01; Control positive = 0.13 ± 0.01; Basal = 0.25 ± 0.02; High Combi = 0.46 ± 0.02, $p < 0.05$, $N = 30$). This research will contribute to the understanding of NEFA-SIRT interactions and their involvement in reproductive dysfunction during NEB in cattle.

AMH blood concentration in intact and neutered male llamas

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Anti-Muellerian hormone (AMH) blood concentration is used in different animal species to diagnose abdominal cyptorchidism. To the best of our knowledge, this has not yet been tested on male llamas, and to date, there is no homologous AMH assay kit available for this species. Even though AMH is a glycoprotein that is conserved across species, heterologous tests may have species-dependent variations in their applicability. Therefore, this study aimed to investigate the applicability of a commercial electrochemiluminescence immunoassay method (Elecsys**)** in male llamas that was originally developed for AMH measurements in human blood samples. In this study, a total of 16 adult llamas were used (8 castrated, 8 intact and proven to be fertile). The mean age of castrated and intact animals was 11.8 ± 1.5 and 5.5 ± 1.5 years, respectively. The measurement of AMH levels was conducted in a commercial laboratory. Serum AMH levels ranged between 1.79–10.05 ng/mL (mean: 4.53 ± 2.83 ng/mL) in intact llamas, and were < 0.01 ng/mL in all castrated llamas ($p < 0.01$). The results from a correlation analysis indicate that in intact llamas AMH levels may be negatively correlated with age ($r = -0.547$; $p = 0.160$). It has been demonstrated that the Elecsys AMH kit is a reliable method for diagnosing abdominal cryptorchism in llamas.

Isolation and characterisation of novel lytic *Escherichia coli* bacteriophage Tig2B

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Isolation and characterisation of novel phage, which could potentially be used in the future for prevention of bovine mastitis or for phage therapy. *Escherichia coli* phage Tig2B was isolated from urban sewage using an enrichment method. Its sensitivity to various physical and chemical factors, host range and growth parameters were determined. Whole genome sequencing was performed using Illumina technology (MiSeq, paired-end technology, 2 × 300 nt). Cutadapt, FastQC, and Spades programmes were used for preliminary data processing. BACPHLIP 23, PhaTYP 24 and PHACTS tools were used for genome characteristics. Average Nucleotide Identity (ANI) was calculated. The whole genome sequencing data were deposited in the GenBank under accession number OR958571. The phage belongs to the Straboviridae family. It shares at least 95% whole-genome ANI with 85 most similar phages deposited in the NCBI database, suggesting they all are representatives of the same species. Phage was classified as lytic. No antimicrobial resistance determinants and virulence factor genes were found in the genome. Preliminary characterisation of Tig2B has shown its potential for use in bovine mastitis prevention and phage therapy.
This work was supported by the Wrocław University of Environmental and Life Sciences (Poland) as part of the Ph.D. research program Innovative Scientist No N060/0010/22.

Semen quality evaluation of boars with low and high genetic reproductive potential: A multiparametric approach

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Although sow genetic potential mainly affects the reproductive prolificacy in a swine breeding program, heritability of service sire effect on litter size is around 0.03. To date, it is not known

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whether breeding boars from ancestors with low and high reproductive traits can be segregated based on their semen quality. This study investigated whether semen quality (basic or advanced, e.g. multicolor flow cytometric assays) can differentiate boars with low (−1 SD) and high (+1 SD) genetic reproductive potential (GRP). Boars (German Piétrian, $n=6$) were grouped based on their GRP (low vs. high, $n=3$/group). Semen was collected weekly ($n=3$, ejaculates/boar), processed into routine semen doses (=1.7 billion sperm/dose), and transported to the IFN Schönow overnight. Basic semen quality traits (sperm concentration, motility, and morphology) were assessed on day (d) 2 of semen storage, and advanced multicolor assays were conducted to evaluate viability and acrosome intactness (VAI), plasma membrane fluidity, intracellular calcium, in vitro capacitation, acrosome reaction, and mitochondrial membrane potential on d4. A thermo-resistance test was performed on d7 (30 and 300 min at 38°C). There were significant differences (independent t-tests) in progressive sperm motility on d2 (low; $77\pm4\%$ vs. high; $83\pm2\%$; $p<0.001$) and d4 ($71\pm7\%$ vs. $80\pm5\%$, $p=0.01$), and VAI sperm populations on d4 ($85\pm3\%$ vs. $88\pm2\%$, $p=0.036$) between the groups, respectively. All other parameters did not differ between the groups. In summary, classical spermatological fertility predictors i.e. progressive motility and VAI were significantly different in boars with low and high GRP.

Prenatal stress leads to a sex-specific modulation of the immune response during an influenza infection in adolescent mice

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Adverse environmental factors during gestation can influence fetal organ development, including the immune system with long-lasting consequences. Numerous studies on developmental causes of health and disease have neglected the sex of the offspring, which affects our understanding of sex differences in immune disorders due to prenatal imprinting. The presented study utilises a mouse model that demonstrates a sex-specific enhancement in disease tolerance to the influenza A virus.
Pregnant C57BL/6 mice underwent sound stress exposure during gestation on gestational days 9.5, 11.5, 13.5 and 15.5. Eight weeks after birth, adolescent offspring were subjected to infection with 10^3 P.F.U. of H1N1 influenza A virus.
Our research indicates that prenatal stress only affects female offspring by mitigating influenza infection severity, mirrored by lower rates of morbidity and mortality (80% vs. 20%) as well as reduced viral titers in the lungs at 6 days post infection (12.05 ± 7.36 P.F.U. /mg lung vs. 0.97 ± 0.92 P.F.U. /mg lung). The latter indicates a more rapid viral clearance, which indeed is reflected by higher frequencies of T-bet$^+$ (27.59% ±15.56 vs. 63.23% ±23.30) and INF-y$^+$ (3.01% ±1.47 vs. 6.98% ±5.10) CD8 T cells.
The evidence we have gathered indicates that female offspring, in particular, exhibit adaptations to unfavourable prenatal conditions, such as stress, since prenatally stressed male offspring did not show differences compared to their control counterparts with regard to the previously mentioned parameters.

The effects of GnRH agonist slow-release implants on Sertoli cell dynamics in male dogs

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GnRH agonist slow-release implants (SRI) are widely used in veterinary medicine for temporary suppression of testicular endocrine, and germinative function. Treatment effects are fully reversible, but the impact on Sertoli cells (SCs) and their niche is still uninvestigated. We analysed markers, including GDNF, bFGF, AR, BMP4, and FSHR, during downregulation and recovery. Healthy male dogs were treated with azagly-nafarelin SRI for 5 months, followed by surgical removal (week 0, W0). At W0 and 3, 6, 9, 12, and 24 weeks post-removal, 3–5 dogs were castrated. Similarly, 3 dogs each were implanted with a buserelin (PG) or deslorelin (SG) SRI and castrated 5 months later. Testes of 5 adult (CG) and 3 juvenile (JG) dogs served as controls. Respective gene expressions were analysed and GDNF-, bFGF and AR-immunopositive SCs in 20 seminiferous tubules counted. Statistical comparisons were made for 1) Downregulation G_{down} (W0, PG, SG, CG, JG) and 2) Recovery G_{recov} (W0, 3, 6, 9, 12, 24 weeks; CG). Numbers of immunopositive SCs (NIPSCs) differed significantly in G_{down} (ANOVA-AR: $p<0.001$, Kruskal-Wallis-GDNF/bFGF: $p<0.05$, each), with the lowest in JG, followed by W0, PG, and SG. Also for G_{recov}, the NIPSCs were significantly different (ANOVA-AR/GDNF: $p<0.05$, each, bFGF: $p<0.001$) with fewest NIPSCs in W0. Gene expressions differed only significantly in G_{down} for GDNF and FSHR (ANOVA-GDNF: $p<0.05$, FSHR: $p<0.0001$). Different to this, for G_{recov} only AR gene expression was significantly different (ANOVA, $p<0.05$). In conclusion, treatment with a SRI reversibly affects SCs and the spermatogonial stem cell niche.
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Preservation of old domestic pig breeds through bilateral exchange with the national cryoreserve

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In addition to the in situ conservation of rare pig breeds, the cryopreservation of germ cells plays a significant role in the conservation of biological diversity. Ideally, there should be an

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exchange between the two forms. In co-operation with the responsible breeding association (Hybridschweinezuchtverband Nord/Ost e.V.) and the German Gene Bank for Farm Animals, based at the Friedrich-Loeffler-Institut, cryopreserved sperm of a German Saddleback boar of the endangered O-line was brought in the purebred Saddleback herd of the Research Institute for Farm Animal Biology (FBN).

Five inseminations were carried out on four multiparous sows. Three inseminations took place after weaning, two after a 15-day synchronisation with altrenogest (Regumate) and all animals received 850 IU PMSG (Pregmagon) and 500 IU hCG (Ovogest). Deep uterine insemination was carried out 40 h after hCG with 3 billion motile sperm diluted in isothermal 50 mL Androstar plus expander. Ovarian activity and pregnancy were monitored ultrasonographically.

The inseminations resulted in two farrowings with 13 and 12 vital piglets (10 male/15 female; 1.39 Kg mean piglet weight; 17.4 Kg litter weight) and 11 weaners each. The unsuccessful inseminations could be attributed by sonography to insufficient follicle development and one post-ovulatory insemination. All offspring underwent intensive testing to determine their suitability for breeding so that boars could be accepted at an insemination centre, ensuring that the genetics are available to all breeders via semen dispatch and the return of semen to the German Gene Bank for Farm Animals.

Associations between inflammatory processes in the uterus and oviduct in postpartum dairy cows

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Excessive inflammation in the reproductive tract may adversely affect early embryo development. Therefore, the objective of the study was to investigate the relationship between inflammatory processes in the uterus and oviduct in postpartum dairy cows. Uterine samples were taken by the cytobrush technique and oviductal samples were collected by transvaginal endoscopy from 31 cows at 28 and 56 days postpartum (dpp). Samples were used for cytology to determine the percentage of polymorphonuclear neutrophils (PMN%). In addition, total RNA was extracted for RT-qPCR of selected pro-inflammatory factors (*CXCL1/2*, *CXCL3*, *CXCL8*, *IL1A*, *IL1B*, and *PTGS2*), receptors (*TLR2* and *TLR4*), and *OVGP1*. Based on uterine PMN% (≥5%) and vaginoscopic findings (pus in the discharge) at 28 dpp, cows were classified as "healthy" (HE; $n=17$) or "endometritic" (EN; $n=14$). At 28 dpp, EN showed 2.4-fold greater uterine PMN% ($p<0.05$) and greater mRNA abundance of *CXCL1/2*, *IL1A*, *IL1B*, *PTGS2*, and *TLR4* (2 to 7-fold; $p<0.05$) compared to HE. In the oviduct, EN showed significantly greater PMN% (1.6-fold, $p<0.05$) and greater mRNA abundance of *CXCL1/2* and *CXCL3* than HE (both 1.4-fold; $p<0.05$) at 28 dpp. Numerous positive correlations were found between the uterine and oviductal mRNA expression at 28 dpp ($p<0.05$). Uterine PMN% at 28 dpp correlated significantly with all pro-inflammatory factors in the oviduct at 28 and 56 dpp ($p<0.05$). In contrast, negative correlations were found between uterine PMN% and oviductal *TLR4* and *OVGP1* mRNA expression at 28 dpp. The results indicate pro-inflammatory processes in the oviduct of endometritic cows, which may impair subsequent reproductive events, such as gamete maturation, fertilisation, and early embryo development.

Zu lang.

Effect of heat stress on the proportion of polymorphonuclear neutrophils and the microbial growth in the uterus of postpartum dairy cows

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Heat stress (HS) has become a significant concern in livestock farming as it affects animal health, welfare, and productivity. The number of studies on the impact of HS on the uterine environment, however, is still limited. The objective of this study was to assess the effect of HS exposure on the proportion of polymorphonuclear neutrophils (PMN%) and the microbial growth density (MGD) in the postpartum uterus. Uterine cytobrush samples were taken from 52 dairy cows at days 7, 14, 28, 42 and 56 postpartum (dpp). Samples were used for cytology to determine the PMN% and for bacteriology to assess the MGD on the agar plates after aerobic cultivation. Ambient temperature and relative humidity were recorded at 30-min intervals with dataloggers installed in the barn and the temperature-humidity index (THI) was calculated. The accumulated HS exposure was calculated as the area under the curve between the THI and the threshold of 68 (Riemann sum). Positive correlations ($r=0.3$–0.4, $p<0.05$) were observed between short-term HS (48 h prior to sampling) and PMN% at 7, 14 and 42 dpp. The MGD correlated negatively with HS 12 h prior to sampling at 14 and 42 dpp (both $r=-0.4$, $p<0.05$). Long-term HS (7, 14, 21 and 28 days prior to sampling) significantly correlated with PMN% and only at 42 dpp ($r=0.3$–0.4, $p<0.05$). Our results demonstrate that the effect of HS on PMN% and MGD depends on the duration and intensity of HS exposure and the day postpartum. Further research is needed to explore underlying mechanisms contributing to impaired uterine health and fertility under HS.

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Altered activity of the HPG axis and prolonged lifetime fertility in superfertile dummerstorf mouse lines

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Research in the field of reproductive biology is largely based on informative mouse models. Almost all of these mouse models (~99%) show a subfertile or infertile phenotype. In contrast, the number of animal models that show an enhanced reproductive phenotype is extremely rare.

We analysed two Dummerstorf outbred mouse lines that have been selected for high fertility for 200 generations (fertility lines 1 and 2; FL1, FL2). In both mouse lines, the number of offspring per litter almost doubled (from 11.5 (ctrl) to 20.6 (FL1) and 21.4 (FL2)), without the offspring showing any signs of growth retardation compared to animals from a non-selected control line (ctrl). We analysed the lifetime fecundity of all three lines and found significant differences. Control and FL1 females gave birth to up to 10 litters, whereas FL2 females only up to six litters per female per lifetime.

We analysed the ovarian phenotype of FL1 females. We noticed a reduced concentration of FSH: serum hormone levels: −60%; pituitary gene transcription: −90%. In addition, hypothalamic GnRH transcription was decreased by 70% compared to ctrl. Gene expression analysis in ovaries revealed that multiple genes associated with follicular development, such as Lhcgr, Esr1, Kit or Foxl2 are differentially expressed. Our results indicate that follicular atresia is decreased in FL1 mice.

These data suggest that the unique ovarian phenotype of FL1 females selected for high fertility provides valuable approaches for the search for molecular mechanisms predisposing to increased fertility.

Pathohistological findings in bilateral removed ovaries of mares with behavioural problems

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Behavioural problems in reproductively healthy mares are a challenging issue, which is successfully treated with bilateral ovariectomy (bOE). Studies evaluating effects of bOE on unwanted behaviour in mares from owner's perspective revealed a high improvement. However, a pathohistological explanation to justify surgical ovarian removal regarding animal welfare is lacking. Therefore, the aim of the present study was to histopathologically evaluate bilateral removed macroscopically unremarkable ovaries in mares with behavioural problems. We hypothesized, that there are non-neoplastic changes in ovaries histologically detectable, which could be responsible for unwanted behaviour in mares. Bilaterally removed, clinically unremarkable ovaries from mares with behavioural problems ($n=20$) were immunohistologically evaluated and compared with pathohistologically confirmed granulosa cell tumours ($n=10$). Evaluation of immunohistochemical markers, Ki-67, Anti-Müllerian hormone, Aromatase, Epidermal Growth Factor Receptor, Calretinin, and Epithelial Cadherin, revealed no clear differentiation between large follicular structures of clinically unremarkable ovaries and cyst-like structures of neoplastic ovaries. Overall success rate after bilateral ovariectomy was 85% comparable to previous studies. Early neoplastic changes could be determined in 15% and anovulatory-like follicles in 30% of mares with bilaterally removed ovaries. These ultrasonographical non-detectable changes might be a pathohistological explanation for behavioural problems of ovarian origin and a reason for the high success rate of bilateral ovariectomy.

Use of a CPAP (Continuous positive airway pressure) helmet in a premature calf with respiratory distress syndrome

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A premature, female Holstein Friesian calf was born on day 235 of gestation because of emergency slaughter of the dam. At initial examination, the calf showed signs of immaturity and respiratory distress syndrome, tachypnea (60 breaths/min) with a rocking movement between the thorax and abdomen, hypothermia (37.1°C) and hypoglycemia (1.8 mmol/L). Initial arterial blood gas (aBG) analysis showed metabolic and respiratory acidemia (pH 7.04, pCO_2 75.7 mmHg, pO_2 29.8 mmHg, lactate (Lac) 4.2 mmol/L). Resuscitation consisted of clearing the upper airways, as well as drying and respiratory stimulation with towels. Initial stabilisation included intranasal O_2 administration, an intravenous (i.v.) bolus of theophylline, infusion with glucose and theophylline supplementation via an i.v. catheter, antibiotics and warming. Due to the respiratory pattern and aBG results, CPAP was commenced 1 h post-delivery for 5 h. After treatment, the calf became active, attempted to stand, drank 100 mL of colostrum and showed improved respiratory pattern, confirmed by aBG (pH 7.2, pCO_2 61.1 mmHg, pO_2 218.7 mmHg, Lac 6.2 mmol/L). Treatment with intranasal O_2, infusions with glucose and theophylline, antibiotics and colostrum were continued. 32 h after termination of CPAP the calf became apathetic with extended apneic phases and deteriorated aBG values (pH 6.8, pCO_2 178.7 mmHg, pO_2 63.6 mmHg, Lac 2.2 mmol/L). CPAP was restarted with no effect and euthanasia was performed. In conclusion CPAP can be an option to improve respiratory function in preterm calves. It is unclear why it worsened again and then became resistant to treatment.

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