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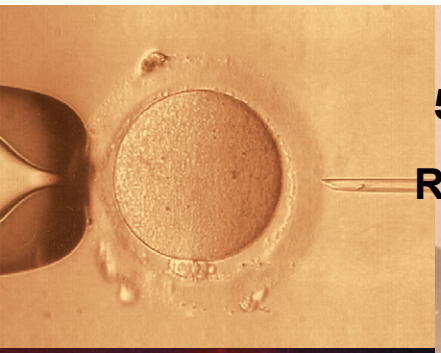

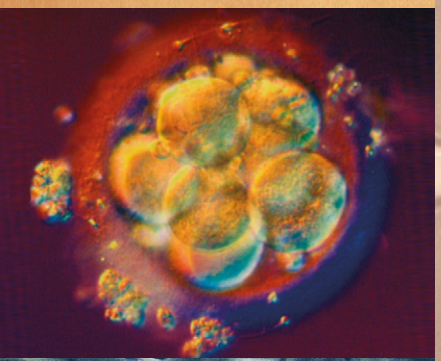

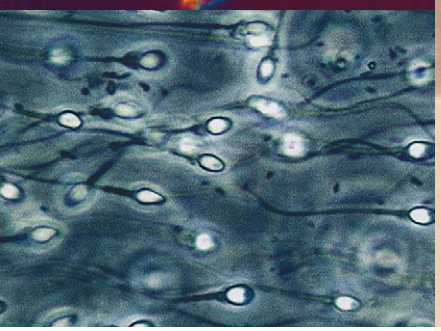

59th Annual Conference Physiology and Pathology of Reproduction and 51st Joint Conference on Veterinary and Human Reproductive Medicine at the Ludwig-Maximilians-University Munich, February 11th–13th 2026

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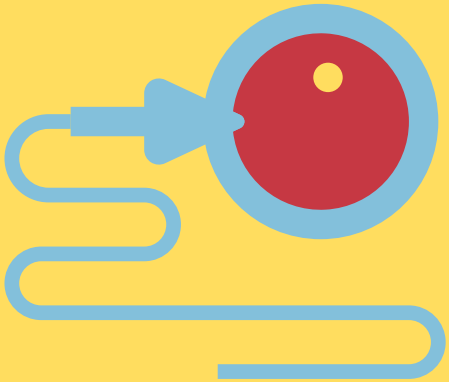

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From Bench to Bedside and Back

59th Annual Conference Physiology and Pathology of Reproduction
and
51st Joint Conference on Veterinary and Human Reproductive Medicine at the Ludwig-Maximilians-University Munich

February 11–13, 2026

"Connecting Perspectives in Human and Veterinary Reproductive Medicine" …

… is the motto of this year's Reproduction Conference in Munich.

On behalf of the Bavarian organizing team from the university, reproductive medicine practices, diagnostic laboratories, and clinics, we are delighted to welcome you to the Ludwig-Maximilians-University Munich from February 11–13, 2026.

The 59th Annual Conference on Physiology and Pathology of Reproduction, held jointly with the 51st Joint Conference of Veterinary and Human Reproductive Medicine, is dedicated to exploring modern developments in reproductive medicine through dialogue and comparison between veterinary and human medicine.

Our central goal is to highlight the fruitful exchange between these two disciplines at the heart of the Munich conference. Across ten thematic sessions, internationally recognized keynote speakers from both fields will present overview lectures, while early-career researchers will share their latest findings in short presentations.

Preparing this conference has been a great pleasure for the Munich organizing team. We were delighted by the strong response and the submission of more than 100 contributions. We expect an excellent attendance and a significant number of presentations from human medicine.

We sincerely thank the **DVG and the DGRM** for their valuable support in organizing this meeting, as well as all keynote speakers and participants for their dedication and interest.

Of course, a major scientific meeting is also a social event – and this will again be the case in Munich: the city itself needs no introduction. The conference dinner at the traditional **Augustiner-Bräustuben** promises to be a memorable evening filled with Bavarian hospitality and music.

Our venue, the **Biomedical Center (BMC)** in Munich-Martinsried, provides an inspiring and fitting setting for this event.

We look forward to welcoming you and to engaging discussions in Munich!

On behalf of the organizing committee
Viktoria von Schönfeldt and Holm Zerbe

Abstracts

Regression models for gestational age prediction and fetal sex estimation in Arabian horses

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This study aimed to establish precise gestational age estimation and evaluate the applicability of prenatal fetal sex determination in Arabian horses, a breed for which existing regression models may be inadequate due to their smaller, refined conformation. Seven maiden Arabian mares were inseminated, and transrectal ultrasonography was performed daily from ovulation to day 35, then weekly until day 91, and biweekly until parturition to monitor embryonic and fetal development. Parameters measured included embryonic vesicle (EV), crown-rump length (CRL), biparietal diameter (BIP), stomach diameter (STD), chest depth (CHD), abdominal diameter (ABD), kidney length (KDL), eyeball diameter (EBD), and eye lens length (ELL). The EV was first detected on day 10.3 ± 0.5, the embryo proper on day 21.3 ± 1.3, organization on day 33.6 ± 2.0, and ossification on day 57.7 ± 3.5 of gestation. All parameters except CRL, ELL, and KDL showed linear growth, with strong correlations with gestational age ($P < 0.0001$). Fetal sex was determined between days 56 and 161, with optimal accuracy between days 105 and 133, achieving 64.9% applicability and 98% accuracy.

These findings provide a chronological reference for fetal development and sex determination in Arabian horses, supporting more precise breeding management in this breed.

Changing patterns of infertility in female dromedary camels in Saudi Arabia: A clinical survey of 633 cases

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This study aimed to update data on the causes and mechanisms of infertility in female dromedary camels in Saudi Arabia. Between September 2022 and March 2024, 633 infertile females were examined following owner complaints, mainly repeat breeding with regular (62.2%) or prolonged (14.5%) intervals, and pseudopregnancy (21.3%). Gynecological examination revealed ovarian hydrobursitis (OH) in 275/633 (43.4%) cases (right: 13.1%, left: 45.1%, bilateral: 41.8%), clinical endometritis in 27.5%, overgrown follicles in 17.1%, vaginal/cervical adhesions or stenosis in 6%, salpingitis in 2.5%, ovarian inactivity in 1.3%, and apparently normal findings in 2.2%. Compared to earlier reports, OH rates increased while vaginal adhesions declined. OH, strongly linked to Chlamydia infection, is a major cause of long-term infertility, embryonic loss, and abortion. Clinical endometritis, mainly caused by *Trueperella pyogenes*, remains an important herd problem, often associated with unsanitary reproductive practices and postpartum complications. Overgrown follicles may result from hormonal imbalances (LH, FSH) or follicular defects. Vaginal/cervical adhesions often stem from trauma, chronic vaginitis, violent mating, or harmful local practices.

These findings highlight that infertility causes in camels are dynamic, emphasizing the need for continuous monitoring to guide prevention and treatment strategies.

Understanding infertility in female dromedary camels: From congenital disorders to management challenges

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This study highlights the main infertility-related reproductive disorders in female

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dromedary camels, outlining their categories, clinical signs, and treatment feasibility. Infertility causes are grouped into four categories: congenital, functional, pathological, and management-related. Congenital defects include ovarian agenesis, mesonephric duct aplasia, endometrial agenesis, double cervix/vagina, imperforate hymen, vulvar atresia, and intersex. Functional disorders comprise ovarian inactivity, overgrown follicles, and ovulation failure. Pathological causes include ovarian hydrobursitis, hydrosalpinx/pyosalpinx, clinical and subclinical endometritis, hydrometra, pyometra, vaginal and cervical adhesions, and neoplasms. Management-related infertility arises from mating errors, outdated practices, improper herder-to-camel ratios, and limited managerial skills. Pathological lesions and poor management practices represent the most significant contributors to infertility. Clinical manifestations typically include repeated breeding (with regular or irregular cycles), refusal to mate, difficulty or bleeding during mating, and male-like behavior.

While several therapeutic approaches for endometritis are effective and carry a favorable prognosis, ovarian hydrobursitis and vaginal adhesions remain challenging to treat and often result in poor outcomes. Improved management strategies and preventive measures are essential to enhance reproductive efficiency in dromedary herds.

Ovarian hydrobursitis and chlamydial infection in dromedary camels: Diagnosis, impact, and management

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Chlamydia is a major cause of reproductive disorders in women and animals. In dromedary camels, Chlamydia abortus has been isolated from bursal tissue and fluid in cases of ovarian hydrobursitis, a chronic reproductive condition characterized by fluid accumulation, ovarian encapsulation, infertility, and complications such as early embryonic death, abortion, and repeated breeding failure. Diagnosis is difficult by palpation but reliably achieved through ultrasonography, where the ovary appears entrapped in fluid of variable echogenicity. Ovarian hydrobursitis contributes to 30–40% of infertility cases in camels and is often associated with endometritis, adhesions, pyometra, or tubal enlargement. Treatment options include surgical ablation in unilateral cases (with ~60% conception success) or oxytetracycline therapy for small bursae, though bilateral involvement remains untreatable. Antibodies to Chlamydia abortus were detected in 13.5% of infertile males, which often presented with testicular and preputial lesions.

Given the difficulty of treating advanced cases, camel immunization against Chlamydia is under consideration.

Surgical management of severe uterine torsion in a mid-gestation dromedary camel

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A 14-year-old multiparous dromedary camel (parity five) at six months of gestation was presented with a rare case of high-degree uterine torsion. The animal had shown inappetence and nervous manifestations, particularly involving the head and neck, for four days, in addition to heavy tick infestation. Vaginal examination revealed a closed cervix under marked tension, while rectal palpation confirmed a severe right-sided torsion, with the left broad ligament stretched across the uterus and cervix. The uterus was twisted approximately four complete turns anterior to the cervix, forming thick layered folds at the caudal uterine region. Ultrasonography demonstrated normal fetal fluids, placenta, and part of the umbilical cord, suggesting fetal viability. Hematological findings indicated leukocytosis with marked neutrophilia, whereas biochemical analysis revealed hyperglycemia, increased creatinine, and minor alterations in electrolyte balance and enzyme activity. Surgical correction was performed via laparotomy, during which the uterus was rotated manually four times to the left to restore its anatomical position. Following detorsion, the fetus was found alive and mobile, and the head advanced toward the cervical os. The dam regained normal appetite, and the nervous signs resolved.

This case underscores the importance of combined rectal and ultrasonographic examinations in diagnosing severe uterine torsion in camels and highlights laparotomy as an effective treatment approach to correct high-degree torsion and preserve fetal viability.

Profound effects of dexamethasone on the proteome of human testicular peritubular cells

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Human testicular peritubular cells (HTPCs) are located between the seminiferous epithelium and the interstitial areas of the testis. They enable sperm transport, produce ECM and exert immunological roles. Expression of the glucocorticoid receptor (GR) indicates that they are targeted and regulated by glucocorticoids (GCs). As the consequences of GC actions are not fully known, we examined the actions of dexamethasone (Dex), a synthetic GC also used in the clinic, which is also used in the clinic, in cultured HTPCs and performed deep proteome analysis. To assess time-dependent effects of Dex on HTPCs, cells were treated for 7 and 14 days. Global proteome changes were identified using high-resolution liquid-chromatography and tandem mass spectrometry (LC-MS/MS). Data were processed and evaluated using sophisticated statistical workflows. Differentially abundant proteins were visualized with Volcano plots, and bioinformatically explored using PROTEOMAPS and REVIGO treemaps. Dex treatment resulted in 177 and 283 differentially abundant proteins at 7 and 14 days, respectively. The analysis revealed strong regulation of proteins related to ECM organization and adhesion, lipid metabolism and redox-response, and stress-response. In contrast, proteins associated with intracellular signaling and transcriptional regulation were reduced.

In conclusion, Dex treatment leads to time-dependent proteomic remodeling of HTPCs, marked by increased ECM- and metabolism-related proteins and decreased signaling and transcriptional regulators.

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Dystocia in alpacas: Retrospective evaluation of 35 clinical cases in Southern Germany

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Dystocia is rare in alpacas, yet uterine torsion represents the most common obstetric emergency and a primary indication for cesarean section. The aim of the study was to analyze the causes, course, and treatment outcomes of obstetric emergencies in alpacas presented to a veterinary clinic in Southern Germany. Data from all obstetric alpaca patients treated surgically or conservatively between January 2017 and August 2025 were retrospectively evaluated. Among the 35 dams included, manual correction was successful in six cases, while 29 were treated by cesarean section. After an average hospitalization of 3.7 days, 32 animals (91.4 %) were discharged; 24 of the 30 long-term survivors recovered without complications. The most common cause for presentation was uterine torsion (12/35), followed by foetal malpresentation (8/35). Retained fetal membranes were frequent but rarely clinically significant, and postoperative wound healing after cesarean section was very good. Thirteen crias (37.1 %) were stillborn, and 22.7 % of the live-born crias died within the first days or had to be euthanized. Of the 25 surviving dams which received a cesarean section, ten were bred again, and eight (80 %) became pregnant.

In summary, rapid decision-making is crucial in alpaca obstetrics. Due to the anatomy of the dam and the cria, manual correction is often challenging. In case of cesarean sections, the left flank approach provides good prognoses for maternal survival and fertility.

The value of bacteriological culture examination in canine breeding: Are vaginal swab results repeatable?

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Vaginal swabs (VS) are demanded by owners and therefore collected during breeding management examinations in healthy bitches. Their diagnostic value depends on the repeatability of bacterial culture findings. This study aimed to assess the intra-individual repeatability of bacteriological results from canine VS using conventional culture examination. Three VS each were collected from 20 clinically healthy oestrous bitches using a tube speculum. Samples were analysed by routine microbiological methods, with semiquantitative evaluation of growth [+, ++, +++]. The isolates identified were in good agreement with the literature. In terms of bacterial quality, all VS yielded identical results in 7/20 bitches, in additional 9/20 bitches 2 of 3 results were identical. For assessment, the VS with most positive findings was used for comparison with the others. The maximum number of isolates per sample and dog ranged from 1 to 5, resulting in 60 findings in all 20 bitches. The respective isolate was detected in all 3 VS of a bitch in 37/60 findings. Noteworthy, in 19 of the remaining 23 findings only low-grade bacterial growth was found in the isolate-detected VS. It was absent in the other(s). Scoring of bacterial growth matched for all 3 VS in 23/37 findings and for 2 of 3 scores in 11/37 findings, but all 3 results differed in the remaining 3/37. In one bitch all 3 samples were different. In terms of quality and quantity, all 3 VS had the same results in 4/20 bitches.

In conclusion, repeatability of bacteriological results from VS obtained from healthy breeding bitches in oestrus was limited. This should be considered to prevent antimicrobial treatment in healthy breeding bitches.

LH-induced differentiation of bovine theca and granulosa cells in a co-culture model

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The luteinizing hormone (LH) surge is a critical regulator of follicular development and ovulation. To investigate the coordinated effects of LH on theca and granulosa cells, we applied a co-culture system mimicking the follicular structure. This system utilizes a permeable membrane insert to separate theca and granulosa cells, culturing them on opposite sides to reflect the in vivo compartmentalization. Earlier, we could outline the physiological nature of this co-culture system. In the present study, we assessed the differentiation potential of this co-culture model in the absence of androstenedione but with supplementation of 100 ng/ml LH. LH treatment induced a shift in steroidogenesis, decreasing estradiol production and increasing progesterone concentrations in the media. Quantitative PCR analysis revealed that LH significantly downregulated CYP19A1 and FSHR expression in granulosa cells and CYP17A1 expression in theca cells. Furthermore, LH upregulated RGS2 expression in both cell types, while HSD3B1, STAR, PTGS2, PTX3 and VNN2 expression increased specifically in theca cells.

These findings demonstrate that this co-culture system has the potential to mimic the LH-induced differentiation of bovine theca and granulosa cells, providing a valuable in vitro model to study the molecular mechanisms underlying the early post-LH phenotype and the process governing ovulation. Furthermore, this model offers a robust platform for investigating the effects of environmental toxicants on ovulatory function.

Follow-up study on outcome and long-term effects after teat amputation in dairy cows

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Teat injuries should be treated promptly to prevent intramammary infections and enhance animal welfare. However, little is known about the production loss in dairy cows with teat injuries. This study aimed to investigate the effects of closed teat amputation on wound healing, milk production, and longevity. Retrospectively, clinical data, DHI records, and culling reasons were evaluated in 196 dairy cows that had undergone closed teat amputation. The data was analysed using linear mixed-effects models (R version 4.3.2). Interestingly, in 57% of cases, the cows were in peak lactation, ranging from day 22 postpartum to day 213 of gestation. When comparing the 305-day milk yield of the previous and subsequent lactations, a closed teat amputation resulted in an average milk production loss of only 9.44%. A total of 87.04% of the operated animals remained in the herd until the end of their lactation. 74.07% remained for one more lactation, and 41.67% for more than one lactation after teat amputation. Furthermore, the teat-amputated animals in the study had an average culling age that was 1.79 years higher than the average reported in Bavaria.

The data reveal that closed teat amputation results in a smaller-than-expected reduction in milk yield. Therefore, this therapeutic measure is not only medically but also economically viable and practically relevant. It contributes to longevity and, consequently, improves sustainability and animal welfare in dairy farming.

Human menopausal gonadotropin is a low-cost in vitro oocyte maturation alternative in cattle

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In cattle, in vitro oocyte maturation (IVM) is performed routinely by adding gonadotropins to the culture such as, recombinant FSH, porcine pituitary extracts (FSH-LH) or equine chorionic gonadotropin (eCG/PMSG). Especially for labs processing numerous oocytes, the cost for these hormones can be substantial. However, in case of eCG for example, which has been an inexpensive and efficient alternative for IVM for many years, animal welfare concerns in Europe have restricted its commercialization. As the product is not available in several countries, substitutes are needed. The use of human menopausal gonadotropin (hMG) has been reported for cattle IVM, but is not used as a standard. Therefore, we compared the effects of hMG (75mIU/ml, Merional® HG 150 IE, IBSA) against eCG (10 IU/ml eCG and 5 IU/ml of human chorionic gonadotropin (hCG) PG600® MSD Animal Health GmbH) supplementation during IVM on embryo developmental rates. Cattle ovaries were collected from a local abattoir. A total of 5653 oocytes was obtained by slicing and matured for 24h. Thereafter, oocytes were fertilized for 19 h and the presumptive zygotes were cultured for 8 days. Higher blastocyst rates were obtained using eCG-hCG (39.2%, p = 3.15e-09) compared to hMC (32.6%). Regarding developmental speed, blastocyst stage was reached likewise on day 7 (47.8% and 46.4% for eCG and hMG respectively) and day 8 (52.2% and 53.6% for eCG and hMG respectively).

Our results indicate that despite a slightly lower blastocyst production, hMG is a suitable low-cost substitute for IVM in cattle.

Teat cleaning in an automatic milking system – risk factor for animal health

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Automatic milking systems (AMS) are now the dominant milking method in new build dairy barns in Germany. Despite their long-standing market presence, challenges remain in the area of animal health – particularly with regard to udder health. Key risk factors for suboptimal udder health in AMS operations are udder preparation and automatic mastitis detection. Our goal was to demonstrate a relationship between suboptimal udder preparation in AMS and udder health. For this purpose, 1,980 milkings were documented over a period of nine months using 16 milking robots on a farm with 855 German-Holstein cows (28.2 kg daily milking yield) in a batch milking system. The quality of teat cleaning (complete, partial, no cleaning) was recorded. In addition, milk flow curves were retrospectively evaluated for 989 milkings, and somatic cell counts from four milk yield tests were included. Descriptive data analysis showed that in 55.3% of milkings, udder preparation was only partially or unsuccessfully performed. Altered milk flow was observed in 20.2% of milkings. Milk flow was more often altered in cows with repeated suboptimal cleaning. Furthermore, the somatic cell count was higher in cows with altered milk flow and in cows

with suboptimal preparation than in cows with optimal cleaning and optimal milk flow. Groups (altered milk flow vs. unaltered milk flow and suboptimal udder preparation vs. optimal udder preparation) were compared using a t-test.

These results highlight the continuing technical challenges of automated milking and further underscore the importance of accompanying management factors for ensuring udder health.

Basic medium composition, but not hydroxypropyl-cellulose, determines the post-warming viability of pooled, in-straw vitrified cattle blastocysts

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Efficient pooled-embryo cryopreservation is essential for polytocous species including the pig, where transferring multiple embryos is required for pregnancy establishment. Using biosecure closed devices (e.g. 0.25 mL straws) is challenging, as they exhibit slow thermal exchange, thereby reducing cryo-survival. Here, using cattle embryos as a model, we evaluated if 100 µg/mL of hydroxypropyl cellulose (HPC) could improve the cryo-survival rates of in vitro-produced (IVP) day 7–8 cattle blastocysts vitrified in pools of 7–11. Embryos (n = 616) were vitrified using six treatments structured by basic medium TCM199 or Tyrode's Lactate (TL) and supplements T1 (TCM199-FBS), T2 (TCM199-FBS+HPC), T3 (TCM199+HPC), T4 (TL-PVA), T5 (TL-PVA+HPC) or T6 (TL+HPC). Fresh, non-vitrified embryos served as controls. No overall significant differences in 48 h re-expansion rates (ranging from 40.7–69.5 %) were observed, although T1 had higher rates than T4 (69.5 ± 5.8 vs 40.7 ± 6.4, respectively; $P < 0.05$). All vitrified groups had lower 48 h hatching rates (range 23.0–43.6%; $P < 0.05$) than controls (96.8%), with no significant differences among treatments. However, pooling data by basic media revealed that TCM199-based media yielded higher re-expansion and hatching rates at 48 h than TL-based media (39.7 vs 25.9%, respectively; $P < 0.05$).

In conclusion, HPC supplementation did not improve post-warming viability. Instead, the choice of base medium was the dominant factor affecting embryo survival. TCM199 offered a more critical protective buffer against cryo-injuries than TL. Optimizing basic medium composition appears more critical than supplementation with HPC.

Expression and pathogen mediated induction of S100 proteins in ovine mammary explants

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Antimicrobial peptides (AMP) are key molecules for the resolution of intramammary infection and are significantly upregulated during mastitis in cows. The AMPs S100A8 and S100A9 have been suggested as diagnostic markers for ovine mastitis, however little is known about their regulation during intramammary infection. Aim of the study was to establish an ovine mammary tissue model based on a successfully established bovine model to investigate immunoregulatory processes during host-pathogen-interaction. Ten healthy sheep (age 4–7 years) in their third month of lactation were applied as donor animals on the day of weaning. Tissue explants of mammary gland parenchyma were taken aseptically immediately after slaughter and were processed for in vitro challenge experiments with lipopolysaccharide (LPS) and heat inactivated *S. aureus*. Abundance of mRNA transcripts for S100A8, S100A9 and the defensin SDB2 were analysed using RTq-PCR. Statistical analysis war carried out using Mann-Whitney-test. LPS but not *S. aureus* significantly ($P < 0.05$) upregulated expression of S100A8 and S100A9. However unstimulated ovine udder tissue displayed comparingly higher expression levels than triton-X-treated ovine and untreated bovine mammary tissue suggesting non-specific upregulatory effects. There was no significant regulation of SDB2 in ovine mammary tissue detectable.

In summary, ovine mammary explants can successfully be cultivated in vitro, however they may not serve as surrogate model for studying bovine mastitis.

Plasma steroidomic profiling in women and cattle during natural and stimulated reproductive cycles using high-resolution mass spectrometry

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Steroid hormones and their metabolites (steroidome) shape female physiology and fertility, yet the functions of many remain incompletely understood. We aimed to comprehensively profile 61 target steroidome and prostaglandin metabolites (PGM) in plasma to uncover their dynamics throughout the reproductive cycle. An ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) workflow was developed and validated for quantitative analysis of the steroidome and PGM. Plasma samples collected daily and twice-daily from three heifers and four women undergoing ovarian stimulation across their natural and super ovulatory cycles (n = 244 and 58, respectively) were profiled. The UHPLC-HRMS workflow quantified the 61 targets within 30 minutes from 250 µL plasma with high sensitivity (limits of quantification 5–200 pg/mL). In total, 36 and 43 targets were detected in heifer and women plasma, respectively. Interestingly, in cattle, three tetrahydro-cortisol/cortisone metabolites significantly peaked one day before ovulation in both natural and stimulated cycles. In women, concentrations of conjugated estrone/estradiol, androsterone, and hydroxy/dihydro-progesterone metabolites increased as ovulation stimulation progressed.

The workflow revealed unique endocrine dynamics across natural and stimulated cycles and has the potential to unravel the origin of heterogeneous ovarian stimulation responses. Leveraging cattle as a valuable model for human reproductive research, this work demonstrates the translational potential of this platform from animal breeding to clinical IVF applications.

Effects of an intrauterine carbon matrix with high adsorption capacity, administered to postpartum dairy cows, on antibiotic use and fertility in a commercial farm

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Dairy cows are susceptible to postpartum metritis. As antibiotic use in food-producing animals must be reduced to limit resistance, alternative treatments are needed. Ultrapure carbon sorbent matrix proved to retain bacteria and toxins in media and in women with uterine infections. This study evaluated the effect of a carbon matrix administered in postpartum dairy cows on: i) antibiotic use due to uterine infection and ii) fertility. A total of 120 Holstein-Friesian cows were included and randomly assigned to carbon (Carb, n = 50) or control (Ctrl, n = 70) group. Treatment consisted of the insertion, 24–48h after calving, through the cervix and under aseptic condition, of two cellulose boluses each containing 19.8 ± 0.2 g of microporous Carb spheres (300 m2/g of adsorbing surface, 0.5–1.0 mm diameter). One case of puerperal metritis was observed in Ctrl group. Treatment did not significantly affect the risk of receiving antibiotic due to metritis diagnosis, nor fertility, in primiparous cows. In multiparous cows, increased odds of receiving antibiotic in Carb (OR = 5.50, $P = 0.05$) was observed, while open days were reduced (157.78 ± 12.51 in Ctrl vs. 117.92 ± 11.83 in Carb, $P < 0.05$). The general linear model excluded the effect of the interaction between treatment and antibiotic on fertility ($P = 0.316$).

Although not optimal, these results suggested that intrauterine microporous matrices may help restore the uterine environment after calving and improve clearance, probably due to initial stimulation of inflammatory response; future studies should aim to mitigate exudate characteristics, which probably influenced the veterinarian decision to administer antibiotic treatment.

Derivation of roe deer embryonic stem cells to model embryonic diapause in vitro

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Embryonic diapause is a reproductive strategy that allows roe deer *(Capreolus capreolus)* to optimize the birth timing of their fawns in spring. In roe deer, diapause is characterized by reduced proliferation and slowed developmental progression, which delays the implantation of the blastocyst in autumn. Although this phenomenon was first reported in 1854, the molecular mechanisms governing this process are still largely unknown. Our research project aims to use an in vitro model to elucidate the regulatory mechanisms governing embryonic diapause and reactivation in roe deer. While pluripotent stem cells have been used for decades to recapitulate the epiblast of the blastocyst, no pluripotent stem cell lines have been derived before from obligate diapausing species, such as the roe deer. Therefore, we collected diapausing blastocysts from hunted roe deer between September and December and isolated the inner cell mass to derive embryonic stem cells. We tested various culture conditions, including plate coatings (geltrex, vitronectin, feeders, laminin) and media supplements, like Activin A, XAV939, FGF2, IWP2, LIF, and serum. Currently, five cell lines have been successfully established on geltrex with either AFX or AFI media, and show promising expression of pluripotency markers (OCT4, NANOG, SOX2, and KLF4) detected by RT-qPCR and/or immunofluorescence, though full characterization and validation are still in progress.

This in vitro model will be used to study the effects of diapause-associated factors on cell proliferation and development progression, providing insights into the regulation of embryonic diapause and developmental timing.

Predictive value of assessment of sperm quality in fresh semen by using flow cytometry assays and CASA for the quality of cryopreserved semen in warmblood stallions

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This study assessed whether routine fresh-semen metrics capture biological determinants of freeze-tolerance and enable pre-freeze prognostication, and whether predictive relationships are conserved across stallions. Four ejaculates from each of 20 stallions were assessed fresh and post-thaw. Multicolor flow cytometry quantified PMAI, MMP, and intracellular Ca^{2+}. CASA measured total/progressive/circular/immotile fractions. Associations were summarized by Pearson's r (two-sided p), stallion-level heterogeneity by ANOVA, and multivariable standardized OLS related post-thaw outcomes to fresh PMAI/MMP/low-Ca and RAPID. Across 80 matched pairs, PMAI showed a moderate fresh to post-thaw link ($r = 0.63$; $R^2 \approx 0.40$; $p < 0.001$). MMP correlated similarly ($r = 0.57$; $R^2 \approx 0.33$; $p < 0.001$), whereas low-Ca was weaker ($r = 0.39$; $R^2 \approx 0.15$; $p < 0.001$). The fertile population (PMAI-hight, MMP-high and low-Ca), approximated from marginals, was moderate CASA RAPID showed the most correspondence ($r = 0.69$; $R^2 \approx 0.48$; $p < 0.001$). Variance-component models attributed substantial post-thaw variance to stallion and ejaculate. Multivariable models improved correspondence.

Fresh viability (PMAI), mitochondrial membrane potential (MMP) and RAPID are biologically coherent yet only moderately predictive of post-thaw quality. Stallion- and ejaculate-level components explain a sizeable fraction of residual heterogeneity. Fresh testing can inform pre-freeze triage and management but cannot replace stallion- and ejaculate-specific strategies to optimize cryopreservation outcomes.

Breed-specific ovarian responses to the GnRH agonist in mares: Implications for estrus and ovulation indices

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This study investigated mares breed-specific effects on key reproductive indices. A total of twenty-seven cycling mares, comprising Thoroughbreds (n = 9), Ponies (n = 8), and Arabians (n = 10), received a single injection of Deslorelin during estrus. Parameters monitored included estrus length, the interval from treatment to ovulation (T-O), diameter of the ovulatory follicle and subsequent corpus luteum (CL) and ovulation rate. T-test and and chi-square were used to statistically compare means and ovulation rate in different groups (SPSS version 0.25), respectively. Results revealed significant differences ($P < 0.05$) among breeds for several critical outcomes. The T-O interval was markedly shorter in Pony mares (24.81 ± 0.61 hrs, $P < 0.01$) compared to both Thoroughbred (96.37 ± 1.29 hours) and Arabian (111 ± 17.19 hours) mares. Furthermore, Pony mares exhibited a significantly higher number of ovulations (1.8 ± 0.17, $P < 0.05$). Although ovulation rates were generally high across all groups (83.3% to 100%, $P < 0.2$), the diameter of the resulting CL was largest in Thoroughbreds (38.87 ± 0.66 mm, $P < 0.05$). Estrus duration also varied, being shortest in the Pony group (3.12 ± 0.65 days, $P < 0.05$).

These results demonstrate a profound breed-dependent ovarian response to exogenous GnRH administration. The markedly accelerated ovulation in Ponies suggests a heightened sensitivity to the agonist. These findings are critically important for developing optimized, breed-specific estrus synchronization and ovulation induction protocols in equine practice, thereby improving reproductive efficiency.

Dynamic changes of small, medium, and large follicles during the estrous cycle in thoroughbred mares

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Follicular growth and regression in mares are a dynamic process, and understanding the interaction among small, medium, and dominant follicles provides insight into ovulatory regulation and reproductive efficiency. This study investigated follicular dynamics in thoroughbred mares (n = 9). Follicles were classified as small (< 20 mm), medium (20–< 30 mm), and large/dominant (> 30 mm). Transrectal ultrasonography was performed daily across the estrous cycle, with Day 0 designated as ovulation. Follicular counts and diameters were recorded, and corpus luteum (CL) development was monitored. Data were analyzed in relation to pre- and post-ovulatory days. Small follicles were consistently present throughout the cycle, showing modest fluctuations but no distinct peri-ovulatory peak. Medium follicles increased in number approximately 8–12 days before ovulation, reflecting recruitment and selection. A progressive decline was observed as a dominant follicle emerged. Large follicles (> 30 mm) developed steadily toward ovulation, with the pre-ovulatory follicle reaching maximum diameter just before Day 0. After ovulation, the dominant follicle regressed, coinciding with rapid CL formation. Subordinate follicles regressed after the dominance shift, with minimal growth in the luteal phase. The CL reached maximal diameter between Days 4–10 post-ovulation before stabilizing. The coordinated rise and fall of small and medium follicles reflect continuous recruitment, while the dominant follicle suppresses subordinate growth, ensuring a single ovulatory event.

Thoroughbred mares exhibit distinct follicular patterns with a clear dominance hierarchy leading to ovulation.

Fetal mummification in an Arabian mare: A rare case with radiographic and histopathological findings

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Fetal mummification is a rare condition in equines. We report a unique case in a six-year-old pluriparous Arabian mare which foaled a healthy colt after normal delivery. Approximately 30 minutes postpartum, the mare expelled a hard, round, handball-sized bony mass, coinciding with the passage of fetal membranes. The mare had experienced disturbed health conditions during the 4^{th} and 10^{th} months of pregnancy but otherwise maintained normal clinical status. Hemogram and blood chemistry analyses were within normal reference ranges, confirming the absence of systemic illness. Radiographic examination revealed a well-organized mummified fetal structure with irregular skeletal outlines. An undefined bony formation was

closely associated with the skull region, while adjacent soft tissues exhibited histological features resembling undifferentiated neural tissue. These findings suggested congenital cranial and neural developmental anomalies that likely contributed to fetal demise and subsequent mummification. Histopathological assessment confirmed degenerative fetal tissue changes and preserved soft tissue architecture resembling brain parenchyma. No signs of infection or toxemia were present, consistent with sterile mummification. The mare recovered uneventfully without systemic complications.

This case highlights the potential role of congenital malformations in the pathogenesis of fetal mummification in horses. It underscores the value of integrating clinical, radiographic, and histopathological findings to reach a definitive diagnosis. Reporting such rare presentations provides new insights into equine reproductive disorders.

Student perceptions of sensor technologies in dairy farming

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Digital technologies are offering advanced tools to monitor and manage animal health, welfare, and productivity. This study aimed to explore how students in veterinary medicine (VetMed) and agricultural sciences (AgriSci) perceive sensor technologies on dairy farms and how well their academic training prepares them for this digital transformation. An online survey was distributed across universities in Austria, Germany, and Switzerland. A total of 428 questionnaires were analysed (295 VetMed, 133 AgriSci). Overall, students demonstrated a high level of acceptance toward sensor-equipped cows. AgriSci students were notably more positive, with 69.2% agreeing that sensors offer benefits for both, animals and workers, compared to 44.2% of VetMed students. However, skepticism was also evident, particularly among VetMed students, who expressed discomfort with practices like automated calf feeding and early cow-calf separation. A significant portion of respondents feared that increasing automation could undermine the human-animal bond. Students emphasized animal welfare as the most important motivation for adopting digital tools, followed by reducing pharmaceutical use. Only 20.7% of VetMed students reported feeling well prepared for the digital shift in dairy farming, versus 37.6% of AgriSci students.

The findings underscore a clear educational gap in preparing students for digital integration in livestock farming. In conclusion, while future professionals recognize the potential of digital technologies to enhance dairy farming, concerns about reduced animal interaction and inadequate training must be addressed.

The uterine microbiome composition and fertility in the mare

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Subfertility in mares is a major problem for horse breeding, due to repeated ineffective treatments. Reduced uterine receptivity is a key cause, but the role of the uterine microbiome remains poorly understood. This study aimed to compare the uterine bacterial microbiota profiles of fertile and subfertile mares using 16S rRNA gene V3-V4 amplicon sequencing. We included 19 fertile mares, 18 subfertile mares with persistent breeding-induced endometritis (PBIE), and 17 subfertile mares without a clear diagnosis. Uterine cytobrush (uCB) and low volume lavage samples (LVL) were collected. RNA and DNA were isolated from eCB using the AllPrep DNA/RNA/miRNA Universal kit. LVL samples were centrifugated at 16,000 x g and the pellet was resuspended in PBS and RLT Plus buffer with DTT. DNA and RNA extraction were performed with the AllPrep DNA/RNA Micro kit. Amplicon generation was performed using the universal Illumina primers Pro341F and Pro805R, as previously described (Dyroff et al. SciRep 2025). After barcoding, amplicons will be sequenced on an Illumina Nextseq2000 platform. Bioinformatic analysis will compare microbial profiles between groups.

Preliminary results suggest that fertile mares show higher microbial diversity than subfertile mares, supporting the hypothesis of a link between microbiome composition and uterine receptivity.

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Comparative RNA-seq analysis of roe deer *(Capreolus capreolus)* and cattle *(Bos taurus)* embryos during peri-implantation development

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Embryonic diapause, the reversible halting or reduction of developmental velocity, is a remarkable reproductive adaptation in mammals. It naturally occurs in the seasonal roe deer but not in cattle, despite shared features of embryo development such as conceptus elongation prior to implantation. To elucidate the molecular basis of these developmental strategies, we first generated a novel roe deer genome annotation. We then performed single-embryo RNA sequencing of roe deer embryos spanning diapause to elongation and compared them with in vivo-derived cattle blastocysts and elongated embryos. Our analyses revealed both conserved developmental trajectories across species and profound transcriptional reprogramming during diapause. Roe deer diapause embryos downregulated oxidative phosphorylation (OP), mitochondrial biogenesis, and protein glycosylation, metabolic pathways active in cattle blastocysts, while maintaining lineage-specific gene expression, suggesting a metabolically quiescent yet developmentally competent state. Lipid metabolism and steroid biosynthesis were enriched supporting diapause. Upon reactivation, roe deer embryos showed a transcriptional switch, with strong induction of cell-cycle, ribosomal, and OP genes reflecting the energetic surge of elongation. Notably, while cattle rely on interferon tau (IFNτ) to sustain elongation, we confirmed that roe deer achieve this transition independently of IFNτ.

Overall, our study reveals the molecular and metabolic basis of diapause and elongation, highlighting plasticity in reproductive timing and offering new perspectives for fertility management and conservation.

Pregnanolone, likely of placental origin, indicates timing of implantation and previous reactivation from embryonic diapause in roe deer *(Capreolus capreolus)*

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The roe deer exhibits a unique reproductive strategy known as embryonic diapause characterized by reduced development at the blastocyst stage. Breeding occurs in July–August, followed by a 4–5-month period of diapause before reactivation and implantation in December–January and parturition in April–May. Following ovulation, the corpus luteum (CL) remains active, producing progesterone (P4) in both pregnant and non-pregnant does until April. We utilised high-resolution mass spectrometry to investigate the role of progestogens in roe deer reactivation and implantation by analysing plasma samples frequently collected from enclosed does (December–April) and CL and placental tissue from regular huntings (September–January). Interestingly, we observed an early decline in plasma P4 concentrations in February–March in both pregnant and non-pregnant females. Notably, the neurosteroid pregnanolone (3α-hydroxy-5β-pregnan-20-one) appeared exclusively in pregnant does in late December/early January. Tissue steroid profiling revealed pregnanolone in placental but not luteal samples, indicating placental origin. The temporal and tissue-specific pattern suggests that pregnanolone reflects the timing of implantation, thereby elucidating

the reactivation phase following diapause and enabling determination of both diapause duration and true pregnancy length in roe deer. The role of pregnanolone in gestated pregnancy as well as whether it, or other steroids, overtakes the role of P4 in pregnancy remains to be unravelled.

This discovery opens new perspectives on neurosteroid functions in implantation and pregnancy.

Bovine cervical explants show lower vitality compared to endometrial explants under identical culture conditions

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Inflammatory processes in the bovine postpartum uterus remain a major cause of poor fertility. To examine underlying mechanisms, an endometrial explant (EE) model was established. This study aimed to adapt it to cervical explants (CE). 13 clinically healthy uteri from cows of common breeds aged 21–153 months were used. Incisions were made in the uterine horn ipsilateral to the corpus luteum and the cervix from a dorsal aspect. 74 EE and 58 CE were collected using a 5 mm biopsy punch and stored in PBS at 6 °C for ~1 h. Under sterile conditions, residual tissue was removed. One explant per well of a 24-well plate, containing 1 mL DMEM with penicillin, streptomycin and amphotericin, was pre-incubated at 6 °C for 1 h, followed by medium change and incubation at 37 °C, 5.5% O_2, and 5% CO_2 for 3 h and 24 h hours. For viability assessment, 100 µL WST-8 was added at 0 h or 21 h and optical density (OD) was measured 3 h later. Transport on ice significantly improved explant vitality after 24 h compared to room temperature (separate dataset, $p < 0.01$). Means and differences between groups were calculated by mixed-effects regressions with R4.5.0. OD was 0.62 ± 0.27 in CE and 0.78 ± 0.28 in EE at 3 h and increased to 0.76 ± 0.36 in CE and to 0.91 ± 0.37 in EE at 24 h. This increase between 3 h and 24 h was significant in EE ($p = 0.048$) only. Vitality tended to be higher in EE at 3 h ($p = 0.062$) and was significantly higher at 24 h ($p = 0.017$).

In conclusion, CE show lower vitality compared to EE under identical conditions. But cervical explants showed acceptable vitalities for further use.

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Combining R848 with microfluidic chips for sperm sexing: From concept to practical application in cattle

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In recent studies, Resiquimod (R848), a toll-like receptor 7/8 agonist (TLR7/8) was used for separating X-/Y-sperm. Following this approach, microfluidic chips (MC) were introduced into the R848-procedure and outcomes were evaluated through CASA and flow cytometry to determine sperm quality parameters and X-/Y-sperm ratio comparing MC to swim-up (SU). Diluted fresh (F), and cryopreserved sperm (CRY), from six bulls were separated by SU (SU-Group) or MC (MC-Group), with (Group R) and without R848 (Group C, control group). Sorting with MC yielded sperm with significantly higher proportions of fast-moving sperm than SU in the upper layer (UL) in comparison to the lower layer (LL) in for F (MC-Group C: UL 80.93 ± 7.11% vs LL 47.62 ± 14.42%; Group R: UL 34.39 ± 11.68% vs. LL 12.19 ± 10.23%, $p < 0.0001$) and CRY (MC-Group C: UL 78.39 ± 5.08% vs. LL 13.77 ± 6.59%; Group R: UL 57.60 ± 8.86% vs. LL 7.22 ± 4.99%, $p < 0.0001$). Sorting with MC also exhibited significantly lower plasma and acrosome membrane damage in UL and LL, compared to SU for F (Group C: 0.68 ± 1.29% vs. 7.55 ± 4.84%; Group R: 1.14 ± 0.80% vs. 9.35 ± 4.58%; $p < 0.0001$) and CRY (Group C: 4.12 ± 4.06% vs. 30.98 ± 12.75%; Group R: 5.37 ± 3.33% vs. 34.89 ± 13.41%; $p < 0.0001$).

In conclusion, despite improving sperm quality, none of the methods achieved a significant enrichment of X- or Y-sperm ($p > 0.05$). Although MC enhanced sperm quality and lowered membrane damage, sex enrichment remained insignificant. Further refinement could expand their use in cattle breeding.

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Comparison of sperm preparation techniques prior to bovine IVF – preliminary data

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Sexed semen is commonly used in artificial insemination and in-vitro fertilization (IVF), but still quite below conventional semen usage. Two preparation techniques of sperm cells were tested prior to IVF. First, 2 conventional and 2 sexed sperm doses were thawed and pooled separately. Progressive motility (Mot), sperm concentration (Con) and cell counts (Cell) were measured using a CASA system. Live sperm cells were determined after eosin staining. Sperm were prepared either via density gradient centrifugation (DGC) or via swim-up technique (SU), using 150 µl sperm suspension each. After preparation, Mot, Con, Cell and live sperm cells were evaluated for every sexing and preparation method. At least 4 groups with doses of 2 bulls were used in 6 repetitions. For DGC, 90% density gradients containing Sperm Filter® (Gynotec) were used. Preparation via SU lasted 1 h after the sperm were layered under SpermTALP. After DGC, conventional and sexed semen showed significantly increased Mot (34.6 vs. 58.2%, 20.8 vs. 50.5%), conventional semen also after SU (57.6%). Regardless of sexing and preparation technique, counts of live cells were equal to all groups. For conventional semen, SU delivered the lowest Con (10.7 Mio/mL), DGC sperm was significantly more concentrated (26.5 Mio/mL), but lower than directly after thawing (56.1 Mio/mL).

Sexed semen showed no differences in Con or Cell and after DGC, both semen types had similar Cell. Due to cell counts and motility results, DGC seems to be the preferrable preparation method.

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Functional and translational effects of canine decidua-derived extracellular vesicles (EVs) on trophoblast regulation and decidualization

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Extracellular vesicles (EVs) from uterine stromal cells are affected by decidualization, resulting in higher concentrations and altered cargo. In the canine endotheliochorial placenta, decidual cells are pivotal as they evade trophoblast invasion and express the nuclear progesterone (P4) receptor (PGR). We hypothesized that decidual cells use EVs to communicate with their environment. Using a dog uterine stromal cell model, EVs were isolated from 3 groups: non-decidualized, decidualized and decidualized cells treated with aglepristone. Decidualization increased EV concentration, which was reduced by aglepristone. Uptake of EVs by decidualizing cells was confirmed by a CD9-reporter, and EVs from decidualized cells enhanced decidualization capacity of non-decidualized stromal cells. Proteomics identified EV-enriched markers and diverse other proteins, e.g., matrix metalloproteinases, signal transducers and activators of transcription, or glucose transporters. Notably, membrane-bound P4 receptor (PGRMC2) and CX43 were present, suggesting horizontal cell-to-cell communication between decidual cells involving responsiveness to P4 via transfer of its receptor or direct cell-to-cell contacts. Among the targets of interest was thrombospondin 1 (THBS1), which localized to decidual cells in vivo and was reduced by aglepristone. In lack of a canine model, functional studies involved human trophoblast (HTR8/SVneo) cells revealing the inhibitory effects of THBS1 on their migratory abilities.

Findings indicate that, decidua derived EVs regulate the function of both maternal and fetal cells in dogs.

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Revisiting the interpretation of mating behavior in roe deer *(Capreolus capreolus)*: Evidence for active female strategies

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Mating behavior in roe deer is traditionally explained by strong dominance selection among males: territorial bucks compete for access to females. We conduct research on embryonic diapause in roe deer under controlled housing conditions with 17 females, housed in groups of one male and up to four females. In 2023–2025 during the rut, we observed behavioral patterns and anatomical features of the female reproductive tract suggesting that female reproductive mating strategies may be more complex and influential than previously assumed. Using behavioral observations, ovarian ultrasound imaging, and anatomical examinations, we identified that (1) does frequently initiate multiple mating interactions, i.e. set the pace in mating races rather than being pursued; (2) does themselves selectively solicit a buck; (3) a constriction of the vaginal tract, located cranial to the urethral opening, delineates a cavity anterior to the cervix; (4) ovulation occurred up to two days after multiple copulations had ceased; and (5) a twin pregnancy following artificial insemination with semen from two bucks confirmed the absence of strict mechanisms preventing multiple paternities. Our findings challenge the traditional male-centered view of roe deer mating behavior. Females appear to employ active reproductive strategies – such as repeated mating, self-initiated approaches toward males, an anatomical site for temporary sperm storage, and delayed ovulation – to ensure fertilization with viable sperm and to reduce the risk of unsuccessful breeding.

Our findings highlight the need to reconsider the reproductive dynamics of this species within a broader, female-inclusive framework.

Dynamic regulation of HIF1α in steroidogenic cells: Evidence-based insights into conserved oxygen-dependent control of steroidogenesis

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Hypoxia-inducible factor 1 alpha (HIF1α) is the major transcription factor that plays a pivotal role in cellular adaptation to reduced oxygen (O_2), including the physiological hypoxia (physoxia). In reproductive biology, evidence has linked HIF1α activity to ovarian and testicular function, particularly in granulosa, luteal and Leydig cells, with variations in O_2 availability dynamically affecting steroidogenic activity. Nonetheless, its dynamics and further functions remain unclear. Using murine models of granulosa (KK1) and Leydig cells (MA-10 and MLTC1), transcriptomic analysis of cAMP-stimulated cells, chronically exposed to different O_2 contents (20% and 1%), was performed. The upregulated differentially expressed genes (DEGs) showed regulation of RNA/DNA transcription (Rela, Sp1, Klf4/5/10/11, Atf3, Egr1, Irf1, Smad7), and epigenetic modification (Tet2, Dnmt3a, Kdm3a/4b/5a). Conversely, downregulated DEGs were involved in cellular response to oxidative stress (Sod2, Txn1, Prdx2/3) and regulation of PI3K/AKT/mTOR signaling (Rptor, Akt1, Igf1r, Map2k2). Interestingly, hypoxia suppressed Hif1α, associated with upregulation of Vhl and Egln1. These findings indicated O_2-dependent regulation of HIF1α activity in steroidogenic cells. Therefore, the accumulation of HIF1A was assessed in KK1 and MA-10 cells at different time points under 1% O_2. Protein levels displayed rapid increase in the first 6 hours, followed by a marked reduction after 24h.

Our findings suggest a dynamic balance in HIF1α regulation during chronic hypoxia, mediated by transcriptional and redox mechanisms that attenuate HIF1α abundance across steroidogenic contexts.

Organoid modelling of hormonal and tumor suppressor interactions in ovarian cancer development

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High-grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy and likely originates from the distal fallopian tube epithelium (FT). TP53 is mutated in vast majority of HGSOC cases, suggesting involvement of tumor suppressor gene in tumor initiation. Germline BRCA1 mutations cause homologous recombination deficiency (HRD), increasing genomic instability and cancer risk, while somatic BRCA1 mutations occur in ~25% of sporadic cases. CRISPR/Cas9-edited fallopian tube organoids were used to model early tumorigenic events in a physiologic 3D context. We propose that TP53 loss disturbs epithelial homeostasis and additional BRCA1 loss amplifies genomic instability of HGSOC initiation. TP53 knockout (KO) organoids were established and characterized by proliferation assays, immunofluorescence, and Western blot (BAX, BCL2, PCNA). TP53 KO organoids displayed phenotype fully consistent with loss of function mutation. Importantly, hormonal stimulation (Estradiol) and Notch inhibition (DBZ) in wild-type and TP53 KO organoids revealed changes in proliferation, ciliogenesis, and differentiation, indicating impaired epithelial plasticity upon TP53 loss. A sequential TP53-BRCA1 double KO line is under validation for DNA repair capacity and PARP inhibitor (Olaparib) sensitivity.

Together, these data establish TP53 loss as an early driver of epithelial dysregulation and set the stage to examine cooperative oncogenic effects of BRCA1 loss in HGSOC.

Using posture classification for detecting calving related behaviour in dairy cows

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Observing cows approaching parturition to predict the onset of calving is a crucial task for farmers, which is time consuming and often requires working at night. Although applying computer vision (CV) to automate calving monitoring is a promising approach, it is still in the early stages of development. In animal monitoring with CV, skeleton-based action recognition is commonly used to infer behaviour through the movement of key body points in video sequences. While this approach has been successfully used for behaviour classification in cattle, it is prone to errors if multiple behaviours occur simultaneously. This is especially the case in cows close to parturition, which are known to express a variety of different behaviours, such as frequent transitions between standing and lying, sniffing the ground and tail raising. Additionally, these algorithms usually require a sliding window method for analysing longer recordings and real-time footage, introducing further challenges if behaviours vary in duration. Therefore, detecting calving-associated postures of cows in individual frames instead of video sequences may help to overcome limitations of traditional algorithms. In this study, two key-point-based posture classification methods were evaluated.

The results indicate that standing and lying behaviour as well as head posture can be classified with high accuracy, while calving specific tail movement can be classified with moderate accuracy. Further research is needed to improve accuracy in detecting subtle behaviours and to assess the applicability of this method in real-time calving prediction systems.

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Quantification of endogenous oxytocin in plasma and saliva of cows during calving

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Oxytocin (OXT) plays a key role in reproductive physiology in cows, mediating uterine contractions during parturition and milk ejection. Plasma measurements have traditionally been used to establish known OXT profiles. The determination of OXT levels in saliva has recently gained increasing attention due to its potential for non-invasive sampling. Yet, little is known about whether quantification

in both matrices yields comparable outcomes, particularly given the uncertainties surrounding the mechanisms and kinetics of OXT transfer into saliva. Hence, the present study aimed at assessing and comparing the temporal and quantitative changes of OXT levels in plasma and saliva of cows during calving. Blood and saliva samples of five multiparous cows were collected 2- and 1-week prepartum, at approximately 10-min intervals during the first 4h after the onset of calving (calf feet out) and at 12 h and 16 h postpartum. Quantification of OXT in SPE-extracted samples was done using a commercial ELISA Kit (Cayman Chemical, 500440). In plasma, OXT peak levels occurred during the expulsion phase of the calf, milking and placenta expulsion, consistent with the pattern described in literature due to known physiological mechanisms. Hence, the commercially available Cayman kit reliably quantifies OXT in plasma.

Preliminary results show OXT levels in saliva which do not correlate with blood levels. This questions the reliability of saliva as a matrix to detect oxytocin levels associated with physiological changes during calving.

From clinic to culture: Do canine uterine myocytes display myometrial properties in vitro?

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The culturing of canine uterine myocytes represents a promising in vitro model for dystocia – a condition where therapeutic options remain limited. Myocytes are commonly defined by the expression of smooth muscle markers such as smoothelin (SMTN), smooth muscle actin-α (ACTA2), myosin heavy chain 11 (MYH11), transgelin (TAGLN), and caldesmon 1 (CALD1). Contraction-associated proteins including connexin 43 (Cx43), oxytocin (OXTR) and prostaglandin F2α receptor (PTGFR) also ensure myometrial function. To validate the model system's suitability, cells at passages 1, 3, and 6 from three dystocic dogs were examined for these markers, as well as for cell-cell contacts (Cx26, JAM-A, ZO-1, CLDN3/11, OCLDN), estrogen (ESR1) and progesterone receptors (PGR), and compared to matched frozen myometrium tissues using qPCR. While OXTR, Cx43, ESR1, SMTN, TAGLN and MYH11 were expressed at lower levels in cultured cells than in tissue, PTGFR, PGR, ACTA2, CALD1, and cell-cell contact markers mainly remained stable. This indicates that the cultured cells seem to be myocytes, which however downregulated several genes in culture. It remains to be investigated, if hormonal or structural adaptions to the culturing conditions might reduce these effects.

In summary, our in vitro system retains important characteristics of the canine myometrium, representing an interesting cellular model system for dystocia, for selected research topics.

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Effect of heat stress on the mRNA expression of pro-inflammatory factors in the uterus and oviduct of postpartum dairy cows

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Previous research has shown that cows exposed to heat stress (HS) exhibit a greater proportion of polymorphonuclear neutrophils in the uterus. Therefore, we hypothesized that exposure to HS also affects the mRNA expression of pro-inflammatory factors in the uterus and oviduct. At day 28 postpartum, uterine samples were obtained by the cytobrush technique and oviductal samples were collected by transvaginal endoscopy. Total RNA was isolated from the brushes for RT-qPCR analysis targeting specific pro-inflammatory factors (CXCL1/2, CXCL3, CXCL8, IL1A, IL1B, and PTGS2), receptors (TLR2 and TLR4), and OVGP1. Ambient temperature and relative humidity were recorded at 30-minute intervals with dataloggers, and the temperature-humidity index (THI) was calculated. To quantify cumulative HS exposure, the area under the curve between the THI and the threshold of 68 (Riemann sum) was calculated. In the uterus, for all selected factors, except for PTGS2 and TLR4, positive correlations ($r = 0.3–0.7$, $P < 0.05$) were observed between short-term HS exposure (12 to 24 hours prior to sampling) and mRNA expression levels. In the oviduct, expression levels of CXCL1/2, CXCL3, CXCL8, IL1A, PTGS2 showed positive correlations ($r = 0.3–0.4$, $P < 0.05$) with short-term HS exposure. The mRNA expression of OVGP1 in the oviduct showed a different pattern and displayed a significant negative correlation with heat stress ($r = 0.4$, $P < 0.05$).

Our findings indicate that short-term HS influences mRNA expression patterns in the reproductive tract. Further research is needed to elucidate the mechanisms responsible for the observed molecular alterations.

Umbilical cord torsion as a rare cause of abortion in a dromedary camel: A case report

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Umbilical cord torsion is a well-documented cause of abortion in equine and bovine species, but it has not yet been described in dromedary camels. This report documents, to the authors' knowledge, the first case of abortion associated with umbilical cord torsion in a dromedary. A six-year-old nulliparous she-camel presented with signs resembling the onset of parturition approximately 89 days before the expected calving date. Two days after the initial signs, the dam expelled the placenta prematurely, followed by complete expulsion of a dead fetus within 8 hours. The camel remained in good general condition, showing normal appetite and water intake. The owner administered penicillin for three consecutive days after abortion. Veterinary examination was performed 5 days post-abortion. Complete blood count revealed monocytosis and lymphocytopenia, indicating systemic stress or inflammation. Clinical assessment and microbiological testing excluded infectious etiologies. Ultrasonographic and gross examination of the aborted fetus revealed evidence of umbilical cord torsion, which likely compromised blood flow and led to fetal death. No other abnormalities were detected.

This case emphasizes the importance of considering non-infectious causes of abortion in camelids, especially mechanical or vascular disturbances such as umbilical cord accidents. While such events are rare in camels, awareness among veterinarians and breeders is crucial to avoid unnecessary antimicrobial use.

Comparative proteome analysis of bovine uterine luminal fluid and secretomes from endometrial explants

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The bovine endometrial secretome contains a wide variety of proteins and plays a key role in early embryonic development and embryo-maternal communication. Furthermore, immediately after parturition, the uterus is susceptible to invading pathogens, triggering an endometrial immune response that largely takes place in the uterine luminal fluid (ULF). Since generating standardized endometrial ULF samples challenged with defined immunological stimuli in vivo is difficult, in vitro endometrial explant models are commonly used instead. In order to assess the extent to which these explant models reflect protein secretion in vivo, we compared ULF samples collected in vivo with cell culture media from in vitro endometrial explants. In vivo samples were collected non-invasively by inserting a Merocel sponge into the uterus using a novel uterine sampling device ($n = 5$). For the in vitro samples, cell culture media from endometrial explant experiments were taken ($n = 6$). To analyze their protein composition, liquid-chromatography and tandem mass spectrometry (LC-MS/MS) analysis was performed. Overall, more than 2000 proteins were identified, with more than 1450 proteins being present in both groups, displaying a

broad overlap of identified proteins in all samples. Bioinformatic analysis of the corresponding proteome profiles using PANTHER led to very similar results.

In conclusion, in vitro samples reflect endometrial secretion in vivo on a highly comparable level, and are therefore a suitable tool for further studies on the endometrial secretome.

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Tumors of the female reproductive tract and mammary gland in small ruminants: A retrospective study

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With the growing importance and lifespan of pet sheep and goats, expectations regarding the diagnosis and therapy of neoplastic diseases are increasing. This retrospective study included all confirmed reproductive and mammary tumors in small ruminants presented at the Clinic for Ruminants, LMU Munich, between 2002 and 2025. Data were manually extracted from medical records. During this period, 10,496 small ruminants (7,200 sheep and 3,296 goats) were admitted to the clinic. Tumors were detected in 68 small ruminants (0.7%), including 28 sheep (0.4%) and 40 goats (1.2%). Fourteen tumors (21%) involved the reproductive tract, while four (6%) affected the mammary gland. Reproductive tumors were markedly more frequent in goats (33%) than in sheep (4%). Of the animals with reproductive and mammary gland tumors, 50% (n = 9) were euthanased, 28% (n = 5) were discharged without treatment, and 22% (n = 4) underwent surgery. Seven uterine and two mammary tumors were examined histopathologically. Uterine adenocarcinomas (n = 4) were the most common tumors in goats, followed by leiomyosarcoma (n = 2). In sheep, one leiomyoma was observed. In the mammary gland of sheep, one adenoma and one papilloma were identified. In small ruminants older than ten years (n = 610), 4.7% (n = 29) were diagnosed with neoplasia. Twenty-eight per cent (n = 8) of these tumors affected the reproductive tract, and 7% (n = 2) the mammary gland.

Neoplasms of the reproductive tract play an important role in geriatric small ruminants and are often a reason for euthanasia due to poor prognosis and animal welfare.

Upper and lower limits in freezing rate, sperm and glycerol concentration for cryopreservation of ram semen

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The cryopreservation of ram semen is one of the tools at the German Gene Bank of Farm Animals to preserve the genetic diversity of rare sheep breeds. Based on the cryopreservation protocol of the Gene Bank, we evaluated tolerance limits for sperm concentration, glycerol concentration, and freezing rates. To this end, individual ejaculates were frozen in a two-step procedure in a basic Tris-Fructose-Egg Yolk extender. Sperm concentrations between 800×10^6/mL to 20×10^6/mL had no effect on total motility ($p > 0.05$; n = 8 rams; 6% glycerol; freezing rate: -10 °C/min; IceCube). However, fewer viable, acrosome intact sperm were noted at 800, 40 and 20×10^6/mL when compared to 100, 200, or 400×10^6/mL ($p < 0.05$). Glycerol concentrations between 4 and 8% yielded highest values for post-thaw motility (44 ± 13%; 200×10^6 sperm/mL; freezing rate: -10 °C/min, IceCube; n = 8 rams). Motility was lower, if ≤ 3% or ≥ 10% glycerol was used ($p < 0.05$). When semen was frozen at 200×10^6 sperm/mL and 6% final glycerol either in an IceCube (-10 °C/min) or 4 cm above liquid nitrogen (-55 °C/min), no differences in motility or viable, acrosome intact sperm were observed ($p > 0.05$; n = 5 rams). A subsequent comparison showed that semen had a similar post-thaw motility irrespectively whether it was frozen 1, 2, 3, 4, or 5 cm above liquid nitrogen, respectively (-141 to -42 °C/min; 200×10^6 sperm/mL; 6% glycerol; $p > 0.05$; n = 7 rams). In conclusion, ram spermatozoa tolerate a wide range of freezing conditions. Optimum results are to be expected when between 100 and 400×10^6 sperm/ml are frozen with 4 to 8% glycerol at 1 to 5 cm above liquid nitrogen.

Granulated vs. homogenous: Does ooplasm appearance predict the protein and lipid landscape in immature bovine oocytes?

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Immature bovine cumulus-oocytes complexes (COCs) that present several layers of tightly packed cumulus cells and a homogeneous, darkish ooplasm are considered to be of good quality. Yet, COCs exhibiting cytoplasmatic granulation are frequently observed and routinely discarded. Therefore, the present study aimed to evaluate and compare the protein and lipid landscape of these two oocyte classes. Oocytes were retrieved from slaughterhouse ovaries from minimum three collection rounds for each omics analysis. Proteomic profiling via LC-MS/MS revealed that only five out of 2,130 total identified proteins were differentially abundant between both oocyte groups (Student's t-test; FDR = 0.05 and $\log2FC > |0.6|$). However, functional enrichment analysis of candidate proteins revealed an enrichment of pathways associated with fatty acid metabolism. Lipid profiling via ESI-IT MS discovered a higher content of phosphatidylcholine (Student's t-test; $p < 0.05$) and a tendency to increased amounts of free fatty acids in granulated compared to homogenous oocytes. To supplement these results, lipid droplet distribution pattern was assessed utilizing Nile Red staining and confocal microscopy. Homogeneous oocytes were mainly characterized by small sized lipid droplets evenly distributed throughout the ooplasm, while granulated oocytes presented increased lipid droplet accumulation, higher heterogeneity in droplet size and formation of droplet clusters.

This study demonstrates that despite minimal differences in overall protein abundance, granulated oocytes exhibit significant metabolic distinctions, primarily related to fatty acid metabolism and lipid content.

Combined analysis on the impact of vaginal discharge on fertility after progesterone supplementation with an intravaginal P4 device in lactating dairy cows submitted to TAI protocols

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Ovsynch protocols combined with progesterone-releasing intravaginal devices (PRID) can improve fertility, but muco-purulent vaginal discharge is often observed at removal of the device. Our objective was to evaluate the effect of vaginal discharge after PRID application in lactating dairy cows on pregnancy per artificial insemination (P/AI) and pregnancy loss (pregloss) when subjected to a timed AI (TAI) protocol. For this, a combined data analysis including data from 3 studies and 2,210 cows was performed. All cows enrolled were submitted either to a 5 d or to a 7 d TAI protocol with P4 supplementation. Vaginal discharge was scored after device removal by using a similar scoring system in all 3 studies. In total, 168 cows were assigned to a vaginal discharge score (VDS) 0 (clear mucus with no debris), 1,461 to VDS 1 (small flecks of purulent debris on the P4 device) and 581 to VDS 2 (abundant amount of purulent debris on the P4 device and vulva). There was no association between VDS at device removal and P/AI at d 35 or d 75 after TAI. In pairwise comparison, cows scored with VDS 0 had less pregloss (1 %) compared with cows scored with VDS 1 (7 %) or VDS 2 (5.2 %), but none of these exceeded the expected values of cows experiencing pregnancy loss in the observed timeframe. Distribution of VDS differed between primiparous (VDS 0: 5.2 %, VDS 1: 65 %, VDS 2: 29.8 %) and multiparous cows (VDS 0: 8.9 %, VDS 1: 66.7 %, VDS 2: 24.4 %).

Role of rare T-cell subtypes in human testicular germ cell tumors

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Incidences of testicular germ cell tumors (TGCTs) are rising worldwide, especially in Europe, North America and Australia. TGCTs affect young men aged from 15 to 44 years and develop from fetal gonocytes that de-differentiate into pre-invasive germ cell neoplasia in situ (GCNIS). After onset of puberty, GCNIS may – or may not – proceed into solid seminomas (SE) or non-seminomas (NSE). Factors driving this development and metastatic spread are still largely unknown, but immune cell infiltration, especially with T cells, is a hallmark in TGCT. Whereas T-cell subpopulations such as regulatory T-cells (Treg) and type 17 T-helper cells (Th17) are well studied in other cancer entities, knowledge is still limited in TGCT. We performed flow cytometry with extended antibody panels for T-cells (resting and activated) and respective cytokine signature analysis on n = 5 TGCT samples. With this, we showed a higher abundance of Tregs and Th17-cells in SE tumor centers. For functional analyses, we retrieved peripheral blood monocyte cells (PBMCs) from n = 45 healthy blood donors and n = 9 TGCT patients for in vitro analysis. With that, we will explore active and passive effects of Tregs and Th17-cells and/or their signature cytokines on seminoma (Tcam2) and Sertoli cell lines (HSerC/HSeC).

Thus, we aim to get a better understanding of TGCT biology and the tumor microenvironment as well as the underlying pathogenesis of TGCT development and spreading. Ultimately, this will pave the way for more precise diagnostics and future immune-modulatory therapies.

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Vascularisation and immune cell infiltration in human testicular germ cell tumours

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Testicular germ cell tumours (TGCTs) originate from germ cell neoplasia in situ (GCNIS) and can evolve into seminomas (SE) or non-seminomas (NSE). In other cancer entities involving immune cells, high endothelial venules (HEVs) recruit lymphocytes and are associated with tumour progression. In order to investigate the role of HEVs and immune cells in TGCTs, testicular biopsies were analysed from azoospermic men. The samples included cases of normal spermatogenesis (NSP), non-neoplastic inflammatory hypospermatogenesis (HYP+LY), GCNIS, SE, and NSE and were stained with haematoxylin/eosin for histological evaluation (HE), as well as for immune cells (CD45+, CD68+, CD20cy+, CD11c+, CD3+, CD25+, FOXP3+) and for vessels (MECA-79, PECAM-1, smooth muscle actin) by immunohistochemistry (IHC). HE revealed increased vascularisation and immune cell infiltration in HYP+LY and GCNIS compared to NSP, with markedly higher levels in SE and NSE. In some SE and NSE samples, blood vessels and immune cells form follicle-like structures around tumour regions. IHC showed a higher density of blood and lymphatic vessels in SE, NSE and GCNIS than in NSP and HYP+LY. HEVs were largely restricted to tumour centres in SE and were rarely observed at the margins or in other patient subgroups. IHC further showed dense infiltration of T cells, B cells and macrophages in tumour centres. Ongoing qPCR analyses are profiling vascular and leukocyte gene expression in SE, NSE, GCNIS+LY and HYP+LY samples.

These findings will be placed in a clinical context to evaluate the role of HEVs in TGCT biology.

Filamin A is essential for proliferation, shear stress-induced cytoskeletal remodelling and motility in the human ovarian granulosa cell tumor cell line KGN

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Filamin A (FLNA) is a multifunctional actin-crosslinking protein with mechano-sensitive properties, which is expressed in different tissues and cell types. Aberrant FLNA expression is linked to the development and progression of various cancers. Recently, we confirmed its presence in primary human ovarian granulosa cells and in the granulosa cell tumor (GCT) line KGN. An immunohistochemical study, analyzing FLNA protein expression in 51 GCT tissue microarray samples revealed heterogeneity, with 62.8% showing moderate staining, 19.6% weak staining, and 17.6% strong staining for FLNA. To examine its pathophysiological role, we generated FLNA-knockout (KO) KGN clones using CRISPR/Cas9. Loss of FLNA resulted in a flattened cellular morphology and enlarged cell size. Mass spectrometry-based proteomic analysis revealed broad changes in abundance of proteins associated with cell adhesion, cytoskeletal organization, proliferation, and cell cycle regulation. Further analyses demonstrated a number of changes in cellular functions of KO cells, including reduced proliferation, cell cycle arrest, impaired migration, and disturbed actin cytoskeleton reorientation in response to shear stress. Moreover, we identified transcriptional changes in genes related to the FLNA protein, which were up- or downregulated under fluid shear stress.

In summary, the results demonstrate an unexpectedly prominent role of FLNA in regulating KGN cells, highlighting the potential of FLNA expression levels to influence the progression and metastatic potential of this ovarian tumor type.

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Testicular and epididymal KCNJ5 expression in boars and its association with sperm morphology defects caused by a point mutation

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The ducts of the testes and epididymis provide an optimal environment for sperm production and maturation. Potassium ion channels play a pivotal role in these processes. Here, we focus on the G-protein-activated inwardly rectifying potassium channel KCNJ5. Immunohistochemistry revealed KCNJ5 expression in the nuclei and cytoplasm of Leydig cells and in all germ cell stages of the testis, except for elongated spermatids. In the epididymis, KCNJ5 was localized in epithelial cells of the epididymal duct, with a stronger signal in the apical region. In addition, KCNJ5 was detected in detached residual bodies in the epididymal lumen. Proteomic analysis of ejaculated and epididymal sperm revealed 2830 and 6968 proteins, respectively. As expected, KCNJ5 was not detected in sperm. Cloned boars with a KCNJ5G151R/+ point mutation (n = 3; age: 10 month) had a > 90% prevalence for ejaculated spermatozoa with a retained cytoplasmic droplet. Even at a mature age (36 months), ejaculates contained > 60% morphologically abnormal sperm. Abnormalities were composed of sperm with a proximal droplet, a distal cytoplasmic droplet, or a bent tail with a droplet. In contrast, semen from wild-type boars (n = 7) contained less than 16% abnormal sperm. Sperm motility after 24 h storage at 15 °C was significantly lower in mutant boars (30 ± 13%) compared to wild-type boars (68 ± 19%). In conclusion, a point mutation in the KCNJ5 gene in testicular and epididymal tissue may severely compromise sperm production and maturation.

Screening a larger number of boars for SNPs in the KCNJ5 gene will be helpful to identify whether a systematic correlation with semen quality exists.

Reciprocal translocation detection in boar sperm using flow cytometry

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Balanced reciprocal translocations (RTLs) are one of the underlying causes of hypoprolificacy in breeding boars. Although RTLs are prevalent in 0.47% to 3.3% of boars, approximately 50% of sub-fertile boars carry RTLs despite having normal semen quality. Early embryonic losses and genetic defects (cleft palate and deformed legs) are among the major effects of RTLs causing significant economic losses. The available methods for detecting RTLs are currently expensive, time-consuming, subjective and unproductive. Alternatively, flow cytometry could offer a more efficient screening method. This talk presents a project to develop such a method for detecting RTLs in boar semen. To achieve this goal, two published flow cytometric methods were considered. In Method 1, the nuclei of spermatozoa are stained with a DNA stain (DAPI) after undergoing decondensation using dithioerythritol and papain. Next, samples with (n = 4 ejaculates) or without (n = 12 ejaculates) RTLs are classified by histogram analysis; two peaks in normal boars. In Method 2, permeabilized sperm are stained with a DNA stain (propidium iodide, PI) without nuclear de-condensation. Histogram analysis is then used to detect RTLs; higher PI intensity in boars with RTLs. Method 1 showed irregular de-condensation of nuclei; multiple DNA peaks in boars without RTLs, and method 2 showed no differences in PI intensity based on histogram overlay analysis; similar PI peaks in boars with or without RTLs.

In conclusion, further research is needed to develop a high throughput flow karyotyping method for effective, rapid and routine screening of breeding boars.

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The efficiency of photodynamic inactivation of bacteria in boar semen is influenced by the preservation medium

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Recently, we established Photodynamic Inactivation (PDI) as an alternative to antibiotics in preserved boar semen. Bacteria are inactivated by singlet oxygen (1 O_2) which is generated by a photosensitizer (PS) upon illumination with low-intensity light. The aim was to examine the influence of semen extenders on antimicrobial efficiency in the PDI system. Two antibiotic-free extenders, the short-term extender Beltsville Thawing Solution (BTS) and the long-term extender Androstar® Premium (APrem), were spiked with ~3 × 10^3 CFU/mL *Klebsiella oxytoca* or *E. coli*. After adding 0.5 µM of the PS 5,10,15,20-tetrakis(1-methyl-4-pyridinio)-porphyrin-tetra-(p-toluaolsulfonat) (TMPyP), samples (n = 12) were illuminated with a blue LED (λ = 415 nm) light source at 2.5 mW/cm² for 90 s. Dark samples (n = 12) were used as control. After 0, 24 and 48 h of storage at 17 °C, bacterial counts were determined after culture on blood agar. In BTS, PDI reduced *K. oxytoca* by 2-log levels throughout storage and decreased E. coli to below the detection limit (< 10 CFU/mL; $p < 0.05$). In APrem, PDI induced an initial 1-log reduction in both bacterial species. After 48 h, *K. oxytoca* recovered to levels in control samples (~104 CFU/mL), whereas E. coli remained at low level (~30 CFU/mL).

In conclusion, the effect of PDI differs depending on the extender medium and the bacterial species. The choice of semen extender for PDI needs to consider both broad spectrum antimicrobial efficiency and sperm compatibility.

Effects of the deslorelin slow-release implant (DSRI) on the endocrine and germinative testicular function in tomcats

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Deslorelin slow-release implants (DSRI) are widely regarded as a safe alternative to surgical castration in dogs. In tomcats, however, despite confirmed efficacy and reversibility, high individual variability in duration of effectiveness limits their clinical significance. Furthermore, data on pharmacodynamic (PD) effects on testicular function and the pharmacokinetic (PK) profile of DSRI are lacking. To address these gaps, a single-center PK laboratory, randomized, controlled, parallel and blinded study was conducted in 20 healthy adult male cats, of which 16 received a DSRI and 4 a placebo. Over an 18-month period, plasma deslorelin and testosterone (T) were monitored, together with testicular volumes (TV), penile spine assessment, and serial semen analyses (total sperm count, TSC). Plasma deslorelin remained quantifiable for at least 308 days post-implantation. Next to that, treated cats showed significant T suppression (pre-/post-GnRH stimulation), accompanied by reduced penile spine scores and TV from Day (D) 42, persisting until D336 and D392, respectively. Compared with controls, post/pre-T ratios were significantly lower from D14 to D364 ($p < 0.0001$). Although TSC tended to decline in treated cats, high variability and small control group size prevented statistical significance. Deslorelin exposure showed a counterclockwise hysteresis with T, reflecting a delayed PD response; T, TV, and TSC were weakly correlated and decreased sequentially in a physiologically consistent manner.

Our data confirm that the DSRI is a safe and suitable option for suppressing T in male cats, with a concomitant reduction of semen quality.

Bacterial killing activity in ejaculates: An emerging immune-physiological marker of bull sperm quality

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Unlike conventional semen quality traits, bacterial killing activity (BKA) is a unique indicator of innate immune defense in semen. While previous studies have demonstrated species-specific bacterial patterns of BKA, the precise relationship with standard semen traits and advanced flow cytometric markers are unclear. This study therefore aimed to characterize these associations in bulls. Semen from 24 bulls (19 Holsteins, three Red Holsteins, one Uckermarker and one Belgian Blue) was assessed for BKA against laboratory-cultured *Escherichia (E.) coli* and *Staphylococcus (S.) aureus*, as well as for standard semen traits (ejaculate volume, sperm concentration, and total, progressive and post-thaw sperm motility at 30 and 120 min) and flow cytometric sperm markers (viability and acrosomal integrity (VAI), membrane fluidity, intracellular calcium levels and mitochondrial activity). *E. coli* BKA was negatively associated with sperm concentration ($\rho s = -0.5$, $P = 0.003$), but positively with VAI ($\rho s = 0.43$, $P = 0.035$) and with the proportion of sperm exhibiting low intracellular calcium ($\rho s = 0.34$, $P = 0.03$), suggesting a link to sperm structural stability. In contrast, *S. aureus* BKA showed positive correlations with total sperm motility ($\rho s = 0.49$, $P = 0.01$) and total sperm motility after 30 min of thawing ($\rho s = 0.67$, $P = 0.001$), indicating a stronger antibacterial capacity in semen that is functionally robust.

Contrasting associations of *E. coli* and *S. aureus* BKA show that seminal plasma immune factors differentially support sperm stability and function, reinforcing BKA as an independent immune marker of semen quality.

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Determination of oxidation-reduction potential in semen and peripheral blood of men with infertility: Pilot analysis using the MiOXSYS system

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Male infertility accounts for approximately half of all infertility cases, yet in up to 50% of affected men no clear cause can be identified. Oxidative stress is a factor impairing sperm DNA integrity and function. The MiOXSYS System enables a rapid quantitative assessment of the oxidation-reduction potential (ORP) in semen. Here we compared ORP levels in semen and peripheral blood to analyze their correlation and determine oxidative stress in infertile men. ORP was measured in

blood serum (ORP-B) and sperm (ORP-S) using the MiOXSYS System (n = 6 men, Ethics approval: LMU 24-0229). Oxidative stress was calculated as ORP-S divided by sperm concentration. Data were analyzed with Wilcoxon signed-rank and Pearson correlation tests (α = 0.05). Median ORP-B exceeded ORP-S by 56.15 mV (p = 0.031), indicating a higher systemic oxidative state. ORP-S values were significantly greater than calculated oxidative stress (median difference = 28.97 mV, p = 0.031). Sperm concentration showed no significant association with ORP-S (p = 0.44) but a near-significant inverse trend with oxidative stress (p = 0.063). Pearson correlation revealed moderate, non-significant coupling between ORP-B and ORP-S (r = 0.56, p = 0.25) and a weak positive correlation between ORP-S and oxidative stress (r = 0.26, p = 0.62).

These pilot data demonstrate distinct redox profiles between blood and sperm and suggest that sperm concentration modulates oxidative stress burden. Future investigations into a larger cohort will elucidate systemic-local oxidative interactions and their relevance to male infertility, potentially allowing for antioxidant-based therapeutic strategies.

Analysis of the rectal temperature drop in periparturient bitches – preliminary results

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Different options to predict parturition in dogs have been described, among others a drop of the rectal temperature of 0.8–1.2 °C 12 hours before birth. A more recent study including 16 bitches did, however, not identify this drop [Geiser et al. Reprod Dom Anim 2014; 49: 109]. To proof the suitability of a rectal temperature drop for prediction of parturition, we retrospectively analyzed temperature data from periparturient bitches (n = 114) taken by breeders between day 55 after ovulation until parturition. Microsoft Excel was used for data analysis of the lowest and highest individual and mean temperatures. Besides, the lowest temperature of every bitch was compared with the temperature 12 to 16 hours before, and the bitches were grouped whether they had a drop of ≥ 0.8 °C or not. Additionally, graphical data presentations were evaluated for the presence of a significant temperature decrease. The lowest and the highest individual rectal temperatures were 35.9 °C and 38.6 °C. The mean temperatures before and during the temperature drop were 37.7 °C (before) and 36.4 °C (during), respectively. Evaluating the drop of rectal temperature, 50 bitches (44%) showed a drop ≥ 0.8 °C, whereas in 64 bitches (56%) the drop was < 0.8 °C. Considering the evaluation of the graphical presentation, an obvious drop of the rectal temperature was visible in 59 bitches (52%), but not in 55 bitches (48%).

Our preliminary results confirm that measuring the rectal temperature in bitches could be used as an additional indicator to approximately predict parturition, but it is not as reliable as most literature suggests and should therefore by accompanied by other parameters.

Smaller endometrial thickness at time of insemination is not detrimental to the conception rate in estrous cows with complete luteolysis

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The aim was to examine if time of ovulation and 1st service conception rate (FSCR) are related to endometrial thickness (ET) at time of AI. In a 1st study, 410 lactating cows were investigated by ultrasound at time of 1st service after parturition and 1 d later, and pregnancy was diagnosed at 28 to 42 d after AI. At 1 d after AI, the dominant follicle was no more detectable in 76.2% of cows (ovulating, OV), whereas in 23.8% of cows it was still there (non-ovulating, NOV). In NOV-cows, FSCR was lower compared to OV-cows (21.6% vs 73.5%; P < 0.05). Ovulation rate was lower in cows with a corpus luteum (CL) > 15 mm at the time of AI (n = 17) compared to cows with no CL > 15 mm (n = 393; 11.8% vs 79.1%; P < 0.05). ET was bigger in OV-cows compared to NOV-cows (8.8 vs 8.4 mm; P < 0.05) but did not differ between pregnant and non-pregnant OV-cows (8.8 vs 8.9 mm; P > 0.1). In a 2nd study, 299 lactating cows submitted for 1st service were examined sonographically, and only those with a follicle 12.0 to 22.5 mm, without a CL > 10 mm, and with no echoic content were inseminated. Pregnancy was determined at 28 to 35 d after AI. FSCR of cows considered suitable for AI (n = 201) was 62.7%. Inseminated cows with ET of 6.6 to 8.0 mm (below lower quartile) tended to have higher FSCR than cows with ET of 9.1 to 11.5 mm (above upper quartile; P = 0.08). The amount of anechoic intrauterine fluid (≤ 2 vs > 2 mm) did not affect FSCR (P > 0.1).

In conclusion, a quarter of cows do not ovulate within 1 d after AI and these cows have smaller ET. However, ET of ≤ 8 mm compared to > 9 mm at time of AI favors conception provided that incomplete luteolysis is ruled out as a complication.

Prediction of DNA fragmentation status of single human spermatozoa using AI technology

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Approximately 40% of infertility cases are classified as male-factor. Sperm selection methods for fertilization are limited. At the time of sperm injection, sperm are selected based on external characteristics whereas intra-cellular quality remains unknown. Our objective is to train and use AI technology to predict the DNA fragmentation status of single human spermatozoa. Sperm samples from 13 patients were fixed and stained for fragmentation using the TUNEL method. Fluorescence images of spermatozoa were analyzed to quantify DNA fragmentation levels. A pixelwise image-to-image translation model based on the pix2pix architecture was trained to predict DAPI and FITC fluorescence patterns directly from brightfield images. The model-generated fluorescence predictions were subsequently used to estimate DNA fragmentation, which was then compared to experimentally measured fragmentation values obtained from the ground truth fluorescence images. A total of 2,450 single sperm cell images from 13 patients were used to train and test the model. The predicted DNA fragmentation index (DFI) values obtained from the model showed a strong positive correlation with the ground truth fluorescence-based measurements, with a Pearson correlation coefficient of r = 0.84, indicating high predictive accuracy of the pix2pix-based image translation model.

There is a high predictability score with using the pix2pix translation model to identify good/poor DNA quality sperm at the time of oocyte injection and thus improve the sperm selection process. This may lead to increased fertilization rates, improved embryonic development and better clinical outcomes.

Sonicated and disrupted dead sperm compromise the function of live bovine spermatozoa after thawing

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The impact of dead sperm on live bovine sperm is unclear, raising concerns regarding artificial insemination, where sperm handling can cause damage. The effects of disrupted sperm on nearby live sperm remain unexplored. This study aimed to assess the impact of sonicated spermatozoa on nearby viable sperm. Semen samples (n = 12) from 12 healthy Holstein bulls were diluted to 80×10^6 sperm/mL in pre-warmed OptiXcell® extender at 38 °C. A 6 mL aliquot was sonicated and mixed with untreated semen to generate the following treatment groups (TG): TG25%, TG50%, and TG75% contained sonicated sperm, while the control group contained no sonicated cells. Post-thaw sperm quality was assessed based on thermo-resistance after 30 and 120 min at 38 °C (CASA; progressive motility; relative variation), as well as on viability and acrosome-integrity (VAI), low membrane fluidity and low intracellular calcium (LMLC), and high mitochondrial membrane potential (HMMP). Generalized linear mixed models revealed a significant difference in relative variation between the control and the TG75% group (P = 0.034). The percentage of VAI-LMLC showed a progressive decline (P < 0.001), along with a reduction in percentage of VAI-HMMP (P < 0.001).

In conclusion, these findings suggest that leakage of cellular content may compromise the functionality of live sperm, affecting pro-

gressive motility at high concentrations and resulting in a significant decrease in mitochondrial activity, which is detectable even at low concentrations. These results highlight the need for further mechanistic studies to evaluate possible damaging pathways.

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Standardization of vaginal cytology in canine reproductive assessment: How many cells are enough?

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Exfoliative vaginal cytology is a common method for evaluating reproductive status in bitches. However, inconsistent cell counts and low reproducibility limit its diagnostic reliability. The lack of standardized quantification criteria may reduce sensitivity and specificity. This study aimed to determine the minimum number of epithelial cells needed for statistically reliable and diagnostically valid cytological assessments. Sixteen smears were collected from bitches (n = 16) of various breeds and ages, sampled from the dorsal vaginal wall using saline-moistened swabs. Eosinthiazine rapid staining (Hämacolor®, Merck) was used for slide preparation. Four smears were analyzed from each reproductive stage: Proestrus, estrus, diestrus, and anestrus. Cell types evaluated included superficial, intermediate, parabasal, basal, metestrus, foam cells, clusters, and sheets. Cytological evaluations were performed at 400 x magnification, and mean cell-type values were calculated. Slides were grouped by cell count levels, and only groups with ≥ 5 comparable samples were analyzed. The number of cells required for 95% confidence in detecting target cell types was calculated based on expected frequencies (5%, 10%, 50%) with tolerable errors (3–10%). Blood samples were also analyzed for progesterone and estradiol-17β to confirm endocrine staging.

Findings suggest that counting ≥ 200 cells is necessary when the target cell type makes up ≥ 5% of the sample. Counting 100 cells is acceptable only if the cell type comprises ≥ 50%. This study offers a quantitative threshold to enhance the standardization of vaginal cytology in canine reproductive diagnostics.

Detection and susceptibility testing of bacteria isolated from the umbilicus of dairy calves

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From September 2022 to April 2024, five dairy farms in Brandenburg (Germany) were visited weekly for three months, respectively, to investigate the bacteria isolated from the umbilicus of dairy calves and their susceptibility to antibiotics commonly used. All calves up to four weeks were examined weekly (n = 1,248). The umbilicus of newborn calves and those with umbilical inflammations (UIs) were sampled using swabs and subsequently cultivated. Susceptibility testing of selected bacteria was carried out by broth microdilution or VITEK® 2 compact (bioMérieux). A total of 144 calves were diagnosed with UIs (11.5%). Over 100 bacterial species were identified in newborn calves and UIs, respectively. Some species were isolated almost exclusively from UIs (e.g., *Bacteroides spp., Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Trueperella pyogenes*). More than two-thirds (69.6%) of all *S. aureus* from UIs were methicillin-resistant. Overall, susceptibility to tetracycline was low across all bacterial species tested. Susceptibility of *streptococci* and *T. pyogenes* to first-line antibiotics for UIs (penicillins, amoxicillin with clavulanic acid) was high. Susceptibilities of *Escherichia coli* from UIs were significantly lower compared to newborns. *E. coli* in UIs from farms using chlortetracycline-spray for navel care showed significantly lower susceptibilities to ampicillin, enrofloxacin, trimethoprim/sulfamethoxazole, and tetracycline compared to isolates from farms using iodine.

In general, the use of first-line antibiotics appears effective. Off-label use of chlortetracycline-spray for navel care, however, is not reasonable and bares the risk of promoting antimicrobial resistance.

Unlocking the canine placenta: First single nucleus insights into the cellular architecture and functionality of endotheliochorial feto-maternal barrier

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In dogs, bulk RNA-seq and targeted studies have provided crucial insights into placental maturation and species-specific initiation of parturition. Yet, the cellular and spatial heterogeneity of the canine endotheliochorial placenta remains a challenge. We performed single-nucleus (sn) RNA-seq to enable cellular and molecular dissection of placental cell populations. Nuclei isolated from mid-term fresh frozen placentae were submitted to 10X Genomics technology and the main maternal and fetal cellular components were identified based on cellular markers established in our laboratory. A previously unrecognized complexity within the tissue was revealed, including the possible existence of distinct populations of cells. A dynamic cellular landscape of decidual cell subclusters was identified, indicating possible alternative differentiation and functional states, supporting their roles in the deciduo-chorial barrier and in modulating trophoblast behavior. The clustering patterns provided insights into syncytialization and its cellular origins. Cytotrophoblast compartments included potential novel clusters and a distinct extravillous lineage, likely linked to invasive properties, and clearly separated from villus-associated cytotrophoblast populations. First insights into luteolysis-derived nuclei suggest reversal of the mesenchymal-epithelial differentiation of decidual cells, associated with the withdrawal of P4/PGR signaling.

Our results highlight the potential of snRNA sequencing in providing a detailed characterization of the canine placental cellular components and open new avenues for deeper, clinically relevant, and targeted analyses.

Long-term development of the oxidative status in growing Holstein heifers depending on early postnatal feeding with transition milk or milk replacer

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In this study, the long-term effects of transition milk on growing Holstein calves were assessed. 50 calves received either 12 L/d of transition milk or milk replacer for the first 5 days of life. Thereafter, all calves received 12 L/d of milk replacer until gradually weaning between weeks 8 and 14. Body weight and oxidative marker in blood were measured until first lactation. Feed intake and behavior, as well as health data were assessed during the rearing period and milk yield, composition, and somatic cell count (SCC) were recorded until d 200. Calves fed transition milk had higher energy intakes and numerically higher starter intake during the rearing period. Both groups had high body weight gains even during weaning. Calves fed transition milk had fewer health incidents and numerically required less medication. The reproductive performance, first lactation milk yield and composition during the first 200 days in milk did not differ. However, a lower somatic cell count was observed in the transition milk

group during the first three weeks of lactation. Heifers fed with transition milk had a lower body weight at calving, but tended to have a greater body condition score than heifers fed with milk replacer. No significant effects on oxidative status were found, apart from early-life differences.

Overall, transition milk did not affect the development and performance of calves and heifers at high feeding allowances. The positive effects of transition milk feeding (health and solid feed intake) were limited to the rearing period; further effects, e.g., growth rate, age at calving and milk yield were not affected.

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How to measure testicular volume in tomcats?

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The testicular size and the gonadosomatic index (testicular weight divided by body weight) are commonly used estimates of active spermatogenesis in different species, as functional seminiferous tubules account for around 90% of the testicular mass. Reference values for cats are however lacking. To identify the most practicable and reliable methodology for testicular volumetry in clinical practice, we applied two manual measurement devices (digital caliper and measuring tape) and formulas (ellipsoid and Lambert) in two cohorts. Cohort 1 included 16 tomcats before and during hormonal suppression by a deslorelin implant, while cohort 2 comprised 20 tomcats of different ages at routine castration. The measuring tape consistently yielded larger length, width and height values than the caliper. Combining tape measurements with the ellipsoid and caliper dimensions with Lambert's formula, however, resulted in comparable volumes, while water displacement volumetry after castration was too imprecise for cats. More precise was the gonadosomatic index, which reliably distinguished between normo- and azoospermic, as well as between juvenile and adult individuals. Further, the identified cut-off could be successfully validated with a third dataset of 8 tomcats from the literature, comparable with cohort 1.

Summarized, the measuring tape with the ellipsoid formula, as well as the caliper combined with Lambert's formula are recommended for testicular volumetry in cats, as they were most comparable, easy to handle and particularly cat-friendly.

Single cell RNA sequencing reveals paracrine interactions in bovine corpus luteum

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The corpus luteum (CL) is a transient endocrine organ on the ovary. Prostaglandin F2α (PGF2α) initiates CL regression under in vivo conditions. However, it neither consistently suppresses progesterone production nor reliably induces apoptosis in luteal cells in vitro, suggesting a significant role for locally acting paracrine mediators in luteolysis. We profiled bovine CL on day 11 with or without PGF2α administration by single cell RNA sequencing and mapped ligand-receptor interactions with CellChat v2, which integrates ligand, receptor, and cofactor signals to quantify strength of paracrine interactions. Our data contained three steroidogenic, five immune, and three endothelial clusters in addition to fibroblast, pericyte, and smooth-muscle cell clusters in CL, with doubling of immune cells during regression. CellChat identified 16,299 and 16,994 interactions in mature and regressive CL, respectively. Among them, extracellular matrix interactions were predominant with COLLAGEN (probability: 0.5335; interactions: 2,986 interactions) > LAMININ (0.2097; 2,224) > FN1 (0.1441; 506) > THBS (0.0833; 385) > SPP1 (0.0454; 373) in mature CL. Comparatively, THBS (0.1674; 403) and SPP1 (0.1709; 417) showed a higher probability of interaction in regressive CL. The specific vascular and apoptotic interactions between THBS1-CD47, VEGFA-VEGFR, and EDN1-EDNR strengthened and FASLG-FAS and IFNG-IFNGR increased from in regressive CL.

Overall, these data deliver a high-resolution atlas of paracrine networks in CL and highlight candidate ligand-receptor interactions to modulate CL function and lifespan.

Pathogen shedding during intramammary infections in dairy cows

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This cross-sectional field study aimed to quantify the extent of selected bacterial pathogens shed in quarter milk samples (QMS) from lactating dairy cows and explored the association between pathogen shedding, udder health, and seasonal influence. Randomly selected dairy farms (n = 305) were visited once and a total of 58,108 QMS were collected from 14,700 cows and examined microbiologically. Pathogen shedding was analysed semi-quantitatively. For statistical analysis with Chisquare-Test (PROC FREQ) shedding classes were categorized into three groups. The level of bacterial pathogen shedding varied depending on the udder health status of the quarter. Healthy quarters exhibited lower or medium bacterial counts compared to subclinically or clinically affected quarters. For *Staphylococcus (S.) aureus*, the probability of low shedding (1–10 cfu/0.01 ml) was significantly ($P < 0.01$) higher in healthy quarters than in clinically affected quarters. *Streptococcus uberis* and *Streptococcus dysgalactiae* were also predominantly shed in medium concentrations in healthy quarters. Additionally, for *S. aureus* significantly higher pathogen quantities were shed in summer than in winter ($P < 0.05$). However, for *Escherichia coli* and *Streptococcus agalactiae*, reliable conclusions could not be drawn due to low detection rates.

The study revealed that even clinically inapparent animals can shed substantial amounts of pathogenic bacteria. This finding underscores the limitations of milk somatic cell count alone in assessing udder health or infection status. Targeted microbiological herd testing is a necessary supplement to somatic cell count analysis.

Determination of individual somatic cell count using single nucleotide polymorphisms in bulk milk samples

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The somatic cell count (SCC) of individual cows plays a crucial role in assessing udder health in dairy herds. Traditionally, individual milk samples were required to determine each cow´s SCC. A novel approach has been proposed that enables SCC estimation using the unique genomic "fingerprint" of each cow. This method involves extracting and analysing the DNA of a bulk milk sample using single nucleotide polymorphisms (SNP). If the genotype and the individual milk yield of each cow contributing to the bulk tank are known, the SCC of every individual can be calculated. Thus, only one bulk milk sample is required to obtain individual SCC results for all cows in the herd. In this study, 36 bulk milk samples from four dairy farms were compared with individual milk samples collected on the same day of a total of 1,884 cows. The correlation between the two methods was highly significant ($p < 0.001$), with a correlation coefficient of 0.83. Furthermore, 81.5% of the samples were assigned to the same SCC category.

Because this method requires only one bulk milk sample to obtain individual results, it substantially reduces sampling effort for farmers and facilitates more frequent testing, enabling earlier detection of SCC changes.

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Non-invasive pregnancy testing applying determination of urinary estrogens and fecal progesterone metabolites in Camelidae species

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In recent years, there has been a growing demand for veterinary services, including pregnancy tests, for South American camelids (SAC) and, to a lesser extent, Old World camels (OWC) in Central Europe. Although

clinical pregnancy diagnosis is also of primary importance in camelids, hormonal methods, especially non-invasive methods, are potentially of considerable interest, particularly in untrained OWC. Pregnancy profiles for total estrogens in urine (uTE) were established in alpaca (n = 10), llama (n = 8), dromedary (n = 11), and Bactrian camel (n = 4) using an in-house radioimmunoassay. In addition, the influence of normalizing uTE concentrations for urinary creatinine concentration (CREA) or specific gravity (SG) on the predictive value for pregnancy was investigated. In all four target species, there is a steep increase in uTE concentrations in late pregnancy, but this can only be used for diagnostic purposes in the last 2–3 months. Normalization to CREA or SG leads to only a slight improvement in predictive value, if any. When measuring fecal progesterone metabolites (FPM) in SAC using a commercial ELISA, highly significant differences ($p<0.0001$) between pregnant and non-pregnant animals were found. However, due to a partial overlap, it was not possible to make an unequivocal diagnosis in all cases.

Preliminary results indicate that FPM determination may be a useful method also in OWC. Although the OWC and SAC lines separated about 11–20 million years ago, the steroid profiles of the target species show clear similarities. However, species differences can also be observed in some aspects.

Simulated transport of boar semen in a warm environment: Pre-cooling of semen doses minimizes the growth of resistant bacteria

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Long-distance transport of liquid preserved boar semen during summertime may affect sperm quality by temperature and vibrations. Moreover, there is an enhanced risk for bacterial growth when temperature surpasses the regular storage temperature of 17 °C. Focussing on biosafety and sperm quality, the aim was to investigate the effects of temperature and vibrations in a simulated real-world transport scenario based on semen shipping in a tropical country. Subsamples of semen from 10 boars extended in Androstar® Premium (Minitüb GmbH) were either routinely processed (17 °C; not pre-cooled) or cooled for 24h to 5 °C (pre-cooled). Transport was then simulated by 6h of orbital shaking (Swip Shaker, Bühler KL-2, Edmund Bühler GmbH) with 200 rpm at 30 °C environmental temperature over a period of 24h. Thereafter, samples were stored at 17 °C. Further samples were held unmoved at 17 °C (control). In not pre-cooled semen, the temperature raised to 25 °C without affecting sperm motility and membrane integrity compared to 17 °C controls ($p > 0.05$). However, if these samples contained *Serratia marcescens*, bacterial counts increased to spermicidal levels within 72h. Pre-cooling delayed the growth of *Serratia marcescens* by 4 log levels and thus maintained sperm quality. A second experiment revealed that shaking of semen doses at 17 °C without warming did not influence the growth rates of *Serratia marcescens* compared to unshaken samples ($p > 0.05$).

In conclusion, pre-cooling of semen before long-distance transport in a hot environment lowers the risk for sperm damage caused by drug resistant bacteria.

Co-culture with cumulus cells during in vitro maturation restores developmental competence of partially denuded bovine oocytes

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This study aimed to evaluate whether developmental competence of immature, partially denuded bovine oocytes can be restored through co-culture with cumulus cells (CCs) during in vitro maturation (IVM). Bovine cumulus-oocytes complexes (COCs) retrieved from slaughterhouse ovaries were subjected to in vitro embryo production (IVP). Prior to IVM, grade 1 and 2 COCs were divided into three experimental groups: COC (intact COC, n = 120), PD (COC partially denuded, n = 105), PD+CC (COC partially denuded and co-cultured with CCs during IVM, n = 107). IVM was performed for 22 h, followed by a standard fertilization and embryo cultivation workflow. Cleavage rate was assessed on day 2 post fertilization, blastocysts rate, stage and quality on day 7 (D7) and day 8 (D8). Three independent IVP-rounds were performed. Data were statistically analyzed using logistic regression model and Tukey test. No differences could be observed in cleavage rate. However, compared to COC group, PD presented reduced blastocyst rate on D7 (COC: 25.00% A; PD: 8.57% B; PD+CC: 19.63% AB; $p = 0.005$) and D8 (COC: 29.17% A; PD: 13.33% B; PD+CC: 24.30% AB; $p = 0.013$), while PD+CC did not differ from both groups. PD resulted in a significantly higher proportion of grade II blastocysts (COC: 5.71% A; PD: 50.0% B; PD+CC: 19.23% AB; $p = 0.005$) compared to COC group. No effect on blastocyst stage could be observed.

Co-culture with CCs during in vitro maturation (IVM) successfully recovers the ability of partially denuded bovine oocytes to develop to the blastocyst stage.

The role of vaginal bacteria and antimicrobials on canine fertility – a retrospective evaluation

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Vaginal bacterial culture examination and antimicrobial use prior to mating are common practice in canine breeding management. However, several studies showed no differences in the microbiota of clinically healthy and reproductive-diseased (e.g., vaginitis) dogs. Thus, antimicrobial use in healthy breeding bitches is questionable due to antimicrobial resistance and dysbiosis development. This study evaluated the pregnancy-outcome in relation to bacteriological findings and antimicrobial use. We retrospectively analysed vaginal swab results from healthy breeding bitches (January 2016–August 2025). Samples were examined by culture. The bacteria were identified by biochemical parameters and MALDI-TOF. The medical records provided data on treatment and pregnancy outcome. 807 samples yielded 53 bacterial species; most were mixed cultures (81.5%). Common isolates included *alpha-hemolytic streptococci, Haemophilus haemoglobinophilus, Streptococcus canis/beta-hemolytic streptococci (Sc. canis/BHS), Escherichia coli (E. coli) and Staphylococcus intermedius* group. Treatment and outcome data were available for 376 cases. There were no significant differences in pregnancy outcome between antibiotic-treated and untreated bitches with monoculture ($p > 0.9999$) and high-grade growth of opportunistic *E. coli* ($p > 0.9999$) and *Sc. canis/BHS* ($p > 0.9999$), respectively. Overall, pregnancy rates were 81.3% (treated) vs. 78.1% (untreated) ($p = 0.5751$). Antimicrobial use did not improve fertility in healthy bitches.

Therefore, treatment in healthy breeding bitches is not in alignment with responsible antimicrobial use and other factors are more likely to influence fertility.

Standardization of vaginal cytology in canine reproduction: Observer variability and diagnostic consistency

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Exfoliative vaginal cytology is widely used for reproductive staging in bitches, yet lacks a standardized protocol. Variations in sampling site, swab type, fixation, and observer expertise can affect diagnostic accuracy. This study assessed the consistency of estrous stage classification from smears prepared using different techniques, interpreted by 107 veterinary students experienced in vaginal cytology. Exfoliatve samples from 332 bitches across all cycle stages (proestrus: n = 81, estrus: n = 139, diestrus: n = 63, anestrus: n = 49) were collected using dry or saline-moistened swabs from either the vestibulum or cranial vagina. Smears were air-dried or spray-fixed (Merckofix®, Merck), then stained with eosin-thiazine (Hämacolor®, Merck). Cytological evaluation was performed at 400 × magnification. Progesterone analysis enabled hormonal staging. Each observer assessed 24 coded smears and classified the estrous stage using standardized criteria. McNemar's test compared classification accuracy across three variables: sampling site, swab type, and fixation method. No significant differences were observed between sampling sites. Dry swabs

improved accuracy in proestrus (p = 0.0016), while moist swabs enhanced reproducibility in diestrus (p = 0.0045) and anestrus (p < 0.0001). In estrus, air-dried smears were more accurately classified than spray-fixed (96% vs. 86%, p = 0.034). Other stages showed no fixation-related differences.

Endometrial organoid based studies indicate WNT-related mechanisms of progesterone resistance in endometriosis

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Endometriosis (EM) affects 5–10% of women and is characterized by endometrial tissue outside the uterus, as well as molecular alterations in the eutopic endometrium. These changes cause severe pain and infertility in many patients. Although its pathogenesis remains unclear, progesterone resistance is a key factor. In this study, we use endometrial organoids from eutopic, ectopic, and healthy tissues to investigate the molecular mechanisms driving EM. Our previous analysis of endometrial cellular composition and differentiation status suggested that WNT/β-catenin signaling, crucial for balancing proliferation and differentiation, is dysregulated in EM organoids. Here, we tested organoid responsiveness to the WNT inhibitor IWR1, which stabilizes AXIN1. AXIN1 is part of the β-catenin destruction complex, and therefore prevents β-catenin accumulation and WNT activation. To assess the efficacy of IWR1, we measured expression of the WNT target gene LEF1 and found that its expression decreased after WNT inhibition in healthy but remained stable in EM samples. This effect was further confirmed by the measurement of protein levels of phosphorylated (inactive) GSK3ß, which is also a component of the destruction complex and promotes β-catenin accumulation when inactive, and β-catenin itself. In healthy samples, WNT inhibition reduced both inactive GSK3β and β-catenin levels, whereas in EM they remained unchanged.

These findings show an abnormal regulation of the β-catenin destruction complex, potentially causing constitutive WNT activation and impaired differentiation, which may contribute to progesterone resistance.

Effect of hypoxia and hypoxia-inducible factor 1α (HIF1α) on cholesterol homeostasis in granulosa cells

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Key female reproductive processes rely on rapid production of ovarian steroid hormones, with granulosa cells (GC) serving as their major source. To meet this demand, cholesterol, the essential precursor for steroidogenesis, is stored within lipid droplets. It is derived either from de novo synthesis or imported into the cell, enabling rapid release of the steroidogenic substrate in response to hormonal stimulation. Physiologically, GC are exposed to reduced oxygen (O_2) tension, and HIF1α has been shown to directly regulate steroidogenesis in these cells by affecting the STAR-mediated transport of cholesterol to mitochondria. Yet, as the focus of the present study, the understanding of the effects of HIF1α/hypoxia on cholesterol homeostasis remains limited. Our ongoing transcriptomic and proteomic analyses of mouse GC cultured under hypoxic conditions indicate the involvement of hypoxia in regulating the expression of key cholesterol transporters, including GRAMD1B and ABCA1. Further, exposure of GC to severe hypoxia resulted in markedly reduced free cholesterol levels within the cells. Similarly, echinomycin-mediated blocking of HIF1α activity reduced free cholesterol levels in control and cAMP-stimulated GC, emphasizing its importance in cholesterol homeostasis. Interestingly, pharmacological stabilization of HIF1α by blocking prolyl-hydroxylases (PHDs), stimulated GRAMD1B and ABCA1 expression, implying HIF1α involvement in controlling cholesterol transport.

Our findings support the hypothesis that HIF1α is a key regulator of cholesterol dynamics and substrate supply in granulosa cells, shedding new light on ovarian follicular functionality.

The placental checkpoint CD24 mucin-like molecule: From biology to pathophysiology

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Cancer and pregnancy share common features, including rapid growth, tissue invasion, and immune modulation. At the fetal-maternal interface, immune tolerance develops between the fetus and the mother. CD24, a checkpoint protein, interacts with Siglec-10 to induce immunotolerance. In the placenta, CD24 is a highly glycosylated mucin-like molecule co-expressed with Siglec-10 on the epithelial cells of the uterine glands. Reduced CD24 mRNA expression has been observed in early-onset preeclampsia compared with normal term and preterm deliveries. Conversely, CD24 protein expression is elevated in term preeclampsia cases relative to term and preterm controls. We examined CD24 expression in BeWo and JEG-3 trophoblast cell line models of preeclampsia, in which the transcription factor STOX-1 is overexpressed. Using RT-PCR, Western blotting, and flow cytometry, we found a significant reduction in CD24 mRNA and total protein levels in STOX-1-transfected BeWo and JEG-3 cells compared with mock controls. Additionally, CD24 and its ligand Siglec-10 were differentially expressed in placentas from patients who had recovered from COVID-19 infection – an effect that was notably influenced by fetal sex.

In summary, consistent suppression of CD24 mRNA and protein expression was observed across preeclampsia placental samples, preeclampsia cell models, and placentas from COVID-19-infected pregnancies. Given the established role of the CD24-Siglec-10 complex in immune suppression, we propose that reduced CD24 expression may reflect a loss of immune tolerance in preeclampsia.

Modulatory effects of GDF9 on gene expression and steroidogenesis in LPS-challenged bovine cumulus-oocyte complexes in vitro

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The oocyte secretes dynamic amounts of growth differentiation factor 9 (GDF9) which acts as a key regulator of follicular development. Preliminary observations showed that GDF9 compensates the detrimental effects of LPS during IVM, rescuing the blastocyst rates. Here we analyzed the expression of potential target genes in cumulus cells and oocytes, as well as steroid production after IVM in presence of GDF9 and/or LPS. Bovine ovaries were collected at the slaughterhouse (n = 5 replicates) and oocytes (≥ 50/group/replicate) underwent following treatments during IVM: control, GDF9, LPS, GDF9+LPS. Following IVM, cumulus cells and oocytes were analyzed for gene expression and IVM media for progesterone and estradiol. Treatment with LPS increased mRNA abundance of inflammatory markers CXCL8 and TNFa in cumulus cells compared to control and GDF9 ($P < 0.05$ in both cases), whereas no difference was observed between GDF9/LPS and control ($P > 0.05$). There was no effect of the treatment ($P > 0.05$) on the mRNA gene expression of IL6, NFkB2, TLR2, TLR4, STAR, HSD3B, BAX, BCL2, TMSB4, TMSB10, FSHR, GATM, HAS2, POU5F1, SOX2 and AREG in cumulus cells. Similarly, the transcriptional levels of HAS2, POU5F1, SOX2 and AREG in oocytes were not affected by treatments ($P > 0.05$). Analysis of the spent IVM media did not show any effect of the treatment on progesterone and estradiol concentrations, or on their ratio.

In conclusion, while GDF9 compensates for LPS in bovine oocytes during IVM, this effect does not alter the mRNA expression of the analyzed target genes, suggesting other genes or pathways may be involved.

Advanced maternal age-induced changes of the insulin/IGF-receptor-system and stress defence system in human granulosa cells

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Advanced maternal age (AMA, ≥ 35 years), is a risk factor for infertility, pregnancy loss,

and obstetric complications. AMA is associated with metabolic and hormonal imbalances that can modify the follicular microenvironment within the ovary. Despite the progress in understanding environmental influences on ovarian function, the molecular mechanisms underlying age-related infertility remain to be fully elucidated. Granulosa cells (GCs) were obtained from women undergoing assisted reproductive technology due to tubal or male factor infertility, to ensure exclusion of confounding pathologies. GCs were analysed in two groups: young donors (24–34 years; n ≥ 8), and women of advanced reproductive age (35–40 years; n ≥ 10). To assess whether AMA affects the oocyte microenvironment, we examined (i) expression of insulin and IGF-receptors (InsR, IGF1R) and (ii) markers of oxidative and glycative cellular stress using Western blotting and RT-qPCR in GCs. Both IGF1R and InsR levels were reduced in GCs from AMA women. While most stress-related markers – superoxide dismutase 2, catalase, glyoxalase-1, and the receptor for advanced glycation end products – remained unchanged, nuclear factor erythroid 2-related factor 2 (NRF2) mRNA abundance was increased.

Our findings indicate that advanced maternal age reduces growth factor responsiveness in granulosa cells through downregulation of insulin and IGF1 receptors. This signaling deficiency may compromise oocyte maturation and contribute to the lower ovulation and pregnancy rates observed in older women.

Extracellular adenosine regulates cell migration and junctional proteins in human testicular peritubular cells

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Several layers of thin, testis-specific smooth muscle cells (SMCs) form the peritubular wall compartment of the human testis. Through coordinated contractions, these cells enable the transport of immobile sperm. Adherens junctions (AJs) and gap junctions (GJs) represent the predominant cell-cell contact structures within this compartment, likely contributing to its functional and structural integrity. As extracellular adenosine is known to modulate cell-cell junctions in other cell types, we hypothesized that adenosine may also be a regulator of AJs and GJs in human testicular peritubular cells (HTPCs). The AJ-protein cadherin 11 (CDH11) and the GJ-protein connexin 43 (CX43) are retained in HTPCs. Western blot analysis demonstrated that adenosine elevated CDH11 protein levels. Consistently, wound-healing assays indicated reduced HTPC migration, while proliferation was not altered by adenosine stimulation (1 mM; 24 h). Adenosine further enhanced phosphorylation of CX43 in a concentration and time dependent manner (Western blot), accompanied by a ~1.5-fold increase in the size of membrane-associated CX43 plaques, as revealed by immunofluorescence labeling. Dye-diffusion assays confirmed functional GJ communication.

The potential modulatory role of adenosine in GJ communication is currently under investigation. If transferable to the in situ situation, the results suggest that adenosine may be involved in the maintenance and functionality of the multilayered architecture of the contractile human peritubular compartment by regulating CDH11 and CX43.

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AI-based health monitoring of boars – practical examples from research

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Artificial insemination (AI) plays a central role in modern pig breeding. In Germany, around 7,000 AI boars produce approx. 12 million semen doses each year. However, up to 30% of AI boars fail due to reduced libido or poor semen quality leading to high replacement rates and considerable economic losses. Therefore, detecting health problems early is crucial. This talk highlights two research projects that use artificial intelligence to monitor and improve boar health and fertility. The "VitalCheck4Pigs" project focuses on recording vital parameters, such as heart and respiratory rates, using sensor-equipped "smart dummies" during semen collection. Additionally, libido scores and semen quality are evaluated. These data are combined in a database that uses machine learning to link physiological stress biomarkers with semen performance. The goal is to identify early signs of health decline before they affect productivity. The "FertiBoar" project uses ultrasonography to monitor testicular development in young AI boars. Convolutional neural networks (CNNs) and supervised learning analyze the testicles' echotexture parameters to predict future semen quality with over 90% accuracy.

Implementing these new technologies allows for more objective, data-driven management decisions, helping to optimize boar selection, lower replacement costs and ensure consistent semen quality. Finally, these innovations strengthen both the economic efficiency and the genetic progress of pig breeding programs.

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Spontaneous pregnancy following bilateral salpingectomy in a queen: A first documented case and insights into potential fertilization mechanisms

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Surgical sterilization is a common method for reproductive control and feline population management, most often performed as an ovariohysterectomy or ovariectomy. In contrast, procedures such as tubal ligation or salpingectomy, which preserve the uterus and ovaries, have gained attention in human medicine as contraceptive options. This report describes bilateral salpingectomy in three queens, with focus on a spontaneous pregnancy following the procedure. On February 10, 2025, three queens of different breeds and ages underwent elective sterilization via midline laparotomy through the linea alba under general anesthesia. Bilateral salpingectomy was performed in all, with uterine and ovarian preservation; in one case, the right ovary was removed due to intraoperative hemorrhage. Postoperative follow-up confirmed contraception in two queens. Unexpectedly, one became pregnant and delivered three healthy kittens on May 22, 2025. The 102-day interval between surgery and parturition makes preoperative fertilization unlikely, suggesting conception occurred post-salpingectomy despite absence of functional oviducts. Possible mechanisms include a residual tubal segment or microscopic fistula at the uterotubal junction permitting sperm ascent, or transperitoneal migration of gametes, where sperm traverse the peritoneal space to fertilize an oocyte followed by intrauterine implantation. Although unreported in queens, such mechanisms remain plausible.

This case underscores the need for detailed evaluation of the uterotubal junction and surrounding tissues after salpingectomy in queens.

Bilateral mastectomy in small ruminants: Clinical indications, surgical outcomes, and long-term development

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In small ruminants, udder health is essential for both animal welfare and farmer motivation. This study evaluates clinical indications, surgical techniques, and long-term outcomes following bilateral mastectomy in small ruminants treated at our clinic since 2022. Fourteen animals were included: 12 goats and 2 sheep, aged 2–14 years. False lactation was the most frequent indication (n = 6), followed by suppurative mastitis (n = 4), gangrenous mastitis (n = 1), mammary neoplasia (n = 1), and teat cistern rupture (n = 1). In three goats with concurrent hydrometra or suspected uterine neoplasia, mastectomy was combined with ovariohysterectomy. All procedures were performed under general anesthesia with animals in dorsal recumbency. Elliptical skin incisions and ligation of regional vessels allowed for safe gland removal. One goat (7.1%) died intraoperatively due to systemic deterioration; no complications occurred in the remaining 13 animals (92.9%). Postoperative follow-up via owner interviews was possible in 10 out of 13 cases (76.9%). At the time of follow-up, five animals (50.0%) were still alive, while the other five (50.0%) had

died 2–12 months postoperatively due to systemic conditions unrelated to mastectomy. Mild, self-limiting wound discharge occurred in two cases (20.0%), whereas eight animals (80.0%) showed no wound abnormalities. All owners interviewed (100%) expressed satisfaction with the procedure and indicated that they would consent to it again if necessary.

Bilateral mastectomy appears safe and effective, particularly for false lactation and chronic mastitis, providing long-term health benefits.

Anti-Muellerian hormone as a potential reproductive biomarker in pregnant and non-pregnant alpacas: A pilot study

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Anti-Muellerian hormone (AMH) is increasingly recognized as a biomarker for assessing reproductive function in domestic species. In South American camelids, however, reference data remain scarce, and species-specific diagnostic tools are lacking, limiting clinical use. Establishing baseline values in alpacas may help clarify the physiological role of AMH and its possible application in reproductive management. This pilot study investigated serum AMH concentrations in pregnant and non-pregnant alpacas using the Elecsys® AMH assay. Eleven adult females of varying ages were enrolled: 6 non-pregnant and 5 pregnant alpacas (gestational age 81–117 days). Blood samples were collected and serum was sent to a commercial laboratory for AMH analysis within 24 hours. AMH concentrations were higher in non-pregnant (mean ± SD: 1.87 ± 1.17 ng/mL; range: 0.50–3.86) compared to pregnant animals (1.00 ± 0.38 ng/mL; range: 0.46–1.44), but the difference was not statistically significant (Mann-Whitney U test, $p = 0.247$). Within the pregnant group, no correlation was observed between gestational age and AMH concentration (Pearson r, $p = 0.823$).

As the first report focusing solely on alpacas, this study provides preliminary reference data on AMH levels and highlights variability between pregnancy states. While no significant associations were found, the results support the feasibility of AMH monitoring in this species and establish a foundation for future studies with larger cohorts to evaluate its diagnostic and clinical potential in alpaca reproduction.

Establishing a six-color flow cytometric panel for the quality control of cryopreserved mouse sperm

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Cryopreservation of sperm provides a reliable method for preserving the genetic material of valuable genetically modified mouse lines. However, before discontinuing breeding, it must be ensured that sperm survive the freeze-thaw process and retain fertilization capacity. Currently, in vitro fertilization (IVF) serves as the routine quality control, but it conflicts with the 3Rs principles as it requires superovulation and euthanasia of female mice. This study aimed to establish a multiparametric flow cytometric assay as an alternative quality control tool for cryopreserved mouse sperm. Spermatozoa from C57BL/6J males were cryopreserved using the Jax Sperm Cryo Kit. After thawing (37 °C), samples were diluted in modified Krebs-Ringer's bicarbonate medium and stained with calcein violet (1.21 µM), Fluo-4 AM (2 µM), PE-conjugated peanut agglutinin (1 mg/mL), Zombie Red (1 µL/mL), MitoProbe™ DiIC1(5) (0.015 µM), and DRAQ5 (1 µM) to assess esterase activity, intracellular Ca^{2+} levels, acrosomal integrity, plasma membrane integrity, mitochondrial potential, and DNA content, respectively. The stained samples were analysed with a CytoFlex flow cytometer. Sperm populations exhibiting up to five functional traits were identified.

Such flow cytometric approach reduces the use of live female mice, aligning with the 3R principles, and holds a potential as an objective tool for sperm quality control in genetic preservation programs. Validation with larger fertility datasets is needed.

Integrated NGS and proteomics identify new hypoxia-inducible factor 1-alpha- (HIF1α) dependent regulators of STAR in mouse granulosa cells

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Steroid hormone production in granulosa cells requires cholesterol transport into mitochondria, mediated by the steroidogenic acute regulatory protein (STAR). Hypoxia-inducible factor 1-alpha (HIF1α), which is highly active under the low oxygen (O_2) conditions typical of the ovarian follicle, regulates STAR expression primarily through the cAMP/PKA signaling pathway. In this study, we used transcriptomics and proteomic profiling to examine the role of HIF1α/O_2 in cAMP-stimulated murine granulosa (KK1) cells. Three experimental groups were analyzed: (i) cells maintained under normoxia (20% O_2) as control group, (ii) cells exposed to hypoxia (1% O_2, HIF1α activation), and (iii) cells cultured under normoxia with inhibition of HIF1α activity (20% O_2, echinomycin). Both the hypoxia-treated and echinomycin treated groups showed suppression of established STAR regulators, including Nr5a1 and Nr4a1, and reduced protein levels of the coactivator NCOA7 when compared to the control group. Additional changes included differential expression of Sp1 and AMPK-related genes (Adk, Prkaa1/2), decreased abundance of PP1/PP2A (cAMP/PKA modulators), and reduced insulin/IGF-related factors (IGFBP7, GRB14). In contrast, inhibition of HIF1α activity enhanced Fos and phospho-c-FOS expression, likely reflecting reduced availability of its functional dimer partner cJUN, shown in previous studies.

These integrated datasets suggest a biphasic role for HIF1α in granulosa cell steroidogenesis and introduce NCOA7 and associated signaling pathways as novel candidate regulators of STAR activity under reduced O_2 conditions.

Development and evaluation of a dynamic decision tree for the selective dry cow treatment of dairy for a digital expert system

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Selective dry cow therapy (SDCT) places high demands on data management. Although time-consuming, this effort is justified by the responsibility to maintain herd udder health while reducing the use of long-acting antibiotics (AB). The objective of this study is to develop a decision tree for SDCT as a knowledge base for a digital expert system that supports farmers with tailored recommendations. An evidence-based decision tree was developed and evaluated during a two-year project phase involving 19 dairy farms. The farms were divided into two groups. In Group A, herd somatic cell counts from the three dairy herd improvement tests prior to the start of the study were consistently below 250,000 cells/ml, and the detection frequencies of *S. aureus, Sc. uberis, Sc. canis,* and *Sc. agalactiae* were not elevated. In total, 1,369 dry-off observations were recorded. Dry-off recommendations differed by herd group. Herds in Group A more often received the recommendation to dry off without the use of AB (58.0%) than herds in Group B (34.7%; OR = 2.59, p-value < 0.01). The proportions of new infections in cows dried off without antibiotics according to the recommendation were similar in both groups (Group A 27.9% vs. Group B 27.4%), as were the proportions of cows that remained healthy without AB (Group A 72.1% vs. Group B 72.6%).

The results indicate that the developed decision tree enables SDCT on dairy farms without compromising herd udder health and providing a reliable knowledge base for a digital expert system aimed at optimizing dry-off management.

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Functional study of neutrophil granulocytes in the blood of equine newborns compared to the dam

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The specific immune system of neonates is still immature at birth, resulting in immune defense relying predominantly on the innate immune system, particularly on neutrophilic

granulocytes. The aim of this study was to characterize neutrophilic granulocytes in the blood of equine neonates in comparison with those of their dams. Blood samples were collected from six mares and seven foals on the first and second day of life, and neutrophilic granulocytes were isolated using density gradient centrifugation. Both neonatal and adult cells were examined for viability, reactive oxygen species (ROS) formation, and phagocytic activity. Statistical analyses were performed using descriptive statistics (mean ± SD) and group comparisons by Student's t-test, with a significance level set at $p \leq 0.01$.

Preliminary results indicate that the number of isolated neutrophilic granulocytes was higher in mares (5037.67 ± 4785.71) than in newborn foals (4731.29 ± 5875.87). The proportion of viable cells after isolation differed only slightly between mares (45.53 ± 35.28%) and foals (48.95 ± 36.10%). Neonatal cells exhibited lower ROS formation (73.87 ± 41.57%) compared to mares (96.33 ± 4.19%). Overall, phagocytic activity was comparable between the two groups, although marked inter-individual variation was observed. The phagocytosis index was 23.12 ± 30.75 in mares and 24.35 ± 36.49 in foals. Foals displayed a high degree of variability across all measured parameters, as reflected by the large standard deviations.

PDK4 orchestrates NEFA-driven metabolic reprogramming in bovine granulosa cells

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Elevated non-esterified fatty acids (NEFAs) during negative energy balance and obesity modulate ovarian cell metabolism and function, yet the underpinning molecular mechanisms, particularly why NEFA-treated cells deprioritize glucose as an energy source remain unclear. We investigated NEFA-driven metabolic rewiring in bovine granulosa cells (GCs) with a focus on pyruvate dehydrogenase kinase 4 (PDK4). We exposed GCs to physiological NEFA mixtures and quantified cellular transcriptome and analyzed bioenergetics and steroidogenesis with or without PDK4 silencing. NEFA increased ATP and mitochondrial membrane potential and reprogrammed 176 genes, upregulating fatty acid oxidation (FAO) related transcripts. This indicates a clear shift toward fatty acids as the preferred energy source. NEFA did not strongly affect the genes of glucose uptake and glycolysis, except for marked upregulating of PDK4. Interestingly, silencing PDK4 redirected substrate use toward glucose by elevating glucose transporters (SLC2A1, SLC2A10) and glycolytic enzymes (GAPDH, ENO1, LDHA) while downregulating FAO genes (SLC27A1, CPT1B). We observed that PDK4 expression is increased by ~1000 fold in large luteal cells compared to granulosa cells. PDK4 knockdown reduced STAR, HSD3B1, and CYP11A1 expression and decreased progesterone production. Together, these findings reveal PDK4 as a central node coupling metabolic flexibility to steroidogenic capacity in granulosa cells.

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Bovine corpus luteum proteomic dynamics during different stages of estrous cycle and pregnancy

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This study aimed to characterize dynamic protein expression patterns in the bovine corpus luteum (CL) model organism during distinct stages of the estrous cycle and at various points throughout pregnancy. CL samples were collected at six stages of the cycle (days 1–2, 3–4, 5–7, 8–12, 13–17, and > 18) and during four stages of pregnancy (months 1–2, 3–4, 5–6, and > 7). To map the proteome expression landscape of the bovine CL over time, we employed high-resolution liquid chromatography mass spectrometry (HR-LC-MS/MS), we identified 3,851 proteins, of which 1,900 were shared across all groups. Furthermore, we implemented SOTA (Self-Organizing Tree Algorithm) classification to detect time dependent regulation dynamics and patterns over all 10 time points, followed by pairwise differential protein expression by group analysis. Changes in protein expression and physiological pathways involved during critical windows such as the CL late stages may be integral to determining whether the CL is maintained to support pregnancy or undergoes regression. Notably, numerous new pathways involved in cell cycle regulation extracellular matrix remodeling like WNT, NOTCH, and Hedgehog, ROBO signaling showed dynamic activity. In addition, proteins traditionally associated with CL development, function, and regression (e.g., PTGES2, PTGR1, PTGR2, CRAB2, ACAT2, PEBP1, FTH1) displayed marked stage-specific regulation.

Together, these findings provide new insights into the molecular mechanisms underlying CL dynamics and advance our understanding of bovine reproductive physiology and ovarian biology.

Intrapartum factors influencing calf morbidity and mortality in dairy cattle: A systematic review of the literature (2000–2024)

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Calf health has a direct effect on the profitability and productivity of dairy farms. Moreover, calf mortality is an indicator of animal welfare. The aim of the study was to conduct a systematic evaluation of literature from the last 24 years and to evaluate the intrapartum factors affecting calf morbidity and mortality. The factors selected were birth induction, calving management (place for calving, calving in individual or group boxes, care for calving boxes), birth monitoring, and the presence of dystocia. A comprehensive search was performed in PubMed, Scopus, and Web of Science using defined keywords, and studies meeting predefined inclusion criteria were evaluated. In total, 40 publications were evaluated and analyzed for the intrapartum period. Birth induction did not influence calf morbidity and mortality. Inadequate calving management was related to higher incidence rate ratio (IRR) of mortality (1.1–3.1) and odds ratio (OR) of morbidity (1.9–10.0). Birth monitoring reduced the risk of mortality (OR 0.1–0.2) and, in one study, also the risk of morbidity (OR 0.7; confidence interval 0.53–0.91). It was found that calves from dystocia had the highest mortality risk (OR 1.9–53.2) and an increased risk of morbidity (OR 1.3–1.7).

The present literature review shows that good calving management, birth monitoring, and prevention of dystocia can have a positive impact on calf losses and calf diseases.

Uterine perforation due to placenta percreta in two bitches with dystocia

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In human medicine, placenta percreta is a well-known placental disorder. It is characterized by the invasion of trophoblasts through all layers of the uterus and in some cases in adjacent organs. In dogs, placenta percreta has so far only been described histologically in one animal on the fourth day postpartum [1]. In the present cases, two bitches were referred due to dystocia, which led to a cesarean section. The first bitch was a three-year-old dachshund. A ruptured uterus was found in surgery. The second dog was a three-year-old miniature bull terrier. In the area of the uterine body only the serosa was intact macroscopically in surgery. After ovariohysterectomy histological and immunohistochemical analyses of the uterine tissue were performed. Epithelioid invading cells were localized throughout the endometrium and within the myometrium. Locally, all endometrial layers were replaced by invading cells. The invading cells stained positive for pan-cytokeratins and negative for vimentin and the nuclear progesterone receptor (PGR), thereby excluding mesenchymal-epithelial transition, as well as maternal origin, as their possible source. Further characterization toward trophoblast lineage revealed positive staining for relaxin (RLN), identifying them as trophoblasts. No syncytia were observed,

and positive staining for COX2 supported the conclusion of an invasive cytotrophoblast origin of the invading cells.

This is the first description of dystocia due to placenta percreta in the canine with immunohistochemical evidence of invading trophoblasts.

Literature:
1. Rosenberg LM et al. Uterine perforation secondary to metritis and placenta percreta in a postpartum bitch. Can Vet J 2020; 61: 584–8.

Real-time extraction of 3D morphological features for single human spermatozoa using holotomographic microscopy

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Evaluation of sperm morphology is essential for assessing male fertility and is limited by conventional microscopy. Holotomographic microscopy enables label-free, 3D imaging of live cells, providing high-resolution volumetric information in real time. The objective here is to demonstrate the use of a rotating-source holotomographic microscope for real-time 3D imaging of spermatozoa and extraction of morphological features. Sperm samples from healthy donors were imaged using a custom holotomographic microscope equipped with a continuously rotating light source and image sensor capable of 360° rotation which enables rapid tomographic reconstruction of cellular structures without staining. Morphological parameters such as head volume, aspect ratio, and midpiece curvature were extracted automatically from 3D datasets. The system achieved 3D imaging at subsecond temporal resolution. Reconstructed refractive-index tomograms provided clear visualization of the head, midpiece, and tail, allowing precise measurement of morphological features.

The proposed technique represents a promising tool for non-invasive sperm analysis and may enhance future diagnostic approaches in reproductive medicine.

Genetic diagnostics in human disorders of fertility and aspects of preimplantation genetic testing

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Chromosomal anomalies or monogenetic disorders as well as complex genetic interactions are common causes of infertility. The clarification of the diagnosis has numerous consequences, such as the explanation of the clinical symptoms, the estimation of the genetic risk for the next generation and the planning of assisted reproduction procedures. We are learning together with our patients and go on defining single genes that are relevant for fertility disorders such as premature ovarian failure and early embryonic arrest. Furthermore clarifying the diagnosis helps couples to take an informed decision in their family planning. Preimplantation genetic testing (PGT) is a diagnostic method aiming at the reduction of spontaneous abortion and implantation failure due to numerical or familial structural chromosomal anomalies (PGT-A; aneuploidy, PGT-SR; structural rearrangements). Preimplantation genetic testing for monogenic disorders (PGT-M) can detect monogenic disease causing variants known in the family that confer a high risk for fetal or postnatal onset disease.

We present the legal background, process of counselling and application for approval through the "Bayerische Ethikkommission für PID", as well as methodological procedures of PGT and clinical case studies.

Pharmacokinetics of amoxicillin/clavulanic acid in healthy cows and cows with metritis

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Puerperal metritis in dairy cows is associated with reduced animal welfare and economic losses. A combination of amoxicillin-trihydrate and clavulanic acid is approved for treatment of metritis in dairy cows. However, evidence supporting its efficacy is lacking. This study aims to investigate the pharmacokinetic profile of both substances at the site of infection, i.e. the uterus. Therefore, twelve healthy dairy cows and twelve cows with metritis received amoxicillin-trihydrate (7 mg/kg) and clavulanic acid (1.75 mg/kg) intramuscularly at nine days ± 5 days postpartum in average. Uterine swabs were analysed microbiologically, and minimum inhibitory concentrations (MICs) were determined. Plasma, lochia, and uterine tissue samples were collected at defined time points until 38 h after administration for plasma and 12 h for lochia and tissue. The drug concentrations were analysed using LC-MS/MS and could be detected in all matrices. The mean maximum concentration (cmax) of amoxicillin in plasma (0.75 µg/ml) and in lochia (4.07 µg/g) was found at 4 h after treatment, and in uterine tissue (1.38 µg/g) at 2 h after treatment. Amoxicillin concentrations exceeded the reported MICs for gram-positive bacteria associated with metritis.

However, MICs for Escherichia coli, a major pathogen in uterine infections, were not reached in any of the sample types at any time point. This raises questions about the suitability of amoxicillin-clavulanic acid as the sole treatment for Escherichia coli-related metritis.

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Hormone levels in pig from birth to the onset of puberty in two races and two husbandry environments

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Knowledge of hormonal development from birth to the onset of puberty is important for pig breeding. In male fattening pigs, the onset of puberty is important because it marks the beginning of the undesirable boar taint. In breeding boars, it is desirable to fully exploit the fertility potential of the animals. Here we analysed different hormone levels at different times in two different breeds (German Landrace and Angeln Saddleback) under two different housing conditions (animal welfare levels 1 and 3). We analysed archetypal sex hormones such as testosterone, estradiol, and progesterone. Testosterone concentrations in male animals range between 0.5 and 1 ng/ml and tend to rise on day 160 of breeding, when the onset of puberty is expected in the German Landrace breed. Concentrations in female animals are about 50 times lower. We also analysed hormones known to alter HPG axis activity, such as INSL3, AMH, and inhibin B. INSL3 concentrations are around 1.5 ng/ml on days 30, 44, and 70 and rise to ~3 ng/ml on day 160 in both sexes. We did not notice any significant differences between the husbandry conditions for the German Landrace. The development of the Angeln Saddleback appears to be delayed compared to the German Landrace; however, the sample size needs to be increased to substantiate this assumption.

In summary, hormone data could be suitable for predicting the onset of puberty, thereby enabling timely prediction of undesirable boar taint in fattening pigs. It could also be helpful in identifying breeding boars that are fully exploiting their fertility potential. In addition, these studies could contribute to individualized husbandry conditions.

Influence of vaginal seeding on the intestinal microbiome of calves born via Caesarean section

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A diverse microbiota is crucial for developing and maintaining a healthy immune system. Compared to children born normally, children born via Caesarean section have an altered microbiome, which can be partially counteracted by vaginal seeding. The aim of this study was to investigate the effect of vaginal seeding on the early development of the gut microbiome in calves born via Caesarean section. Half of the 14 Holstein-Friesian calves born via Caesarean section underwent vaginal seeding, whereby their muzzle, nose, and body were swabbed with a towel previously incubated in the maternal vagina. Fecal samples were collected from the calves on the day of birth, on day 3, and then weekly until day 35. Bacterial community profiling was performed by sequencing the V4 region of the 16S rRNA gene on an Illumina MiSeq platform. Data analysis was conducted using QIIME2 and RStudio. Time influenced all assessed metrics of alpha and beta diversity and taxonomic composition, respectively ($p < 0.05$). On day 3 seeded calves showed a higher Faith's phylogenetic diversity

($p < 0.05$). *Prevotella* (after birth), *Subdoligranulum* (2nd week) and *Faecalibacterium* (5th week) were more prevalent in the seeded group than in the control group ($p < 0.05$). Otherwise, vaginal seeding showed no influence on analysed parameters ($p > 0.05$).

Vaginal seeding appears to have only a limited effect on the early microbiome development. Further studies should consider other factors, such as colostrum quality and birth conditions.

Prevalence of *Taylorella equigenitalis* in an Icelandic horse population in Germany

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Contagious equine metritis (CEM), caused by *Taylorella equigenitalis (T. equigenitalis)*, remains a notifiable disease of global relevance due to its impact on fertility and international horse trade. Despite strict control programs, recent data suggest an underestimated prevalence in non-Thoroughbred breeds using natural cover. Two studies assessed the prevalence of *T. equigenitalis* in Icelandic horses in southern Germany. In the first, Icelandic stallions showed significantly higher *T. equigenitalis* positive qPCR result (14.2%) compared to Draft or Haflinger stallions (2.5%, $p < 0.01$), with non-breeding Icelandic stallions displaying the highest prevalence (42%, $p < 0.001$). A second study involving 361 Icelandic horses (134 broodmares, 122 maiden mares, 105 geldings) revealed an overall prevalence of 14.4%. Unexpectedly, geldings had the highest prevalence of *T. equigenitalis* positive qPCR results (36%) compared to broodmares (2%) and maiden mares (9%, $p < 0.001$).

These findings indicate that Icelandic horses, especially non-breeding males, may represent an underestimated reservoir for *T. equigenitalis*. Pasture mating systems and close herd contact may facilitate transmission. Systematic testing of all horses in contact with breeding animals, including geldings, and separation of breeding from non-breeding stock are recommended. Future studies should determine optimal sampling sites and evaluate potential Icelandic-specific *T. equigenitalis* strains to improve surveillance and control.

Pathohistological findings in bilateral removed ovaries of mares with behavioral problems

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Behavioral problems in reproductively healthy mares are a challenging issue, which is successfully treated with bilateral ovariectomy (bOE). Studies evaluating effects of bOE on unwanted behavior 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 behavioral problems. We hypothesized, that there are non-neoplastic changes in ovaries histologically detectable, which could be responsible for unwanted behavior in mares. Bilaterally removed, clinically unremarkable ovaries from mares with behavioral problems (n = 20) were immunohistologically evaluated and compared with pathohistologically confirmed granulosa cell tumors (n = 10). Evaluation of immunohistochemical markers, Ki-67, Anti-Muellerian 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 with 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 behavioral problems of ovarian origin and a reason for the high success rate of bilateral ovariectomy.

Assessing the effects of punicalagin and oleuropein on thawed ram semen

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Improving the cryopreservation of ram semen is an ongoing research task at the German Gene Bank of Farm Animals. Recently, punicalagin and oleuropein have been suggested to be very promising additives regarding the preservation of sperm motility during freezing and thawing [1]. In order to verify these results, different concentrations of each antioxidant were tested on ram semen (n = 7 rams) in analogy to the study. A Tris-based freezing extender (4% glycerol, 15% egg yolk) served as control. Either punicalagin (0.025 mg/ml, 0.125 mg/ml, 0.250 mg/ml) or oleuropein (0.250 mg/ml, 0.625 mg/ml, 1.250 mg/ml) were added prior to freezing at 3 cm above liquid nitrogen. Motility, viability, acrosome integrity, and mitochondrial activity were assessed on the thawed samples. There was no difference in post-thaw motility, either immediately after thawing or after 15 minutes incubation at 37 °C (punicalagin: $F(1.829, 10.98) = 0.492$, $P = 0.6082$; $F(1.983, 11.90) = 0.1295$, $P = 0.8782$; oleuropein: $F(1.919, 11.51) = 2.247$, $P = 0.1511$; $F(2.827, 16.96) = 3.055$, $P = 0.0592$). The percentage viable, acrosome intact sperm remain unchanged in the presence of punicalagin ($F(1.645, 9.870) = 1.962$, $P = 0.1934$ or oleuropein ($F(1.781, 10.69) = 0.05563$, $P = 0.930$). Likewise, the mitochondrial activity in viable sperm was not influenced by punicalagin ($F(1.917, 11.50) = 0.9602$, $P = 0.4079$) or oleuropein supplementation (Friedman's test, $P = 0.5098$).

In conclusion, a positive effect of punicalagin and oleuropein on post-thaw ram semen quality could not be confirmed.

Literature:

1. Shehab-El-Deen M et al. Effects of adding punicalagin or oleuropein to TRIS diluent on quality of frozen–thawed semen from rams. Animals 2025; 15: 1242.

Age-related differences in hormonal responsiveness of human fallopian tube organoids

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The epithelium of the female reproductive tract is tightly regulated by hormones, and its aging is a key factor affecting fertility and reproductive health. To better understand age-related differences in hormonal responsiveness and underlying mechanisms, we established 3D organoid models of human fallopian tube (FT) to assess phenotypic changes driven by follicular and luteal phase hormones. From a biobank of over 20 healthy donor-derived organoids, three FT lines per age group (≤ 36 and ≥ 48 years) were analyzed. Based on an established protocol recapitulating in vitro menstrual cycle phases, organoids were pretreated with estradiol (E2) and subsequently stimulated with either E2 or a combination of E2, progesterone (P4), cAMP and the WNT inhibitor XAV939. Expression of progesterone receptor (PGR), the ciliary transcription factor FOXJ1, and the secretory marker OVGP1 was quantified by qPCR, and cilia formation was evaluated by acetylated α-tubulin staining. Compared with young organoids, aged FT organoids showed stronger PGR induction, higher FOXJ1 and OVGP1 expression, and enhanced ciliogenesis following E2 stimulation. When exposed to combined treatment with E2, P4, cAMP, and XAV939, epithelial differentiation and hormonal responsiveness were reduced, with a more pronounced decline in the aged group. Aged FT organoids exhibited stronger E2 responsiveness but enhanced P4-mediated suppression, suggesting compensatory receptor upregulation or dysregulated feedback during reproductive aging.

These findings indicate altered homeostatic control rather than functional decline, with further mechanistic investigations ongoing.

Partial hysterectomy following surgical delivery of a mummified foetus and prolonged gestation in a sheep

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A 6-year-old White Alpine ewe, weighing 80 kg, was presented due to colic. The ewe was meant to be pregnant almost 3 years ago but subsequently never lambed. It showed a severely distended abdomen, restlessness and pasty faeces. A highly distended uterus containing copious amounts of hypoechoic fluid was seen upon abdominal ultrasonography on both sides. No foetal membranes or placentomes were detected, but bony structures were visible on the right hand side. Radiography confirmed a foetus in the ventral abdomen. As the foetus was only visible on the right hand side and difficulties were expected regarding exteriorization of the uterus, a right flank laparotomy was performed in left hemilateral recumbency under deep sedation and local anaesthesia. However, the uterus was not accessible following this approach. The abdomen was closed and further surgery was planned for the following day, when a left ventrolateral incision was performed in right lateral recumbency under an extended anaesthetic protocol, and 17 L of musty-smelling, reddish-brown uterine contents were withdrawn by suction. The right uterine horn was exteriorized and incised. The mummified foetus could not be removed from the uterus without loss of substance from the uterine wall. A partial hysterectomy of the right uterine horn was performed following removal of the foetus. Postoperatively, the sheep weighed 64 kg and recovered quickly.

In summary, mummified foetuses can remain in the uterus for years without clinical disease in the dam, but can lead to severe damage to the uterine wall. Future fertility is questionable and the long-term outcome is yet to be determined.

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