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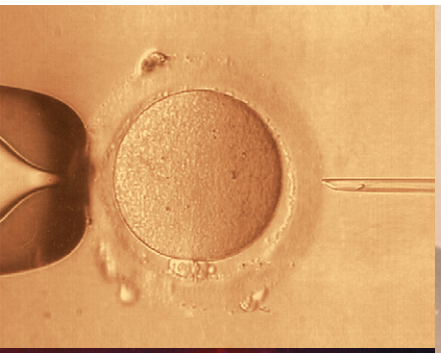

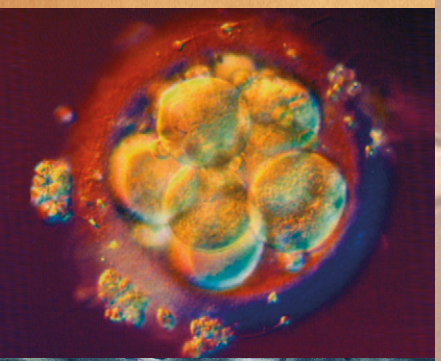

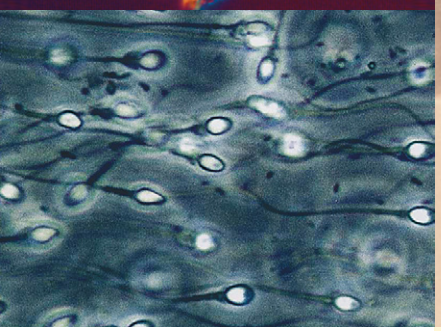

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53rd Annual Conference of Physiology and Pathology of Reproduction
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Abstracts*

1

Is the presence of overgrown follicles in dromedary camel a pathological phenomenon?

Ist das Vorhandensein von übergroßen Follikeln im Dromedarkamel ein pathologisches Phänomen?

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The objective of this study was to test whether overgrown follicles (OVGF) are a pathological phenomenon in dromedary camels. Female dromedaries with OVGF (n = 125) were examined by manual palpation and ultrasonography. The OVGF were subdivided into those with thin walls and clear hypoechogenic content (OVGF-TH, n = 18) and those with thick walls and fibrous trabeculae (OVGF-TK, n = 107). Transvaginal follicle aspiration was performed in females with OVGF and from a control group with growing follicles (GF group, n = 5). Serum was collected and analysed for FSH, LH, P4 and E2. The follicular fluid (FF) was analysed for E2 and P4. The results showed that mean E2 concentration in FF and serum were lower in OVGF-TH and OVGH-TK groups than in the GF group ($P < 0.05$). Mean FSH concentration in serum was higher in OVGF-TH and OVGH-TK groups than in the GF group ($P = 0.03$). Mean LH concentration was not significantly ($P = 0.1$) greater in OVGF-TH and OVGH-TK groups than in the GF group. It seems that the high FSH and/or LH concentrations stimulated the continuing growth of the developing follicles to reach these large sizes with the inability to ovulate, suggesting that the phenomenon of OVGF in camels is a pathological finding.

2

Accidental progesterone ingestion by a neutered male dog

Versehentliche Progesteronaufnahme durch einen kastrierten Rüden

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A 10 years old neutered Petit Basset Griffon was introduced to our clinic three days after ingestion of 12 capsules Famenita 200 mg progesterone capsules (Exeltis Germany GmbH, Ismaning, Germany). The usual dosage of the owner, who took the medication against menopausal problems, was 200 mg per day. The dog consumed 2400 mg in total in one dose. Clinical examination revealed that the dog was in good general health condition. Blood samples were taken to measure the progesterone concentration (with Immulite Progesterone Assay (LKPW), Siemens, Berlin, Germany) and other blood parameters to assess a potential toxic effect. Progesterone concentrations were 4.7 ng/ml three days after ingestion, 1.16 ng/ml after seven days and were below 0.2 mg/ml after 11 days. All other blood parameters were almost within reference ranges. Based on these findings it can be assumed that the progesterone concentrations may have been high directly after ingestion. However, the owner had not observed any clinical side effects. Information in the literature about potential progesterone intoxication is scarce. According to one case report, a cat showed signs of sedation after a potential progesterone intoxication [Dhumeaux, et al. JFMS 2010; 12: 811–3]. In addition, high progesterone doses may modify sleep patterns and be sedative and anxiolytic in humans [Bitran, et al. J Neuroendocrinol 1995; 7: 171–7]. In the present case, the dog seemed to be able to metabolize and excrete progesterone within a few days without any negative implications on his health.

3

HIF1A driven gene expression is essential for granulosa cell function in bovine

Die *HIF1A*-getriebene Genexpression ist essentiell für die Funktion der Granulosazellen bei Rindern

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Hypoxia inducible transcription factor 1 (HIF1) is a pleiotropic transcription factor consisting of a constitutively expressed β-subunit *(HIF1B)* and a regulatory α-subunit *(HIF1A)*. In the present study, we analysed the expression and function of *HIF1A* in bovine granulosa cells (GC) for further understanding of follicular biology. Treatment of GC with FSH and IGF1 hormones resulted in the dose-dependent up-regulation of *HIF1A* expression. Affirming this gene expression, immunohistochemistry of bovine ovarian sections showed distinct staining of *HIF1A* protein in the GC layer of growing ovarian follicles. These data suggest an essential role of *HIF1A* in FSH and IGF1 induced follicular functions. Suppression of HIF1 function using echinomycin and gene knockdown procedures resulted in the down- and up-regulation of *VEGFA* and *VNN2* expression, respectively, indicating the angiogenic and anti-inflammatory roles of HIF1 in GC. Importantly, *HIF1A* could be able to regulate the expression of critical steroidogenic genes such as *STAR, HSD3B1*, and *CYP19A1*. Our data indicate that *CYP19A1* is one of the plausible direct downstream targets of HIF1 in GC as shown by chromatin immunoprecipitation analysis. We could also show that HIF1 plays a distinctive role in cell proliferation under normoxic and hypoxic conditions. Knockdown of *HIF1A* resulted in decreased and increased GC proliferation under normoxic and hypoxic conditions, respectively, by regulating *PCNA* and *CCND2* expression. Based on these results, we propose that *HIF1A* driven transcriptional activity plays a key role in GC of bovine ovarian follicles.

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4

Case report: Perineal urethrostomy in a 1-day old *alpaca cria*

Fallbericht: Anlegen einer Harnröhrenfistel bei einem 1-Tag alten *Alpaca cria*

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Congenital abnormalities and malformations are relatively common in alpacas. In this case, a 1-day old cria (10.1 kg) was presented with strangury and bladder distention. Although exterior male genitals were present, urination through the prepuce was not possible. Slight pressure on an obvious bulging of a pouch in the perineal area revealed drip-like urination from a 1 mm opening. Abdominal ultrasonographical examination of the urinary tract showed a dilated bladder (diameter 6 cm). Blood urea nitrogen and creatinine levels were increased [16.4 mmol/L (4.5–11.5 mmol/L), 155.8 µmol/L (91–203 µmol/L)]. Under general and local anaesthesia, the opening in the perineal area was extended. A Rüsch ureter catheter (No 6 Teleflex Medical, Ireland) was placed through this opening into the bladder and the mucosa was adapted to the skin (Monosyn metric 3/0, B. Braun surgical, Spain). Post-operative care included antibiotic treatment, pain management, daily cleaning of the wound and control of the urination through the catheter for seven days. One day after catheter removal (day 8) ultrasonographical imaging revealed an empty bladder and blood urea nitrogen and creatinine levels were normalized (5.50 mmol/L, 89.6 µmol/L). The cria was discharged after a total of 13 days of hospital treatment and had developed satisfactorily (weight at discharge 13.0 kg). Two months later, the cria shows normal behaviour and normal urination via the urethrostomy. In conclusion, the congenital partial vulvar atresia of the intersex cria was surgically resolved via perineal urethrostomy.

5

L-lactate – a signalling molecule altering the gene expression profile in a genome wide manner during granulosa cell differentiation

L-Lactat – ein Signalmolekül verändert das Genexpressionsprofil genomweit während der Granulosazell-Differenzierung

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L-lactate was shown to influence the differentiation of cultured estrogen-active bovine granulosa cells (GC) leading to an early pre-ovulatory phenotype. Subsequently, we examined, whether L-lactate treated GC showed a significant alteration in their genome wide expression profile. Therefore, GC were cultured serum-free with FSH and IGF-1 for 8 days. Additionally, the cells were treated with 30 mM L-lactate or 30 mM NaCl (vehicle control). The ensuing microarray analysis was performed with the Bovine Gene 1.0 ST Array. 487 clusters (= 461 annotated genes) were identified as differentially expressed in L-lactate treated GC compared to the vehicle control. Two thirds (= 333 clusters) were up- and one third (= 154 clusters) down-regulated. The top up-regulated genes were *TXNIP* (22.0), *H19* (12.4) and *AHSG* (8.5), whereas *VNN1* (-2.8), *SLC27A2* (-2.7) and *CYP19A1* (-2.3) were among the top down-regulated genes. Subsequent pathway analysis by IPA revealed the involvement of 'cAMP-mediated signalling' as well as 'Axon guidance signalling'. Further, estradiol and progesterone were identified as potential upstream regulators of gene expression. An effector network analysis provided first hints that processes of "angiogenesis" and "vascularization" appeared to be activated, whereas "organismal death" was predicted to be inhibited. Our data reveal that L-lactate can act as a signalling molecule by altering the gene expression profile in a broad but specific manner. Moreover, angiogenic processes but also migratory events like cell movement and axonal guidance signalling are initiated.

6

Effects of follicle stimulating hormone- (FSH-) induced controlled ovarian hyperstimulation and nutrition on gene expression in ovine endometrium

Effekte der mit Follikel-stimulierendem Hormon- (FSH-) induzierten kontrollierten ovariellen Überstimulation und Ernährung auf die Genexpression im Endometrium bei Schafen

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Overweight and underweight are commonly known risk factors for fertility disturbances. Moreover, controlled ovarian stimulation with exogenous gonadotropins during assisted reproductive technologies may alter endometrial receptivity in cycles of in vitro fertilization (IVF). Here, using an ovine model, we investigated the effects of ovarian hyperstimulation under different feeding regimes, on expression of selected genes involved in uterine receptivity. Several (n = 14) genes were screened for their mRNA levels in caruncular endometrial areas of adult sheep in 12 experimental groups (n = 3–5 each): hyperstimulated with FSH or non-treated (naturally cycling), in normally fed (NF), overfed (OF) or underfed (UF) animals. In each feeding group, samples were collected at the early- and mid-luteal phases (days [d]5 and 10 of the luteal life-span). Within groups, more effects were observed at d10. When compared with d5, FSH-treatment at d10 revealed, i.a., decreased expression of MUC1 in NF animals, with concomitantly increased FGF10 and FN1 ($P < 0.05$). At d10, FSH increased FGF10 in NF while decreased it in UF ($P < 0.05$). Also at d10, levels of ITGB3 and ITGA4 were increased in OF ($P < 0.05$) and UF ($P < 0.001$) compared with NF control animals. Their expression was also increased in OF animals ($P < 0.05$) treated with FSH. Overall, imbalanced nutrition affects uterine expression of genes responsible for intercellular communication, cell adhesion, growth factors, and impacts the uterine responsiveness to exogenously applied hormonal stimulation and, likely, implantation.

7

Bioassay for early bovine pregnancy detection by using epithelial cells

Bioassay zum frühen bovinen Trächtigkeitsnachweis unter Verwendung epithelialer Zellen

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An early pregnancy detection (before day 21 after artificial insemination; AI), for diagnostic purpose or in research projects, is still not available for cows. However, to examine the underlying reasons for early embryonic mortality (day 7–16 after AI), an earlier pregnancy detection method is necessary. We previously described a bioassay using serum/plasma of a pregnant cow incubated with leucocytes of a donor cow, following measurements of pregnancy induced gene expression. However, due to differences in leucocyte batches, standardization of the test was poor. Therefore, the aim of the present study was to test three different cell types (bovine trophoblast cells [F3], bovine caruncular epithelial cells [BCEC], Madin-Darby bovine kidney cells [MDBK]) in a pregnancy bioassay. After incubation (24h) with serum of pregnant (n = 38) and not pregnant (n = 65) cows (day 1/16 after AI) and a control (recombinant interferon-τ; IFNτ), qRT-PCR was performed to determine mRNA expression of two IFNτ induced genes (MX2, ISG15). In all three cell types IFNτ lead to induction of MX2 and ISG15 mRNA expression. However, a threshold for pregnant and not pregnant cows

cannot be found. A transcriptome analysis of gene expression of with pregnant serum stimulated BCEC and MDBK was performed. Remarkably, the transcriptome data revealed that SPINK5 and HSD17β were solely upregulated in cells stimulated with serum of pregnant cows. Therefore, these genes are interesting candidates for further testing of an early pregnancy bioassay.

8

Teaching artificial insemination in cattle: assessment of stress level

Beurteilung des Stresslevels von Rindern im Rahmen praktischer Übungen zur künstlichen Besamung

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Artificial insemination (AI) is the most important biotechnology in modern animal breeding. As the public interest in animal welfare rises in the last years, the questions were addressed, if and how the stress level of cows used for teaching artificial insemination can be assessed. For that purpose, a herd of 32 cows were divided into three groups: Experienced (E, completed more than 8 sessions), Not Experienced (NE, completed less than 8 sessions) and Control (C, not used for AI training). All animals were monitored during 15 training sessions. In each session, blood and saliva samples for cortisol analysis were taken 60 and 30 min before and 30 as well as 60 min during handling and 30 min after the end of the training. Cortisol was measured using a validated immunoassay. Furthermore, heart rate variability (HRV) was analysed to determine the activity of the parasympathetic (PNS) and sympathetic nervous system (SNS). Intervals of five min length were analysed 60 and 30 min before starting, in the beginning, the middle and at the end, as well as 30 min after finishing a training session. Cortisol increased during training ($P < 0.005$) and decreased within 30 min after finishing a session ($P < 0.001$). During training, only NE had higher Cortisol levels than C ($P < 0.05$). HRV showed no differences between the several analysed five-minute intervals. The results show an immediate return of cortisol to basal levels after finishing a session and a reduced cortisol rise in experienced animals. In addition, the training sessions did not result in a significant shift of the autonomic nervous system to an activation of the SNS or a downregulation of the PNS.

Grants: Supported by FBF.

9

Does testicular greyscale analysis during puberty predict future reproductive performance of AI boars?

Ermöglicht eine testikuläre Graustufenanalyse während der Pubertät eine Vorhersage der zukünftigen Reproduktionsleistung von Besamungsebern?

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New ways of predicting fertilizing performance in young artificial insemination (AI) boars are very important for breeding companies to ensure genetic dissemination in the field. The aim of the current study was to characterize the testicular development of 218 Piétrain boars (Line 408, Pig Improvement Company, PIC) for commercial use in AI at defined ages throughout pubertal development. Scrotum, testes, and epididymis were examined at day (d) 100 and 170 using B-mode ultrasound with LOGIQ®e R7 (GE healthcare) and a 5–13 MHz 12L-RS linear transducer. Greyscale analysis (GSA) was performed with Image-Pro® premier (Mediacybernetics). Statistical analysis showed significant ($P < 0.05$) differences between 100 and 170 d for *Maximum grey value, Minimum Grey value (MIN GV), Mean Grey value, Standard deviation of Mean grey value, Heterogeneity, Normalized grey scale histogram width, Area under the curve (AUC)*, and *Mean Gradient value (GRAD)*. Furthermore, *AUC* ($P = 0.037$) and *GRAD* ($P = 0.030$) measured at 100 d revealed significant differences between boars with high and low sperm producing capability (SPC). *MIN GV* was higher for boars with low SPC at 170 d ($P = 0.046$). Segmental nonlinear regression analysis was used for determining breakpoints for GSA. Boars meeting defined breakpoints, i.e. noticeable shifts in ultrasonic image composition, had a higher risk for a low SPC, calculated as odds ratio (OR) at 170 d (OR = 2.4 [1.18, 5.00]). These results seem promising for predicting future SPC. Now it will be important to validate these preliminary results on a larger boar population.

10

Dystocia and stillbirth on four East German dairy cattle farms

Schwergeburten und Totgeburten auf vier ostdeutschen Milchviehbetrieben

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Dystocia and stillbirth can be of considerable economic importance for the farmer. Not just because of direct losses, but also because of negative effects on the following production period. Furthermore, it has relevance for animal welfare. Still around 6–8% of dairy calves are born dead or die shortly after delivery. Sex, parity of the dam, season of birth and monitoring intensity are a few factors influencing stillbirth and dystocia rates on a farm. In a retrospective observational study on four farms in eastern Germany we analysed calving data submitted by the farmers to the herd management software "Herde". 12,922 calvings over two years were included, and data to parity of the dam, stillbirth rate and ease of calving were collected. Dystocia occurred in 1–6% (mean 3%) of the calvings and stillbirth rate was calculated to be 4–7% (mean 6%). In male calves, dystocia rates were 2–7% (mean 4%) and 47–70% (mean 59%) of all stillbirths occurred in male offspring. Female calves were born in 1–3% (mean 2%) under dystocic conditions. In heifers dystocia occurred with 1–17% (mean 8%) of the calvings. In comparison in cows with 1–4% (mean 2%) ($p = 0.16$). Interestingly stillbirths in heifers occurred less often with 31–48% (mean 38%) of the stillbirths than in cows with 54–69% (mean 63%) ($p = 0.002$). Similarly surprising, stillbirth occurred more often in eutocic calvings (mean 44%, range 35–57% of the stillbirths) than in dystocic calvings (mean 17%, range 13–23% of the stillbirths) ($p = 0.025$). In calvings with assistance, not rated to be dystocic, stillbirth occurred in 20–52 % (mean 39%). In our preliminary study, in a limited number of farms, classical factors favouring stillbirth as parity of the dam and dystocia might not be the main risk factors, and herd specific factors need to be studied in more detail. Further studies with a larger number of farms are necessary.

11

Glyphosate affects in vitro maturation of bovine cumulus-oocyte-complexes

Glyphosat beeinflusst die In-vitro-Maturation boviner Kumulus-Oozyten-Komplexe

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Glyphosate (Roundup®) is a non-selective systemic herbicide widely used. There is some evidence that Roundup® (R-Gly) can act as an endocrine disruptor. Therefore, the aim of this study was to evaluate the effect of different Roundup® concentrations supplemented during in vitro maturation (IVM) on maturation rates and subsequent embryo development as well as on the concentrations of steroids (progesterone-P4, estradiol-E2) and metabolites (glucose, lactate, pyruvate, glutamate, glutamine, citrate) in maturation medium (MM). Embryos were generated using a standard IVP protocol (at least 3 replicates). MM (supplemented with 0, 30,

300 µg/mL R-Gly) was collected after 24 h of maturation and used for steroid analyses via RIA and photometric metabolite measurement. Maturation rates (MR) were determined after Hoechst staining and cleavage/developmental rates (CR, DR) were recorded. MR did not differ significantly between oocytes. CR/DR were similar between embryos generated with 0 and 30 µg/mL R-Gly supplementation during IVM (73.9% ± 11.1, 80.3% ± 7.1; 31.7% ± 11.2; 29.5% ± 11.5). However, cleavage and developmental rates were significantly decreased for embryos generated with 300 µg/mL during IVM (36.2% ± 16.6; 4.9% ± 4.5) compared to both other groups. A significant P4 and E2 increase was detected in MM from the 300 µg/mL group (P4: 26.0 ± 6.0 ng/mL; E2: 147.4 ± 45.1 pg/mL). No differences could be determined for the MM metabolites. These data indicate that a supraphysiological R-Gly concentration during IVM affects steroid synthesis of cumulus cells and subsequent embryo development.

12

Influence of breed on endocrine, metabolic and ethological parameters of sows during farrowing in consideration of husbandry conditions

Einfluss der Rasse auf endokrine, metabolische und ethologische Parameter im Geburtsverlauf von Sauen unter Berücksichtigung der Haltungsbedingungen

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The shift of the birthing conditions of sows from farrowing crates to farrowing pens is intended to improve animal welfare. Concerns have been raised whether modern breeding lines will be capable to fully adapt to the new environment. This study evaluates the spontaneous farrowing process in both farrowing crates and farrowing pens in German Landrace (GL; n = 28) and Angeln Saddleback (AS; n = 13), i.e. pig breeds which differ fundamentally in performance and breeding history. Beginning from the birth of the first piglet until one hour after the last delivery, half hourly blood samples were collected via an ear vein catheter. The duration of farrowing (AS: 199 ± 85 min; GL: 227 ± 57 min) as well as the rate of prolongations during farrowing (birth interval > 60 min, AS: 7.7%; GL: 46.4%, p = 0.03) have been considerably dependent on breed, regardless of the type of housing. Birth intervals were comparable between breeds (GL: 17.1 min; AS: 15.1 min). Differences in piglet losses during lactation (GL: 11.6%; AS: 16.2%) occurred mainly due to crushing (GL: 24.3%; AS: 41.7%). Preliminary results show an impact of breed on metabolic and endocrine parameters of the birthing process. Cortisol for example shows rather higher concentrations for AS vs GL under both housing conditions, whereas GL show rather higher Estradiol levels throughout the farrowing process. Results retrieved from this study form a further basis for the public discussion on improving animal welfare in pig production.

Grants: The project is supported by the Hessian Ministry for the Environment, Climate Protection, Consumer Protection and Agriculture.

13

Lipid accumulation and mitochondrial activity during *in vitro* maturation of bovine oocytes

Lipidakkumulation und mitochondriale Aktivität während der In-vitro-Maturation von Rindereizellen

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The aim of this study was to measure lipid content and mitochondrial activity during *in vitro* maturation (IVM) of bovine oocytes. Therefore, ovaries were collected in a local slaughterhouse and transported to the laboratory in 0.9% saline plus antibiotics at 30°C. The follicles were aspirated and the cumulus-oocyte-complexes (COCs) screened and classified. IVM was performed in BO-IVM medium (Bioscience) for 24 hrs at 38.8°C, 6% CO2 and maximum humidity. COCs (n = 102) were collected at 0, 4, 8 and 24 hrs after *in vitro* maturation, and time 0 was considered the moment immediately after follicle aspiration. Cumulus cells were removed and oocytes stained with MitoTracker® (300 nM) for 40 min (mitochondrial activity) and Bodipy® (3 µg/ml) for 10 min (lipid detection). After washing and fixation, the stained oocytes were analysed in a confocal microscope coupled to an inverted Axiovert 200M microscope. The fluorescence intensity was quantified using ImageJ software and statistical analysis was performed using SAS software (version 9.4). No difference was found of mitochondrial activity ($p > 0.05$) between immature and in vitro matured oocytes for 4, 8 and 24 hrs. However, after 4 hrs of maturation, the lipid content was higher ($p < 0.001$) than that observed in immature oocytes and was similar at all maturation time points studied. It was concluded that oocyte lipid accumulation occurs largely in the first 4 hours of *in vitro* maturation without variation of mitochondrial activity, suggesting that mitochondrial beta oxidation is not the main pathway involved in this process.

14

Examination about bacterial colonization on prolapsed vaginal tissue in ewes with Prolapse *vaginae ante partum*

Untersuchung zur bakteriellen Besiedlung von prolabiertem Vaginalgewebe bei Schafen mit Prolaps *vaginae ante partum*

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The prolapse of vaginal tissue ante partum is a common disorder in sheep. The treatment usually consists of reposition of prolapsed tissue and a temporary vulval lock. Whether an antibiotic drug needs to be administrated or if antimicrobial therapy is unnecessary remains doubtful. The aim of this study was to get information about bacterial colonization of the prolapsed tissue by examining smears from the prolapsed tissue (P) using an aerobe bacterial culture (n = 15). As a control, vaginal smears from healthy high-pregnant sheep were examined in the same way (K) (n = 5). In group P average 5.9 different bacterial species were detected, in group K 2.4 different species. The most frequently isolated bacteria in P were *E. coli* and alpha-haemolytic *Streptococcus*, and in K group were *E. coli* and aerobic *Bacillus*. In P, 37 cases of high-grade bacterial growth were found, in K only one such case. In conclusion the antibiotic treatment seems to be a suitable metaphylactic procedure to prevent ascending transcervical infections of placenta and fetuses.

15

Changes in seminal Semenogelin I and II of asthenozoospermic camels

Veränderungen des seminalen Semenogelin I and II von asthenozoospermen Kamelen

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Semenogelin I (SEM I) and II (SEM II) are two proteins of the seminal plasma in human and animals. They are mainly secreted from the seminal glands and required for preventing premature capacitation of the sperm. The objective of the present study was to evaluate the changes in SEM I or II concentrations of the infertile dromedary males relative to the percentage of motile sperms in their semen. Total of 47 male dromedaries were assigned for the present investigation (39 infertile and 8 control). The main complaint of these camels was the inability to impregnate fertile

females for at least one season. History and signalment, clinical examination and semen analysis were carried out for each individual animal. Based on the semenogram, asthenozoospermia (ASTH) was diagnosed and categorized into light (motility 31–50%, n = 9), moderate (11–30%, n = 11) and severe (≤ 10%, n = 13). Normal motility was set at ≥ 50% (6 infertile and 8 fertile males). Seminal plasma was harvested and analysed for Semenogelin I and II. Both biomarkers were significantly lower in ASTH than control males ($P < 0.05$). It can be concluded that seminal SEM I and II concentrations could be used as indicators for deteriorated sperm motility in male dromedary probably due to premature capacitation. Future work on the source of both SEM I and II in camel and its actual mechanism in the process of capacitation are to be investigated.

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Does glyphosate affect the expression of developmental genes in bovine oocytes?

Beeinflusst Glyphosat die Expression entwicklungsrelevanter Gene in bovinen Oozyten?

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The aim of this study is to determine, whether the active ingredient glyphosate alone or in formulation with Roundup affects the expression of developmentally important genes in bovine oocytes. Ovaries of healthy cows were collected at a slaughterhouse. Glyphosate and Roundup were added to the maturation medium in active agent concentration of 10 µg/ml each. After 24 hours of incubation (39 °C, 5% CO_2), oocytes were denuded individually for separate treatment of cumulus cells and oocytes (n = 12 for each group). Total RNA was isolated with TriZol and cDNA was synthesized. The relative expression of the gene transcripts was determined by qPCR with EvaGreen detection. The samples were quantified according to the method of Pfaffl [Pfaffl, Nucleic Acids Res 2001; 29: e45]. The expression levels were analysed via ANOVA and Tukey's HSD test. Bone morphogenetic protein 15 was significantly higher expressed in the oocytes of the Roundup group compared to the control ($p < 0.05$). Zygote arrest 1 was significantly higher expressed in oocytes of the glyphosate group ($p < 0.01$) and of the Roundup group ($p < 0.001$) compared to the control. Estrogen receptor 1 was significantly higher expressed in cumulus cells of the glyphosate group than in the control ($p < 0.05$). No significant differences in expression could be observed for growth differentiation factor 9, nuclear progesterone receptor and hydroxy-delta-5-steroid dehydrogenase. In this study, negative effects of glyphosate and Roundup on bovine oocyte development could not be detected.

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The expression of genes involved in chronic inflammation and extracellular matrix composition in mare endometrium

Die Expression von Genen, die an chronischen Entzündungen beteiligt sind, und die Zusammensetzung der extrazellulären Matrix im Stutenendometrium

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Chronic endometritis (CE) is a serious medical condition implicated in 12–46% cases of infertile mares. In these cases the pro-inflammatory molecules, including monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) act on cells resided in the extracellular matrix (ECM), and probably affect the hyaluronic acid (HA) content in ECM via changes in the activity of three different hyaluronan synthases (HAS1-3). The aim was to compare the expression of genes of *MCP-1, IL-6, HAS1-3* in the endometrium between health mares (HE) and mares with CE. Total RNA was extracted from all endometrial biopsy samples (HE: n = 10 and CE: n = 10). RT-PCR amplification was done using specific oligonucleotide primers (TaqMan). The semi-quantitation of target gene expression was performed using two independent endogenous reference genes *(GAPDH, HPRT1)* in the comparative CT method (ΔΔCT method). The high ($p < 0.05$) expression of mRNA (mean ± SD) of *MCP-1* and *IL-6* was observed in CE (*MCP-1:* 3.85 ± 1.31 AU; *IL-6:* 3.01 ± 2.42 AU) in comparison to HE (*MCP-1:* 2.25 ± 2.04 AU; *IL-6:* 1.46 ± 1.24 AU). The expression of mRNA of *HAS1* and *HAS3* was higher ($p < 0.05$) in CE (*HAS1:* 2.89 ± 1.49 AU; *HAS3:* 5.74 ± 3.13 AU) than in HE (*HAS1:* 1.30 ± 0.90 AU; *HAS3:* 1.58 ± 1.43 AU), whereas of *HAS2* was lower ($p = 0.032$) in CE (*HAS2:* 0.48 ± 0.34 AU) than HE (*HAS2:* 1.81 ± 1.31 AU). The activity of hyaluronan synthases, responsible for the production of HA which is considered as a major macromolecular component of the ECM, undergoes dynamic regulation during chronic inflammation in mare's endometrium.

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Clinical and spermatological findings in male dogs with acquired infertility: a retrospective analysis

Klinische und spermatologische Befunde bei Rüden mit erworbener Infertilität: eine retrospektive Analyse

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A variety of causative factors of infertility in male dogs have been reported. In the study, the results of clinical examination and semen evaluation of 61 infertile stud dogs were described. The dogs were presented at our clinic from 2012 to 2019 because of infertility, defined as conception failure at least 3 matings with different bitches. The dogs belonged to various breeds, were 4-8 years old and had a history of prior normal fertility. The dogs were subjected to clinical examination including ultrasonography of prostate and testes. Semen was collected by manual manipulation. The sperm concentration and motility parameters were evaluated using computer assisted sperm analyser. The morphology of spermatozoa and the percentage of live and dead spermatozoa were assessed microscopically. In all dogs semen parameters were outside of reference range, mostly oligoastheno-teratozoospermia was found. Thirty dogs showed no clinical abnormalities of genital organ and no signs of systemic diseases and the testicular degeneration was assumed as the possible cause of infertility. In 20 dogs benign prostatic hyperplasia was diagnosed, in 3 dogs infertility was associated with hypothyroidism. Three dogs had a history of babesiosis, 1 dog was previously treated with ketoconazole. Moreover, one case each chronic prostatitis, prostatic adenocarcinoma, epididymitis and retrograde ejaculation was diagnosed. The results showed that the cause of acquired infertility could not be identified in almost half of the dogs. In other dogs infertility was often associated with prostate diseases.

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IVF with oocytes from ovum-pick-up or complete dissociation of the ovary results in similar embryo development rates in the common marmoset monkey *(Callithrix jacchus)*

Vergleichbare Entwicklungsraten bei IVF-Embryonen von Ovum-pick-up Eizellen und von Eizellen aus kompletter Präparation der Ovarien beim Weißbüschelaffen *(Callithrix jacchus)*

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Common marmosets are a valuable animal model in biomedical research, particularly since their genetic modification was invented. Essential for the generation of genetically modified animals is access to early, i.e. single cell embryos, which can be obtained by assisted reproductive technologies. This includes oocyte retrieval after hormonal stimulation of the ovaries in vivo with FSH and hCG. We monitored the stimulation of the ovaries by ultrasound in 9 wake female common marmosets. Follicular development was assessed and numbers of visible follicles were documented. Comparison of the ultrasound-based data with the in vivo findings during subsequent laparotomic oocyte retrieval revealed accurate representation of the in vivo situation by ultrasound. In addition, we used two oocyte retrieval methods: (1) ovum-pick-up (OPU) by puncturing the surface of the ovaries and (2) ovariohysterectomy (OvEx) with complete dissection of the ovaries. OPU provided an average of 12.4 oocytes per round. In contrast, in the OvEx group the outcome was on average 30.1 oocytes. After fertilization with freshly prepared marmoset sperm, 11.3% of the oocytes in the OPU group developed to multicellular embryos, respective 11.6% in the OvEx group. In summary, (1) ultrasonography of marmoset ovaries is a reliable tool for the exact determination of follicular development under hormonal stimulation and (2) full dissection of the ovary after OvEx provides significantly more oocytes than OPU by needle aspiration and (3) the relative embryo outcome after IVF is almost equal irrespective to the method applied for oocyte retrieval.

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Mimicking maternal stress *in vitro:* Using a compartmentalized oviduct epithelium model to investigate transepithelial cortisol distribution

Maternaler Stress *in vitro*: Verwendung eines kompartimentierten Eileiter-Epithelzellmodells zur Untersuchung der transepithelialen Cortisolverteilung

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High levels of the stress hormone cortisol in the early embryonic environment may impair maternal fertility. Aim of the study was to use a compartmentalized in vitro model of porcine oviduct epithelial cells (POEC) to explore the distribution of cortisol across the oviduct epithelium. In experiment 1, different levels of cortisol (0, 50, 100 and 250 nM) were applied to the basolateral compartment of inserts with or without POEC (n = 5) for 3d. Levels of cortisol in the apical and basolateral compartments were quantified by ELISA. Results showed that in the absence of cells, all cortisol concentrations were stable for 3d. However, in the presence of POEC, cortisol in all groups was decreased to < 28 nM in both basal and apical compartment on d3. RT-qPCR analysis revealed generally high abundance of *HSD11B1* and *-2* (converting cortisone to cortisol and *vice versa*) and a down-regulation of *HSD11B1* in treated cells. In experiment 2, POEC (n = 2) were treated with 250 nM cortisol for 12, 24, 48 and 72h, respectively. Cortisol levels in cells, apical and basal compartments were assessed. The cortisol concentration of cells was low (< 0.4 nM in all groups). In the apical compartment cortisol increased to 84 ± 33 nM within 12h and then gradually decreased to 21 ± 6 nM after 72h. The basolateral cortisol concentration steadily decreased over time from 256 ± 10 nM (0h) to 25 ± 9 nM (72h). We hypothesize that basolateral cortisol is rapidly metabolized by the epithelium, thereby stabilizing the luminal cortisol level. The transepithelial distribution profile of cortisol provides the basis for investigating the impact of maternal stress on early embryo development.

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Excessive proliferation of spermatogonia in an azoospermic patient treated with clomiphene – an immunohistochemical approach

Massive Proliferation von Spermatogonien in einem azoospermen Patienten nach Behandlung mit Clomiphen – ein immunhistochemischer Ansatz

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Clomiphene citrate is used as pre-treatment for infertile men with non-obstructive azoospermia (NOA) prior to testicular sperm extraction (TESE). We report on a 33-year old azoospermic patient with a previous history of TESE and clomiphene citrate therapy before undergoing microscopically assisted, bilateral testicular biopsy (M-TESE). For histological analysis, four biopsies of each testis were fixed with Bouin's solution, processed and evaluated according to standard protocols. Protein detection for germ cells, germ cell neoplasia in situ, proliferation and apoptosis, and Sertoli cell markers has been performed using specific antibodies. Histology revealed mixed atrophy of spermatogenesis, predominantly with spermatogonia or primary spermatocyte arrest, respectively. Foci with qualitatively preserved, quantitatively severely reduced spermatogenesis were found in the right testis. In the left testis, seminiferous tubules were massively packed with cells proved to be normally differentiated spermatogonia with slightly increased proliferative activity and a higher degree of apoptosis. Sertoli cells proved to be correctly matured. Formation of blood-testis barrier (BTB), however, seemed to be disturbed, as the expression of BTB specific proteins was absent or dislocated in the tubules with proliferation of spermatogonia. To our knowledge this is the first observation of excessive, non-malignant proliferation of spermatogonia in an NOA patient, maybe related to impairment of Sertoli cell function and a spermatogonial dysfunction caused by high-dose clomiphene citrate treatment preceding testicular biopsy.

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Challenges of a tail mounted calving sensor

Herausforderung von Abkalbesensoren, die am Kuhschwanz befestigt werden

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Objectives Calving sensors can help to recognize onset of parturition and thus in-time calving assistance if needed. A good calving sensor must not only detect onset of calving reliably but must ensure easy handling and be harmless for the dam. Tail mounted calving sensors measure increasing tail raising of a dam as a sign of imminent parturition. This study was to test functionality and compatibility of a tail mounted calving sensor.

Methods 180 dairy cows and heifers were enrolled in this study. The study took place in a Transition Maternity Facility on a commercial dairy farm milking 2500 cows. Five days before the estimated calving date (275 post insemination) a tail mounted calving sensor was attached to the cow's tail. Compatibility and position of the sensor was controlled in every hour until calving.

Results In only 25 cows the sensor stayed on tail until calving, in 114 animals sensor fell off or slipped down > 3 cm, in 31 animals sensor was removed, because the tail was swollen or painful, in 10 cows the sensor messaged technical default.

Conclusion In this study the calving sensor could not detect parturition in 86% of the cows because it did not stay in position or caused irritation at the cow's tail. The device weighs more than 300 grams. It is a great challenge to fixate such an object safely at the cow's tail, without producing irritations, swellings or even wounds on the tail.

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Serum progesterone level but not luteal blood flow is increased 6 days after ovulation in mares with intrauterine fluid accumulation after AI with frozen semen

Bei Stuten, die eine intrauterine Flüssigkeitsansammlung nach künstlicher Besamung mit Gefriersperma aufweisen, ist der Serum-Progesteronspiegel im Gegensatz zum lutealen Blutfluss 6 Tage nach der Ovulation erhöht

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The objective of the study was to investigate the effect of an intrauterine inflammation on luteal blood flow (LBF) and serum progesterone (P4) concentrations in mares. Therefore, 40 Standardbred mares referred for artificial insemination (AI) were bred with frozen semen of proven fertility for a total of 53 estrous cycles. Ovulation (Day 0) was induced by application (iv) of 2500 IU hCG at the first time when a follicular diameter of at least 30–35 mm was observed in the presence of endometrial oedema and a positive response to a teaser stallion. At 30–36 h after application of hCG, all mares were inseminated and additionally treated (iv) with 50 mg dexamethasone. Ovulation and the presence of intrauterine fluid accumulation (IUFA) as an indicator for endometritis were detected by ultrasonography at 12 h after AI. Mares with IUFA received a uterine lavage followed by 20 IU oxytocin (im), whereas mares without IUFA received only 20 IU oxytocin. Blood sampling was performed on Day 6 to analyse serum P4 concentrations. Ultrasonography was used on Days 3 and 6 to assess LBF, and on Day 14 for pregnancy diagnosis. In 53% of the estrous cycles, IUFA was detected and negatively affected the per-cycle pregnancy rate (35% vs 68%; $P < 0.05$). Serum P4 levels on Day 6 were higher ($P < 0.05$) in mares with IUFA compared to mares without IUFA (9.3 ± 5.7 vs 6.6 ± 2.4 ng/mL; mean ± SD), whereas LBF increased ($P < 0.001$) between Days 3 and 6 but did not differ ($P > 0.05$) between mares with and without IUFA. Results indicate that an intrauterine inflammation does not affect luteal perfusion but increases the secretory function of the equine corpus luteum at 6 d after ovulation.

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Gene editing in cultured bovine trophoblast cells and IVP embryos using CRISPR/Cas9

Gen-Editierung in kultivierten Rindertrophoblastzellen und IVP-Embryonen mittels CRISPR/Cas9

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The CRISPR/Cas9 technology is well suited for the targeted introduction of mutations into genomes and is used in a wide variety of organisms, including mammals. We have adapted the method to the requirements of bovine cell cultures and embryos to generate gene knockout mutants that enable the investigation of gene functions. A) For genome editing in cell cultures, cells were transfected with plasmids that co-expressed target-specific guide RNAs and Cas9. In mutant cells (crispants), we usually found deletions or insertions (indels) of a few base pairs (bp). Unexpectedly, however, some crispants had incorporated larger insertions of up to 100 bp derived from endogenous repetitive DNA or the expression plasmid used. B) In order to allow analysis of knockout phenotypes during ontogenesis, it was necessary to perform gene editing experiments in zygotes. The aim was to inactivate both alleles of the target gene in all body cells of the developing individual. To this end, pre-assembled CRISPR/Cas9 complexes were microinjected into the cytoplasm of *in vitro* produced (IVP) zygotes. *In vitro* production of embryos was carried out according to a standard procedure using oocytes from slaughtered cows and the BO media system. The mean blastocyst rate of our IVP experiments was 45%. The mean blastocyst rates of microinjected embryos were inversely proportional to the amounts of injected CRISPR/Cas9 complexes and ranged from 44% to 19%. About 14% of blastocysts derived from CRISPR/Cas9-microinjected zygotes had mutated target sequences. However, since crispant blastocysts were mosaics, the procedure must be further optimized.

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The new four-channel EMG telemetry system used to determine uterine activity in different phases of the estrous cycle

Das neue 4-Kanal-EMG-Telemetriesystem dient zur Bestimmung der Uterusaktivität in verschiedenen Phasen des Östruszyklus

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The new four-channel telemetry transmitter was applied to measure the EMG activity of reproductive tract in sows. Four silver bipolar electrodes were surgically positioned in the muscle layer of the caudal and cranial part of the cervix (CdC, CrC); right and left uterine horn (RUH, LUH). The new digital software platform (Ponemah) was used to record the EMG signals during a continuous *in vivo* experiment. The data series were obtained from a single, representative day in the follicular phase (FLP) and mid-luteal phase (MLP). The semiautomatic analytical software (NeuroScore 3.3.1) was used to extract the EMG signal features: amplitude (A), root mean square (RMS) and duration (D), and the data from all animals of each day were pooled, compared and presented further as mean ± SD. In CdC no differences ($p > 0.05$) in A (FLP: 0.26 ± 0.10 mV; MLP: 0.15 ± 0.06 mV), RMS (FLP: 0.04 ± 0.01 mV; MLP: 0.02 ± 0.01 mV) and D (FLP: 4.11 ± 2.88s; MLP: 5.32 ± 2.51s) were noted. In CrC, RUH and LUH the voltage-dependent features (A, RMS) were higher ($p < 0.01$) in FLP (A: CrC: 1.55 ± 1.01 mV; RUH: 2.01 ± 0.85 mV; LUH: 1.81 ± 0.54 mV; RMA: CrC: 0.99 ± 0.76 mV; RUH: 1.95 ± 1.25 mV; LUH: 1.79 ± 0.94 mV) than in MLP (A: CrC: 0.18 ± 0.09 mV; RUH: 0.22 ± 0.14 mV; LUH: 0.21 ± 0.03 mV; RMS: CrC: 0.02 ± 0.01 mV; RUH: 0.02 ± 0.01 mV; LUH: 0.02 ± 0.01 mV), whereas the time-dependent feature (D) was comparable ($p > 0.05$) on FLP (CrC: 3.85 ± 3.21s; RUH: 4.85 ± 2.88s; LUH: 4.07 ± 2.91s) in comparison to MLP (CrC: 6.25 ± 3.39s; RUH: 4.42 ± 4.05s; LUH: 4.35 ± 3.44s). The new telemetry system is accurate to determine the EMG signal features including estrous cycle phases.

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Influence of thermal treatment of colostrum on triglyceride and cholesterol serum concentration of bovine neonates

Einfluss einer thermischen Behandlung von Kolostrum auf die Serumkonzentration von Triglyceriden und Cholesterin boviner Neonaten

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Colostrum intake influences not only the immunoglobulin but also fatty acid and cholesterol concentration in the blood of newborn calves. Pasteurization of colostrum is gaining importance in killing certain microorganisms. The aim of this study was to investigate the influence of pasteurization of first colostrum on the concentration of triglycerides and cholesterol in the serum of newborn calves. For this purpose, 20 newborn bull calves were

randomly divided into two groups (n = 10). The experimental group received 6 liters at 63.5 °C for 30 minutes pasteurized colostrum within the first 12 hours post natum (Pasteurizer HT 250, Förster), the control group 6 liters of untreated colostrum. Blood samples were taken from the V. jugularis at three time points (before and 24 or 48 hours after colostrum intake) and examined for the concentration of triglycerides and cholesterol. No statistically significant difference in triglycerides could be observed between the groups ($p > 0.05$). The calves of the control group had significantly higher cholesterol concentration ($p = 0{,}041$). The results indicate that the absorption of ingredients other than immunoglobulins is also influenced by pasteurization.

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Results of transvaginal ultrasound-guided reduction of twin pregnancies in mares

Ergebnisse zur transvaginalen ultrasonographisch geleiteten Reduktion von Zwillingsträchtigkeiten bei der Stute

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Twin pregnancies cause economic losses in mares. They terminate in abortion, stillbirth or in the delivery of dead, weak or deformed foals. Transvaginal ultrasound-guided twin reduction techniques are described for twin pregnancies that advance to the phase of fixation and nidation. In total 64 twin pregnancies were ultrasound-guided managed by aspiration of embryonic fluid. Mares with twin pregnancies from day 23 to day 37 after breeding were directed to the ultrasound-guided procedure. All mares got a therapeutic dose of Romifedin (Sedivet Fa. Böhringer Ingelheim/Vetmedica) for sedation and furthermore Flunixine Meglumine (Finadyne Fa. MSD Tiergesundheit) before and for 3 days after aspiration. The aspiration was done with an ultrasound equipment by Pie Medical Dorsten/Germany (Parus Vet 240) using a probe of 5 MHz and aspiration with a needle of 0.9 mm diameter and a length of 80 mm. The location of the twins in the uterus was characterized by 29% in unilateral and 71% in bilateral position. There was a significant influence ($p < 0.001$) of the age of the embryos and of the duration of the aspiration procedure on the survival rate of the remaining embryo. A further influencing factor was the amount of aspirated fluid. In total the success rate was 89%.

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The role of endometrial claudin-3 for implantation, decidualization and embryo development

Die Rolle des endometrialen Claudin-3 für die Implantation, Dezidualisierung und Embryoentwicklung

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The composition of cell contacts in the endometrium plays an important role in the process of embryo implantation and the establishment of pregnancy. In previous studies, we showed an induction of the tight junction protein claudin-3 in the decidua during murine trophoblast invasion from 6.5 dpc onwards. Here we evaluated the function of claudin-3 in implantation and embryo development with the help of a claudin-3 knockout (KO) mouse. Claudin-3 KO mice were fertile and their litter sizes did not differ significantly from those of control mice. Despite the presence of claudin-3 in the luminal and glandular epithelium of murine endometrium during the estrous cycle, KO mice revealed normal cycle phases. However, the weight of the implantation sites on 6.5 and 8.5 dpc as well as the weight of the embryos on 17.5 dpc, but not of the placentas, was significantly reduced in claudin-3 KO mice. This was shown to be a maternal effect. During postnatal development the differences in weight adjust between KO and control mice. In the claudin-3 KO mice, an upregulation of claudin-4 during the early stages of pregnancy was shown which could partly compensate for the lack of claudin-3. Since the main mass of the implantation sites at these stages of early pregnancy consists of decidual tissue, the reduced weight of implantation sites in KO mice hints to an impairment of decidualization possibly subsequently causing placental malnutrition of the embryo.

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Progestogen profiling in the European roe deer *(Capreolus capreolus)* during diapause and the reactivation period

Progestogen-Profiling im europäischen Reh *(Capreolus capreolus)* während der Diapause und der embryonalen Reaktivierungsphase

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The European roe deer *(Capreolus capreolus)* is the sole ungulate species exhibiting a period of temporary embryonic arrest during pregnancy referred to as embryonic diapause. Fertilization takes place in July and August. At the 20-30 cell stage, the embryo enters diapause for approximately five months from August to December. During diapause, the developmental pace of the embryo is reduced. After diapause in late December or beginning of January, the embryo resumes growth and its development resembles that of other ruminant species. The molecular regulation of diapause and the subsequent reactivation of embryonic growth in the roe deer is not fully understood. Unlike in other mammalian species exhibiting diapause like the mink or the mouse, progesterone and estradiol levels do not change in the roe deer during diapause and the reactivation of embryonic growth [Drews et al. RB 2019; 19: 149–57]. Nevertheless, it is conceivable that bioactive metabolites of the classical ovarian steroids such as 5α-dihydroprogesterone contribute to the regulation of diapause. A recently developed LC-MS based approach allows the profiling of 12 progestogens in plasma. A first subset of five roe deer plasma samples spanning the period of embryonic diapause was analysed for progestogens and revealed that at least eight bioactive metabolites are present in roe deer plasma. More than one hundred additional samples across diapause and reactivation are currently under analysis. Thereby, we aim at unravelling a possible contribution of progestogens to the regulation of uterine gland secretion to understand the regulation of embryonic reactivation in the roe deer.

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R123 and JC-1 staining of mitochondria in domestic cat sperm before and after freezing

R123- und JC-1-basierte Mitochondrienfärbung von Spermien der Hauskatze vor und nach Gefrierkonservierung

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To assess the mitochondrial activity of epididymal sperm from domestic cat, rhodamine 123 (R123) and a carbocyanine dye (JC-1) were comparatively applied before and after cryopreservation. Propidium iodide (PI) was used to label the dead sperm. Both dyes generate similar results for the total number of sperm with active mitochondria: 62 ± 11% and 66 ± 10% for R123 and JC-1, respectively, before freezing and 33 ± 8% and 38 ± 10% after thawing (n = 10). As additional information, JC-1 reveals two viable sperm populations with either high or low mitochondrial activity. The ratio between these populations is strongly dependent on incubation time and requires exact timing of flowcytometric analysis. The percentage of sperm with low mitochondrial activity was 16 ± 14% before freezing and 8 ± 7% after thawing. These numbers correspond to about ¼ of all active sperm in both cases. The percentage of all active fresh sperm stained by JC-1 is correlated to the percentages of motile (CASA, mean: 28 ± 13%), progressive (25 ± 13%) and fast

progressive (20 ± 11%) as well as R123 positive sperm after thawing. The percentage of R123 positive fresh sperm correlates to the percentage of fast progressive sperm after thawing (Spearman, $p < 0.05$). The percentages of R123 as well as JC-1 positive sperm determined after thawing correlate to the percentages of motile, progressive, and in case of R123, fast progressive sperm (Spearman, $p < 0.05$). We conclude that the predictive potential of JC-1 staining in fresh samples to post-thaw motility is superior to that of R123 staining but the relation to fertility remains to be investigated.

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Hepatic transcriptome analysis after intramammary pathogen challenge of cows carrying the cholesterol deficiency haplotype

Analyse des Lebertranskriptoms nach intramammärer Pathogen-Inokulation von Kühen mit dem Cholesterindefekt-Haplotyp

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The identification and elimination of hereditary diseases, especially lethal ones, is essential for high reproductive performance in cattle population. The cholesterol deficiency (CD) defect on *Bos taurus* autosome (BTA) 11 results in obligatory sick homozygous animals (CDS) and heterozygous carrier animals (CDC) [Kipp, J Dairy Sci 2016; 99: 8915–31 and Menzi, Anim Genet 2016; 47: 253–57]. In our study, we analysed the hepatic transcriptome of cows after intramammary challenge with common udder pathogens (*Staphylococcus aureus, S. aureus* or *Escherichia coli, E. coli*) early in their first lactation. In the *S. aureus* group, 4 CDC were compared to 20 wildtype homozygous animals. In the *E. coli* group, we evaluated 3 CDC to 8 animals not carrying the defect. RNA sequencing generated 3.8 billion reads (Ø 109 million reads per sample) with 98% of reads mapping at least once to the reference genome UMD3.1. Comparing CDC to the control revealed 577 (*S. aureus* group) respectively 75 (*E. coli* group) tentatively ($p < 0.1$) differentially expressed loci in the hepatic transcriptome. In both challenge groups, the *APOB* (Apolipoprotein B) gene was significantly ($p < 0.05$) differentially expressed between CDC and the control. From our data, we can conclude that *APOB* gene expression in liver is altered not only in CDS animals, but also in heterozygous carrier animals (CDC) – even under conditions of an intramammary pathogen challenge.

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Low temperature preservation in boar semen: Are lipopeptides a possible alternative to conventional antibiotics?

Niedrigtemperaturlagerung von Ebersperma: Sind Lipopeptide eine mögliche Alternative zu konventionellen Antibiotika?

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Antimicrobial resistance is a steadily increasing problem and poses a serious threat to the public health. Therefore, it is highly necessary to question the standardized supplementation of boar semen extenders with conventional antibiotics and to advance the development of novel antimicrobial compounds. The aim of this study was to evaluate a low-temperature, antibiotic-free storing procedure using Androstar® Premium extender (Minitube) combined with antimicrobial peptides (AMPs) as alternative supplements. The effect of 5 °C storage without antibiotics on sperm quality was compared to control samples using a BTS extender (Beltsville Thawing Solution, Minitube) with antibiotics at 17 °C. Secondary, seven lipopeptides were tested on sperm-compatibility at 17 °C. Two AMPs, Pal-KKK-NH_2 and Pal-KKKK-NH_2, did not negatively affect sperm quality and were evaluated for their efficiency of bacterial growth inhibition at 5 °C. As expected, 5 °C-storage affected some sperm quality parameters, however, the total motility after a fertility-relevant thermo-resistance test was not compromised ($P = 0.429$). Addition of Pal-KKK-NH_2 and Pal-KKKK-NH_2 did not impede sperm quality compared to the control at 5 °C ($P > 0.130$) and both AMPs managed to significantly ($P \leq 0.05$) reduce the amount of bacterial contamination at all tested time points (0, 24, 48 and 72 hours) during storage. Therefore, both tested AMPs are promising prospects in view of the current situation regarding antibiotic resistance. Planned in vivo trials will show if there is any effect on fertility.

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Loss of connexin-43 in Sertoli cells leads to altered prepubertal Sertoli cell maturation and impairment of the mitosis-meiosis switch

Abwesenheit von Connexin-43 in Sertoli-Zellen führt zu einer Veränderung der juvenilen Sertoli-Zell-Reifung und des Übergangs zwischen Mitose und Meiose

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In recent years, a significant decrease of male reproductive function has been reported but many underlying causes are still unknown. Generation of a conditional Sertoli cell (SC)-specific connexin43 (Cx43) knockout mouse line (SCCx43KO) has provided a translational model. As the predominant testicular gap junction protein, expression of Cx43 between SC and between SC and germ cells (GC) is known to be essential for initiation and maintenance of spermatogenesis in different species. Adult SCCx43KO males show altered spermatogenesis and are infertile. Thus, the present study aims to identify molecular mechanisms leading to testicular alterations in SCCx43KO mice. Transcriptome analysis of 8-, 10- and 12-day-old mice was performed by next-generation sequencing (NGS). Additionally, candidate genes were examined by qRT-PCR and immunohistochemistry. NGS revealed many significantly differentially expressed genes in the SC-Cx43KO mice. For example, GC-specific genes were mostly downregulated and found to be involved in meiosis and spermatogonial differentiation (e.g. *Dmrtb1, Sohlh1*). In contrast, SC-specific genes, implicated in SC maturation and proliferation, were mostly upregulated (e.g. *Amh, Fshr*). Moreover, time-specific gene ontology (GO) analysis yielded ten significant GO terms at all three time points and eight of these can be related to meiosis. In conclusion, Cx43 in SC appears to be required for normal progression of the first wave of spermatogenesis, especially for the mitosis-meiosis switch, and also for the regulation of prepubertal SC maturation.

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Fractional semen collecting: A chance to reduce the damaging effect of seminal plasma on boar spermatozoa during long-term storage

Fraktionelle Samengewinnung: Eine Chance zur Reduktion der schädigenden Wirkung von Seminalplasma auf Eberspermien während der Langzeitlagerung

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Long-term exposure of boar sperm to autologous seminal plasma can cause a dramatic loss in motility. The aim of this study was to examine whether the selection of the first 150 (± 20) ml of the ejaculate (E1) compared to the total ejaculate (TE) increases the seminal plasma tolerance of sperm from sensitive boars during long-term storage. E1 was collected separately from the remaining ejaculate (E2) of seven boars. Semen samples from E1 only and from the remaining E1 + E2 (representing the total ejaculate (TE)) were diluted to 18×10^6 sperm/ml ad 100 ml in modified pH-stabilized Beltsville Thawing Solution. After 144 h storage at 17 °C, the motility between E1-samples and TE-samples did not differ ($p > 0.05$) but they variated more in the TE-group (82.2 ± 8.4%) compared to the E1-group (87.6 ± 2.4%). Two boars with the lowest motility in TE-samples (Boar 1: 78.6%, Boar 2: 64.8%) showed a higher motility in E1-samples (Boar 1: 86.3%, Boar 2: 86.2%). Spermatozoa of all boars showed a high level of sperm with intact membranes assessed with flow cytometry with propidium iodide and FITC-PNA (E1: 85.2 ± 1.3%, TE: 83.1 ± 1.8%, $p > 0.05$) and a high proportion of viable sperm with high mitochondria membrane potential assessed with propidium iodide and JC-1 (E1: 91.6 ± 3.7%, TE: 92.4 ± 2%, $p > 0.05$). In conclusion, the selection of the first 150 (± 20) ml of the ejaculate may present a possibility to encounter the damaging effect of seminal plasma on liquid preserved sperm of sensitive boars during long-term storage.

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Effects of beta-Hydroxybutyrate on the metabolism and motility of bovine endometrial gland cells in vitro

Einfluss von beta-Hydroxybutyrat auf den Metabolismus und die Motilität boviner endometrialer Drüsenzellen in vitro

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Ketosis is a common metabolic disease in dairy cows leading to decreased reproductive performance in the transition period. Besides other factors, beta-Hydroxybutyrate (BHBA) blood concentration is significantly higher in these animals. The present study aims to investigate the effects of BHBA on bovine endometrial gland cells in vitro. Functional uterine glands are essential for uterine receptivity, implantation and placental development. An established permanent bovine endometrial gland cell (BEGC) line was used for the experiments. BEGC were stimulated with different concentrations of BHBA: 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM for 24h. Cell motility was examined by live cell imaging and cell metabolism was determined using the MTT assay. Serum free medium (SF) and modified Ham's F12 medium (FM) served as controls. There were no significant differences in cell metabolism after incubation with BHBA. Additionally, cell motility was not affected when BEGC were incubated with BHBA concentrations of 0.6 mM, 1.2 mM and 1.8 mM. However, when incubating BEGC with 2.4 mM, cells began to get apoptotic after approximately 13h. These data show that BHBA concentrations of 0.6 mM, 1.2 mM and 1.8 mM seems to have no impact on metabolism and motility of BEGC cells, though live cell imaging showed that incubation with 2.4 mM BHBA results in apoptosis after a certain time. The apoptosis of endometrial gland cells might be one of the reasons for lower fertility in ketotic cows. Further investigations are planned regarding the influence of BHBA on endometrial gland cells, for instance steroid receptor expression and structure of the cytoskeleton.

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Influence of corpus luteum stage on diameter of isolated steroidogenic cells in domestic cat

Der Einfluss des Gelbkörperstadiums auf den Durchmesser von isolierten steroidogenen Zellen bei der Hauskatze

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Corpus luteum (CL) is a transient gland on the mammalian ovary which produces progesterone. It is composed of small (SLC) and large (LLC) steroidogenic cells, as well as fibroblasts, endothelial cells and immune cells. To study the physiology of the gland, *in vitro* culture of luteal cells is a useful tool. However, the isolation step may hold limitations, because of high fragility of steroidogenic cells towards mechanical forces and enzymatic digestion. To investigate whether the stage of CL has an impact on diameter of isolated cells, we have isolated cells from CLs at formation (n = 2), maintenance/development (n = 11) and early regression (n = 5) stage. During isolation, luteal tissue pieces were incubated with collagenase 0.1% (type I and II, SERVA Electrophoresis GmbH) and 0.005% DNase (Merck) for 55 min at 39 °C. Afterwards, the pieces were gently smashed through 40 µm cell strainer and then, isolated cells were purified on 40% Percoll layer. We found that the enzymatic method isolates SLC only, with an exception for formation stage where differentiating SLC or still growing LLC were present too. The diameter for isolated steroidogenic cells was 12.9 ± 3.7, 11.9 ± 2.3, 9.3 ± 2.1 µm for formation, maintenance/development and early regression stage accordingly. By comparing the diameter of cells isolated in maintenance/development and early regression stage we detected significant difference ($p < 0.05$). We found that the average diameter of isolated SLC is decreasing with ongoing age of CL.

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DNA integrity in viable and non-viable bull sperm – a direct analysis after sorting of sperm by flow cytometry

DNA-Integrität in vitalen versus non-vitalen Spermien von Bullen – eine direkte Analyse nach dem Sortieren der Spermien mittels Durchflusszytometrie

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Previous studies revealed only poor or no correlations between sperm viability and DNA integrity. As in those experiments DNA integrity was analysed on the whole sperm population, we aimed to analyse the percentage of sperm with a high DNA fragmentation index (%DFI) separately in viable and non-viable sperm. Cryopreserved semen samples from 24 bulls were thawed and sperm viability was determined based on their mitochondrial membrane potential (MMP) using MitoProbe™ DiIC1(5). The viable and non-viable sperm were separated by a flow cytometric cell-sorter (S3e™ Cell Sorter). Non-sorted stained counterparts were used as controls and their overall MMP was determined using a flow cytometer-analyser (CytoFLEX). Afterwards, %DFI was measured using the Sperm Chromatin Structure Assay in the sorted subpopulations and their controls. The viable sperm showed a lower %DFI (0.74 ± 0.49%) compared to the non-viable sperm (5.51 ± 2.09%) and their non-sorted controls (2.48 ± 0.59%). No correlation ($P > 0.05$) was detected between MMP and %DFI in non-sorted samples. These preliminary results prove that DNA integrity is related to sperm viability, but the analysis of the entire sperm population containing a mix of viable and non-viable sperm conceals it. In further studies we will investigate if there is a relationship between DNA integrity of viable sperm and fertility.

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Tumour infiltrating T lymphocytes in human testis cancer – identification and functional analysis

Tumor-assoziierte T-Lymphozyten im humanen Hodenkrebs – Identifizierung und funktionelle Untersuchung

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In human testicular germ cell tumours (TGCT), i.e. seminoma and pre-invasive germ cell neoplasia in situ (GCNIS), infiltrating immune cells are frequently found with T cells representing a major component of the tumour-infiltrating lymphocyte (TIL) population. Recent studies indicate that functional polarization/subtypes of TIL including regulatory T cells (Treg) and T follicular helper cells (Tfh) play an important role in cancer development and immune surveillance. Therefore, we aim to characterize subsets of TIL in seminoma and GCNIS. Human testis samples (seminoma, GCNIS with lymphocytic infiltrates vs. normal spermatogenesis) were processed for immunohistochemistry (IHC) and RT-PCR, including quantitative analyses (RT-qPCR). Single cell suspensions were obtained from fresh TGCT specimens and used for flow cytometry. IHC confirmed the presence of $CD8^+$ and $CD4^+$ cells in immune cell infiltrates in GCNIS and seminoma with a scattered distribution in the latter, and revealed that $CD4^+$/$FOXP3^+$ Treg are present in seminoma in high number. These results were supported by flow cytometry, which also showed presence of Tfh. Moreover, increased transcript levels of corresponding markers and cytokines (RT-qPCR) were detected in seminoma. Further functional characterization of TIL in testicular neoplasia will help to elucidate "immune editing" during TGCT development.

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Eligibility of boars for hypothermic, antibiotic-free semen storage under field conditions

Eignung von Ebern für hypotherme, antibiotikafreie Spermalagerung unter Feldbedingungen

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Hypothermic storage of boar semen at 5 °C is an innovative method for reducing the usage of antibiotics (AB) in semen extenders and facilitates temperature stability during semen transport. The aim of this study was to examine the quality of boar semen stored at 5 °C compared to 17 °C (control) in a boar population of an AI center in preparation of a field insemination trial. Semen (n = 34 normospermic boars) was diluted to 1.5×10^9 sperm/dose in Androstar Premium®, adapted at 22 °C for 3 ± 1 h and stored at 17 °C with AB or at 5 °C without AB, respectively. At 72 h, total motility for 17 °C-samples was 87.7 ± 7.3% and 77.9 ± 10.4% for 5 °C-samples ($p < 0.05$) and at 120 h it was 87.8 ± 7.4% in 17 °C-samples and 76.5 ± 10.6% in 5 °C-samples, respectively ($p < 0.05$). At 72 h in 5 °C storage, 4 samples (11.8%) failed the requirements for usable semen (total motility ≥ 65%) and 5 samples (14.7%) at 120 h. In 17 °C storage, 1 sample (2.9%) did not pass the threshold at 72 h and 120 h. Acrosome integrity remained high (≥ 90%) for all samples and time points. Then, 6 semen pools from selected boars (n = 15) were used for postcervical insemination (2.5×10^9 sperm/dose) up to 72 h with AB-free at 5 °C stored doses (n = 99 sows) and with AB-containing at 17 °C stored doses (n = 95 sows). Total motility for both pool groups remained high (> 88.9%) for 120 h. Preliminary data revealed high pregnancy rates of ≥ 98.9% in both groups. In conclusion, based on the threshold for semen quality, for the majority of boars (85.3%) the antibiotic-free semen storage at 5 °C seems to be applicable. Confirmation in field insemination trials under different conditions must be obtained.

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Bacteriocins – a possible alternative for antibiotics in boar semen extenders?

Können Bakteriozine im Verdünnermedium für die Konservierung von Ebersperma eingesetzt werden?

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As boar spermatozoa are usually stored at 16-18 °C for several days and a sterile production is impossible, a reasonable inhibition of bacterial growth is recommended. Due to rising numbers of antimicrobial resistance, the conventional use of antibiotics in semen extenders is under current debate. As a possible alternative to conventional antibiotics, four different bacteriocins (B1-4) with known bacteriolytic activity against *Escherichia (E.) coli* were investigated for their tolerance by boar spermatozoa and their impact against bacterial contamination in BTS-extended semen w/o gentamicin. The results showed a 50% reduction of *E. coli* by B4 compared to the control. No impact on spermatozoa was found in lower concentrations of 0.01 and 0.25% bacteriocin in the extender for storage up to 72 hours, the higher concentrations of 0.5 and 1% bacteriocin led to a significant ($P < 0.05$) reduction in semen quality for B1-3. B4 with no impact on semen quality even at higher concentrations was also tested in different short- and long-term extenders as well as at 17 and 6 °C storage temperature. For all tested extenders and temperatures, no significant ($P > 0.05$) differences between samples with and w/o B4 could be shown. Therefore, the targeted use of bacteriocins in semen extenders against specific bacteria seems a reasonable alternative to conventional antibiotics. Planned *in vivo* trials with semen diluted in extenders with bacteriocins will give the information, if there is any impact on fertility.

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Progestagen-profiling in pregnant Asian elephants *(Elephas maximus)* using a novel method combining ultra-high-performance liquid chromatography (UHPLC) with high resolution mass spectroscopy (HRMS)

Progestagen-Profiling tragender asiatischer Elefanten *(Elephas maximus)* durch Anwendung einer neuen Methode, die Ultra-Hochleistungs-Flüssigchromatographie (UHPLC) und hochauflösende Massenspektrometrie (HRMS) kombiniert

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The supervision of pregnant elephants in zoos includes routine endocrine measurements. Various matrices and different methods were

used. In some assays, a specific progesterone (P4) metabolite such as 5α-pregnane-3,20-dione (5α-DHP), 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP) or 5α-pregnan-3β-ol-20-on is determined whereas other approaches made use of antibody cross reactivity to different progesterone metabolites. It was shown that the group of 5α-reduced metabolites are the most adequate metabolites to monitor CL function or predict the calving date. The aim was to determine patterns of progesterone metabolites for the first time in more detail using a novel UHPLC-HRMS based approach. Blood samples were available weekly for 5 weeks and daily in the week before the expected date of parturition from six zoo housed elephants in seven pregnancies. Ten reduced progestagens (20α-DHP, 20β-DHP, 3α,5α-THP, 3α,5β-THP, 3β,5α-THP, 3β,5β-THP, 3α-DHP, 3β-DHP, 5α-DHP and 5β-DHP), P4 and pregnenolone (P5) were determined with defined standards, but only P4, P5, 5α-DHP, 3α-DHP, 5α-THP were detected. The most abundant metabolite was 5α-DHP as expected from literature, but in contrast to previous studies, much higher levels of 5α-DHP were observed in relation to P4. Interestingly, the 5α-DHP/P4 ratio was highly individual for each elephant. In conclusion, the novel method using parallel metabolite assessment promises to be advantageous with respect to diagnose pathological pregnancies in mammals.

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Pro-angiogenic alterations in the uterus of porcine von Willebrand disease (VWD)

Proangiogene Veränderungen im Uterus bei porcinem von-Willebrand-Syndrom (VWS)

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Clinical studies show that women affected by von Willebrand disease (VWD) are more likely to miscarry, possibly because of an impaired angiogenic effect of von Willebrand factor (VWF), due to reduced (type 1, T1) or non-measurable (type 3, T3) levels of VWF. As Endothelin *(EDN1)* and associated molecules could contribute to the impaired vascularization, the aim of this study was to analyse the expression of EDN1, Endothelin Converting Enzyme 1 *(ECE1)* and connective tissue growth factor *(CTGF)* in a porcine model of VWD. The phenotype and genotype of the model is identical with the human VWD. Uterine samples were taken from 14 sows in estrus, representing T1 (n = 2) and T3 (n = 2) and compared to controls (n = 10). Genetic expressions were measured via RT-qPCR and analysed with the ΔΔCT method using *PECAM* and *PROCR* as reference genes. The mean expression level of *ECE1* was four-fold (T3) to six-fold (T1) the mean expression of WT. *EDN1* was upregulated 30% (T1) to 40% (T4) in the affected individuals. *CTGF* showed the strongest upregulation with ten-fold (p = 0.05) expression in T1 and five-fold in T3 pigs compared to WT. We demonstrate an upregulation of three pro-angiogenic factors in individuals with VWD, which might play a role in the impaired blood vessel formation and resulting angiodysplasia seen in VWD patients. This might be a possible link to the higher risk of miscarriage suggesting defective angiogenesis as causative for poor pregnancy outcome. A larger sample size and further angiogenic factors are warranted.

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Conservation genetics: Elucidating gene expression dynamics and regulatory pathways during primary to secondary ovarian follicle transition in *Felis catus* by RNA-seq

Dynamik der Genexpression während der Follikulogenese in *Felis catus*

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The *Felis catus* (domestic cat) animal model is utilised to study artificial reproductive technologies (ART) for translation to threatened and endangered felids. Currently, *in vitro* growth of female felid germ cells remains sub-optimal. Additionally, an inadequate understanding of the early stages of folliculogenesis in felids has abated conservation efforts. Here, we collected primary and early secondary follicles from domestic cat ovaries for RNA sequencing. For this study, we aimed to identify and characterise differentially expressed genes (DEGs) involved in the early stages of folliculogenesis (primary to secondary follicle transition). We identified a total of 154 significantly DEGs comparing primary versus secondary follicle groups which included 122 up-regulated and 32 down-regulated genes. Gene ontology conditional enrichment analysis functionally annotated a number of DEGs associated with biological processes involved in folliculogenesis. For example, gene expression of furin paired basic amino acid cleaving enzyme (FURIN), WEE1 homolog 2 (WEE2), phospholipase C zeta 1 (PLCZ1), and G protein-coupled receptor 149 (GPR149) was shown to increase with ongoing follicle development (5% FDR, 2-fold difference in expression). In conclusion, we have identified genes which promote folliculogenesis through early ovarian follicle growth and through the positive regulation of transforming growth factor β1 activation and positive regulation of cytosolic calcium ion concentration involved in egg activation.

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MicroRNAs of bovine sperm and their relation to sperm fertility after sex-sorting

Spermien microRNAs beim Bullen und ihr Zusammenhang mit dem Befruchtungspotential des gesexten Spermas

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Our study aimed to investigate the relation between the microRNA (miRNA) profile of unsorted sperm and bull fertility after artificial insemination (AI) with sex-sorted semen. For this purpose, 18 proven sperm donors were selected. The annual 56-day non-return rates of the 18 sires after AI with unsorted (NRR_{conv}) and sex-sorted sperm (NRR_{ss}) were 69.1% ± 2.83% and 58.4% ± 5.79%, respectively, thus showing a relative reduction of 5.25% to 31.44% after sex-sorting. Post-thaw motility and functional status (viability, mitochondrial function, intracellular Ca^{2+} levels) of unsorted sperm were assessed through computer-assisted sperm analysis and multicolour flow cytometry. Total RNA was extracted with a modified TRIzol® protocol from a pool of four cryopreserved ejaculates per bull. Small RNA libraries were prepared (NEXTflex™ Small RNA-Seq Kit v3, Bioo Scientific) and sequenced with an Illumina® HiSeq 2500 system. Unique sequences were mapped to a collection of non-coding RNA sequence databases using BLAST to identify miRNAs. The relation between miRNA expression levels and NRR was assessed using Pearson's correlation coefficient (r). In total, 85 miRNAs were identified. The expression levels of nine miRNAs (miR-9-5p, miR-423-5p, miR-34c, miR-449a, miR-1246, miR-92a, miR-2483-5p, miR-21-5p, miR-5193-5p) were related to the NRR_{ss} ($0.515 \leq |r| \leq 0.693$, $P < 0.05$) but not to NRR_{conv} ($P > 0.05$). None of these miRNAs was related to sperm motility and functional traits, except for miR-1246 that was related to the percentage of viable sperm with functional mitochondria and low intracellular Ca^{2+} levels ($r = 0.541$, $P < 0.05$). Concluding, our results show that the investigation of miRNAs in conventionally cryopreserved bovine sperm provides valuable information on their fertility after sex-sorting.

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Ruminant binucleate trophoblast cells release exosomes into the maternal caruncular stroma

Diplokaryozyten der Wiederkäuer setzen Exosomen in das mütterliche Karunkelstroma frei

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It is well known that Binucleate Trophoblast Cells (BNC) in the ruminant placenta fuse with cells of the maternal caruncular epithelium. One function of this fusion is the exocytotic release of BNC-derived secretory proteins at the basement membrane of the caruncular epithelium. The BNC-granules contain a variety of proteins, which are released into the maternal organism by this complex process. We use transmission electron microscopy to reveal that the BNC granules in several ruminant species from two different clades (bovidae, cervidae) contain intraluminal microvesicles of about 65 nm diameter. These microvesicles, together with the secretory proteins, are released by exocytosis into the maternal stroma basal to the caruncular epithelium. The released exosomes can be seen at the basement membrane of the caruncular epithelium and in the underlying stroma. This finding suggests the presence of an additional avenue of feto-maternal communication in ruminants. The released exosomes might function as local mediators of intercellular communication in the placenta and might have also systemic functions in pregnant ruminants. Typically, exosomes are generated by the exocytosis of multivesicular bodies. The origin of exosomes from intraluminal microvesicles is a new, so far unknown, process.

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Impact of a maternal supplementation of 10% inulin/FOS to high-fat or high-protein diet in mice on pregnancy outcome and offspring development

Einfluss einer maternalen Supplementierung mit 10 % Inulin/FOS zu einer Hochfett- oder Hochproteindiät in Mäusen auf den Trächtigkeitsverlauf und die Entwicklung der Nachkommen

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Nutritional imbalances or malnutrition, e.g. maternal high-protein or high-fat diets, during pregnancy are important factors for infant development disorders. Maternal supplementation of prebiotics (e.g. inulin, oligofructose) promotes gut health and reduces body weight. Prebiotics may be beneficial during pregnancy and lactation and may thus influence pregnancy outcomes. The aim of our study was to elucidate the impact of 10% inulin/oligofructose (FOS) supplementation to maternal high-fat (HF) or high-protein (HP) diet on pregnancy rate, litter size and pup's development in mice. Five-week-old female C57BL/6NCrl mice were fed control diet (CD) for 2 weeks (n = 128). Thereafter, full siblings of litter mice were randomly assigned to 6 feeding groups: CD, CD with 10% inulin/FOS (1:1, CD+I; Orafti®HP inulin, Orafti®L95), HF (40% calories from fat), HF with 10% inulin/FOS (HF+I), HP (40% calories from protein) or HP with 10% inulin/FOS (HP+I). At 8 weeks of age, female mice were mated. Pregnancy rate, pregnancy time, stillbirth, pup survival, litter size and pup weight until day 20 were evaluated by a proc mixed model in SAS. Inulin/FOS supplementation alone did not significantly affect the measured parameters. Pups of HF and HF+I-fed dams had higher body weight than pups of all other treatments at 20 days ($P < 0.05$), whereas liver weight was lower in HF+I than HF pups ($P < 0.05$). Pregnancy outcomes were not directly affected by the supplementation of inulin/FOS. Lighter liver weight of HF+I pups indicated beneficial effects on pup health, potentially reducing the risk of obesity-related diseases.

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Comparison of different thawing methods for bovine semen

Vergleich verschiedener Auftaumethoden von Rindertiefgefriersperma

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In dairy reproduction management different methods for thawing semen are practically relevant. To identify the most successful procedure the aim of the present study was to verify how different thawing regimens influence conception rate in dairy cattle. The study was conducted at a large dairy farm in Brandenburg with about 1460 milking cows from October 2017 to January 2019. Animals in heat were automatically detected by an accelerometer system with neck straps. Three thawing methods were randomly tested: (A) thawing in water bath (38 °C) for 11s, transportation of the portion to the cow with a gun warmer at 35 °C, (B) thawing in water bath (38 °C) for 35s, transportation of the portion to the cow with a gun warmer at 35 °C and (C) transportation of the portion to the cow at ambient temperature and thawing the semen in the reproduction tract of the cow for 30s. In total 3337 inseminations were evaluated. For statistical analysis logistic regression was performed. No statistically significant effect of thawing method on conception rate and no interaction between method and insemination or lactation number, insemination code (natural heat vs. Ovsynch) or season could be determined. However, independently from method, lactation number, insemination code and season significantly affected conception rate. The reason for thawing semen in water bath is to reach a higher thawing rate to inhibit osmotic damage of the sperm cells and to prevent the formation of ice crystals, which could also harm sperm cells. Under field conditions at large dairy farms other factors than thawing method are more important to reach high conception rates after artificial insemination.

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Endometritis in mares – diagnostic and treatment practices in Germany

Endometritis bei der Stute – Diagnostik- und Behandlungsverfahren in Deutschland

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Endometritis is the major cause for impaired fertility in mares (up to 60%). The aim of this study was to gather information on diagnostic and treatment practices performed by veterinarians in Germany. Practitioners (n = 550, focus: equine) were asked to fill out an online survey (34 questions). Statistical analysis of the fully completed surveys (n = 119) included descriptive statistics and χ^2-test ($p < 0.05$). Most responders came from Lower Saxony (44.8% of participants) and treated < 20 mares per year (29.0%). For diagnosis of chronic bacterial endometritis, uterine microbiology samples were obtained manually with a uterine swab by 61.2%, whereas 35.4% used the speculum technique. Most practitioners relied on systemic antibiotic treatment (77.7%) with trimethoprim-sulfonamide (57.5%). Only 17.9% used intrauterine antibiotic therapy. Uterine lavages were performed routinely by 44.8% in case of positive cultures mostly with sodium chloride solution (88.8%), while some also used abrasive solutions like iodine (33.35%), chlorhexidine (4.9%) or kerosine (2.9%). Control swabbing after treatment was performed by 87.9%. Only few influences of geographical region (e.g. on selection of mares for uterine swabbing, $p < 0.05$) were observed, whereas the number of treated mares affected the answers notably (e.g. for use of abrasive intrauterine treatment, $p < 0.05$). In summary, while endometritis treatment strategies by veterinarians in the field vary considerably and include non-evidence-based methods, most practitioners apply the recommended diagnostic techniques.

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Quantitation of bovine PAG profiles during pregnancy

Quantitative Bestimmung boviner PAG-Profile während der Gravidität

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Pregnancy-associated glycoproteins (PAGs) are commonly used molecular markers for early pregnancy in cattle, but there are only a few studies about the quantitation of the 22 known bovine PAGs during pregnancy [e.g. Green et al. Theriogenology 2004; 63: 1481–503]. New targeted proteomic methods like the parallel reaction monitoring (PRM) offer an approach to determine protein abundance in complex samples with high reproducibility and sensitivity. The aim of this study is to quantitate bovine PAG profiles with the PRM method during different stages of pregnancy. To evaluate the impact of post-translational modifications of the PAGs on the measurement method, the proteins are analysed glycosylated and deglycosylated. Cotyledon tissue was collected from an abattoir and clustered into three different stages: 1. day 91-180, 2. day 181-calving and 3. afterbirth. Gestation stages were estimated by measuring the crown-rump-length of the fetuses. PAGs were purified from all gestation stages by FPLC. Identification and quantitation of PAGs (1 pmol, 2.5 pmol, 5 pmol) was achieved by mass spectrometry with the PRM method. It was possible to quantitate all 22 known boPAGs for the examined pregnancy stages. There was no significant difference in relative PAG quantity for each PAG and pregnancy stage between the used amount of protein. However, a significant decrease in relative PAG quantity could be observed in the first pregnancy stage in deglycosylated samples in comparison to the glycosylated samples ($p < 0.05$). The study has shown that the application of PRM is a useful method for quantitative proteomics.

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Identification of phospholipid binding partners of the boar spermadhesin AWN using a lipid vesicle-based binding assay

Identifizierung von Phospholipid-Bindungspartnern des Eber-Spermadhesins AWN unter Verwendung eines Lipidvesikel-basierten Bindungsassays

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The sperm binding protein AWN, a member of the spermadhesin family, is supposed to form a multilayer coat around the acrosomal cap of boar sperm cells due to protein-protein and protein-lipid interactions. The molecular mechanisms of these interactions are not well understood. In order to investigate the interplay of the protein with lipids of the sperm membrane in more detail, binding experiments were performed using multilamellar vesicles (MLVs). For that purpose, MLVs comprising different compositions of phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and cholesterol were incubated with recombinantly expressed AWN (recAWN) at varying pH values and salt concentrations. After centrifugation, the supernatant and the pellet (containing MLVs) were checked for AWN by Western blot analysis. The presence of AWN in the pellet fraction indicates a binding of the protein to the vesicle membrane. Signals of AWN were solely detected in pellets of MLVs containing PA or PS while the protein was absent in the pellets of all other MLVs. Changing the pH from 7.4 to 7.0 or 8.0 had no influence on the results. However, in the presence of a high salt concentration (1 M NaCl) a decreased protein signal was observed in the pellet. These findings indicate a binding of recAWN to PA or PS containing membranes which is attenuated at high ionic strength. It remains to be investigated whether PA or PS which are normally located on the cytoplasmic membrane leaflet, mediate the binding of AWN to porcine sperm cells.

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Preliminary results of the therapeutic effects of cystic ovarian follicle aspiration in addition to hormonal treatment in dairy cows

Erste Ergebnisse zum therapeutischen Nutzen einer Aspiration von ovariellen Zysten zusätzlich zu Hormontherapien bei Milchkühen

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Cystic ovarian follicles (COFs) are a cause of subfertility in dairy cattle especially in the post-partum period. Therapies include hormonal as well as ablative treatments with highly variable results. The aim of this study was to characterize late post-partum occurring COFs and the therapeutic effect of an ultrasound guided transvaginal aspiration of COFs in addition to a hormonal treatment. Therefore, in an ongoing investigation cows with cysts and > 45 days in milk are randomly divided in four treatment groups. Two groups are treated either with a GnRH injection or the insertion of a progesterone releasing intravaginal device (PRID) for 7 days. In the other two groups an aspiration procedure of the COF is performed parallel to the GnRH or PRID treatment (GnRH+A, PRID+A). Besides the treatments, ultrasound examinations of ovaries and uterus, and vaginoscopy are done. Until yet, the COFs had a mean diameter of 30 ± 7 mm and 52.5% showed a vascularized wall (Power-Doppler-Mode). Included cows had in 27.5% multiple COFs (≥ 2) and 78.4% had an opened cervix. The PRID group showed in 17% (2/12 cows) luteal structures one week after insertion whereas PRID+A animals developed in 69% (9/12 cows; χ^2 $p < 0.05$) luteal structures. The GnRH+A group developed in 83% (10/12 cows) luteal structures while GnRH animals did in 100% (6/6 cows) so far. In conclusion: A COF aspiration leads to elimination of the COF and in most cases to the formation of a luteal structure. For further insights, hormone analysis of milk and cysts aspirates will be performed.

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Volume responses of equine oocytes in anisotonic and cryopreservation solutions

Volumenreaktion von Pferdeoozyten in anisotonischen und Kryokonservierungsmedien

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Oocyte preservation by vitrification requires knowledge of osmotic tolerance and membrane permeability properties. Equine oocytes were exposed to anisotonic media and solutions containing cryoprotective agents (CPAs), using an inverted microscope with a micromanipulator setup for holding the oocyte in place while adding a particular solution and recording the volume response. Oocyte volumes were analysed, from extracted video frames. The osmotically inactive volume (Vb) was determined to be 30% of the isotonic volume (Vo). The rate at which water flows into the cell under hypotonic conditions (i.e. volume change with exposure time) was found to be slower than cellular dehydration under hypertonic conditions. When oocytes are exposed to permeating CPAs, the cell volume first decreases, and then slowly returns to its original volume, which respectively can be attributed to water moving out of the cell and water and CPAs entering into the cell. Exposure to glycerol results in severe dehydration, followed by a very slow recovery to Vo, whereas exposure to propylene glycol results in lesser dehydration and a more rapid recovery to Vo. Intermediate behaviour was observed with dimethyl sulfoxide and ethylene glycol. The two-parameter-formalism will be used to fit oocyte volume versus time plots and derive the membrane permeability to water (Lp) and the various CPAs (Ps). In conclusion, the biphasic cell volume response of oocytes exposed to CPA solutions strongly depends on the type and concentration of the CPA.

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Factors of female high-fertility – a study of two outbred mouse lines selected for increased female reproductive performance

Faktoren der weiblichen Fruchtbarkeit – eine Studie zweier Auszucht-Mauslinien, welche auf erhöhte weibliche Fruchtbarkeit selektiert wurden

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Factors that are associated with increased fertility are poorly described. The majority of animal models with a reproductive phenotype are sub- or even infertile. In contrast, the Dummerstorf high fertility mouse lines FL1 and FL2 are selected for increased litter size and total birth weight of the litters for more than 180 generations. Today, they almost doubled the number of pubs per litter, as well as the total birth weight of their litters compared to the unselected control line (CTRL) and show no signs of growth retardation in the offspring. We found differences in estrous cycle of FL1 and FL2 mice compared to CTRL. Due to the fact that the ovulation rate of these mice is increased, we investigated hormonal alterations in the hypothalamic-pituitary-gonad axis (HPG axis) throughout the estrous cycle. We found alterations in mRNA expression of GnRH (down-regulated 3.0-fold in FL1), FSH (down-regulated: 3.6-fold in FL1; 2.4-fold in FL2), LH (up-regulated 2.0-fold in FL1) and Oxytocin up-regulated by the factors 2.5 (FL1) and 2.3 (FL2). In contrast to previously published data we did not observe differences in numbers of ovulated oocytes per cycle between FL1 and FL2. Furthermore, we performed a holistic gene expression approach of ovarian transcripts using next generation sequencing. Although not finally analysed, first data confirm the observation that FL1 and FL2 developed different strategies in order to increase litter size during the selection process. Analysing the 2 fertility mouse lines should give basic insights into molecular biology of high fertility.

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Assessment of foal health: differences in surface temperature distribution between foal and adult horse

Beurteilung der Fohlengesundheit: Unterschiede in der Oberflächentemperaturverteilung zwischen Fohlen und erwachsenem Pferd

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Thermography is a non-invasive technique for diagnostic imaging, which shows a physiological state of organism. The aim was to evaluate the foal and adult horse's body surface temperature. Study was conducted on 20 foals (SK Dobrzyniewo) aged 212.7 ± 44.8 days and 5 adult horses (Horse Clinic SGGW) breed Polish Konik using thermographic camera FLIRTherma CAME25 (FLIRSystems, Brasil) with emissivity 0.99–1.0. Pictures were taken from 4 sides: front, back and two flanks in equal environmental condition (closed stable, ambient temperature 19.0 ± 0.5 °C). Obtained thermograms were used for surface temperature measurement (T) in 13 Regions Of Interest (ROI) assigned on head, neck, flank, front- and hindlimbs (Flir Tools; FLIRSystems, Brasil). Differences of T between groups and ROIs were considered statistically significant for $p < 0.05$ (GraphPad Prism 6, USA). In all considered ROIs surface temperature (mean ± SD) of foals (T_f) was significantly higher than in adults (T_a). No differences were found between T_f in eye (30.5 ± 1.27 °C), nose (29.4 ± 1.03 °C) and loin (27.4 ± 2.42 °C) regions and T_a in eye (28.5 ± 1.01 °C) and nose (28.3 ± 0.98 °C). T_a in loin (23.5 ± 1.72 °C) was lower ($p < 0.01$) than in eye and nose. In foals no T_f differences ($p > 0.05$) between fore- and hindlimbs were observed in stylopodium (23.7 ± 1.25 °C/23.2 ± 4.53 °C); zeugopodium (22.5 ± 1.45 °C/23.2 ± 1.60 °C) and autopodium (metapodium: 24.7 ± 1.65 °C/23.5 ± 1.46 °C; fetlock: 24.4 ± 1.65 °C/23.4 ± 1.22 °C), while in adults significant differences ($p < 0.001$) were found. Temperature distribution in foals is significantly different from adults, both in amplitude of T and pattern in ROIs. In thermographic examination foal should not be considered as „little horse".

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Change of breeding indicators in herd of sows during period of susceptibility to mycotoxins in fodder

Änderung der Zuchtindikatoren in der Sauenherde während der Anfälligkeit für Mykotoxine im Futter

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A serious issue encountered in feeding pigs is mycotoxins: zearalenone (ZEN) and ochratoxin A (OTA). The aim was to analyse the breeding factors (e.g. changes in numbers of sows: inseminated, confirmed pregnancies, farrowed, aborted (NSa); number of piglets: alive (Pa), dead, weaned; conception rate, fertility of sows) in a herd of hybrid sows (n = 220) with high SPF health factor between 2013–2016. During this time periodic disturbances of production results and reduction in economical outcome due to fodder contamination with mycotoxins occurred. In comparison to the rest of the year, during which in both fodder and blood only trace amounts of ZEN and OTA were detected, a statistically important mycotoxin concentration increase (mean ± SEM; $P < 0.05$) in fodder and blood serum of sows was found in autumn (7.21 ± 0.34 ppb ZEN; 5.97 ± 0.51 ppb OTA and 2.70 ± 0.67 ppb ZEN; 2.02 ± 0.29 ppb OTA, respectively) and in fodder in spring (3.28 ± 0.87 ppb ZEN and 0.99 ± 0.26 ppb OTA, in blood serum: 0.63 ± 0.12ppb ZEN and 0.14 ± 0.08 ppb OTA). Significantly higher values in NSa and Pa (4.89 ± 1.05; 18.11 ± 4.22) during the autumn period of higher exposure to mycotoxins were observed amongst sows inseminated in October/November compared to spring period (NSa and Pa: 2.00 ± 0.89; 6.44 ± 2.744) and the rest of the year (NSa and Pa: 2.21 ± 0.37; 8.73 ± 1.42). A statistically important increase of abortions in sows inseminated during autumn period of exposure to mycotoxins overlaps with high concentration of ZEN and OTA in fodder and blood serum of examined sows.

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Comparison of bovine milk cell composition *in vivo* and *post mortem*

Vergleich der bovinen Milchzellzusammensetzung *in vivo* und *post mortem*

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Somatic cell count (SCC) is a key indicator for the detection of subclinical and clinical mastitis in dairy cows. Following the 3-R principle, a highly defined explant-model has been established to study immunological mechanisms associated with intramammary infections. Suitable donor cows had formerly been selected based on SCC in milk obtained *in vivo*. To reduce *in vivo* examination and to allow for a selection of donor cows *post mortem*, the aim of the present study was to define suitable donor cows *post mortem* via flow cytometric cell composition analyses. Recently, milk cell differentiation via flow cytometry attracted significant interest as a technical progress for early mastitis detection. Therefore, quarter milk samples of cows were obtained *in vivo* (n = 9) and within 20 minutes after slaughter (n = 10). Samples were analysed for SCC, major pathogens and milk differential cell count. Percentages, absolute numbers and morphology of lymphocytes and polymorphonuclear neutrophils (PMNs) were compared between the two groups. Samples of animals taken after slaughter displayed significantly higher percentages of PMNs among milk cells (74% vs

50%, P < 0.01) with PMN being significantly larger when compared to samples obtained *in vivo*. Separate milk analysis of a third group of euthanized animals (n = 3) did not show differences in SCC in milk until 45 minutes *post mortem*. In conclusion, the present data shows that SCC and milk differential cell count cannot be applied as indicators for udder health in dairy cows immediately after slaughter.

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Correlation between cold and thermo-resistance in boar spermatozoa

Zusammenhang zwischen Kälte- und Thermoresistenz in Ebersperma

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Boar spermatozoa are usually stored at 17 ± 1 °C in extenders with antibiotics. Cold storage at 6 °C could restrict bacterial growth and reduce antibiotics utilization, but may deteriorate sperm quality, as boar spermatozoa are sensitive to cold temperatures. In order to fertilize, however, sperm have to be resistant to the warm temperature in the female genital tract (38 °C). Thermo-resistance test (TRT) is used to simulate the conditions in the female genital tract and can help explaining variations in boar fertility. The present study therefore aimed at analysing the relation between cold- and thermo-resistance in boar semen. Total sperm motility was measured in 166 BTS-extended ejaculates of young Pietrian AI boars (aged: 267 ± 26 d) using computer assisted sperm analysis (CASA, AndroVision®, Minitube). In a split-sample procedure, semen was stored for cold stress at 6 °C for 3 d and incubated 10 min at 38 °C before CASA and for TRT at 17 °C for 7 d and 300 min at 38 °C. Samples with a minimum of 65% motile sperm (cutoff for use in AI) were considered cold- or thermo-resistant, respectively. Based on this cutoff, 17% of the ejaculates were cold- and 31% were thermo-resistant. Chi-square test revealed that cold- and thermo-resistance are dependent factors (P = 0.03). Furthermore, total motility after thermal and cold stress showed a positive correlation ($r_s = 0.39$, $P \leq 0.01$, Spearman's rho). Analysis of additional sperm characteristics and cellular components will enable us to comprehend inter-boar variability in temperature-related behaviour.

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Establishment of a murine 3D cell culture model of the endometrium

Etablierung eines murinen 3D-Zellkultur-Modells des Endometriums

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In vitro models can contribute in understanding the mechanisms of embryo implantation. This study aims to establish a murine 3D endometrial co-culture, which can be combined with a trophoblast body or blastocyst. This may at least partially mirror early implantation and the embryo-endometrial interface. Endometrial cells were isolated from murine uteri 3.5 days after plug detection [De Clercq et al. JOVE 2017; e55168]. Stromal cells were seeded in Matrigel® or GrowDex®, epithelial cells onto the gel surface [Wang et al. MHR 2012; 1833–43]. The 3D co-culture was cultivated for several days. Following cryo-, paraformaldehyde (PFA) or methanol-carnoy fixation, matrix structure and cellular conservation were examined macroscopically and histologically. Cells were immunophenotyped via cytokeratin, vimentin, Ki67, and caspase antibodies (n = 4). Murine trophoblast stem cells (TS) were passaged and banked. For first TS body generation, previously cultivated TS cells were reseeded in suspension plates and cultured on a shaker. Only Matrigel® provided stability in handling and, together with PFA-fixation, histological structure preservation, whilst methanol-carnoy and cryo-fixation led to a loss of matrix. Epithelial cells were positive for cytokeratin and negative for vimentin, some of them were also positive for Ki67 and – slightly fewer – for caspase. Yet, epithelial cells lacked monolayer formation and high prismatic morphology. TS shaker cultures formed aggregates within 48 hours with consecutive attachment and loss of aggregation. Hence, the 3D co-culture and TS body set-up requires further optimization. However, preliminary results may indicate the feasibility of combining the endometrial co-culture with a TS body.

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Minimum inhibitory concentrations of different antibiotics against pathogens isolated from uteri of postpartum dairy cows

Minimale Hemmstoffkonzentrationen verschiedener Antibiotika gegen Erreger aus postpartalen Milchkuh-Uteri

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After a comprehensive amendment in 2018, German drug regulations restrict the use of third-generation cephalosporins that were commonly used for the treatment of acute puerperal metritis (APM) in dairy cows. Therefore, the objective of this study was to evaluate minimum inhibitory concentrations (MIC) of ceftiofur, as well as of ampicillin and amoxicillin/clavulanic acid as possible alternative treatment options for APM. A total of 37 *T. pyogenes* and 85 *E. coli* isolates were evaluated for determination of MIC. The broth microdilution method was carried out in accordance with the Clinical and Laboratory Standards Institute. The concentrations that inhibited the growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were calculated. For *T. pyogenes*, the MIC_{50} of ceftiofur was 0.25 µg/mL and the MIC_{90} was 0.5 µg/mL. For *E. coli* the MIC_{50} of ceftiofur was 0.5 µg/mL and the MIC_{90} was 1.0 µg/mL. The MIC_{50} of ampicillin for *T. pyogenes* was ≤ 0.015 µg/mL and the MIC_{90} was 0.06 µg/mL. The MIC_{50} of ampicillin for *E. coli* was 4 µg/mL and the MIC_{90} was ≥ 128 µg/mL. The MIC_{50} of amoxicillin/clavulanic acid for *T. pyogenes* was ≤ 0.015 µg/mL and the MIC_{90} was 0.06 µg/mL. The MIC_{50} of amoxicillin/clavulanic acid for *E. coli* was 4 µg/mL and the MIC_{90} was 8 µg/mL. No MIC clinical breakpoints to classify pathogens into susceptible, intermediate, or resistant have been established for bovine uterus-borne *E. coli* or *T. pyogenes*. In general, *T. pyogenes* isolates exhibited low MIC. The growth of some *E. coli*, however, could not be inhibited by the highest concentration of ceftiofur and ampicillin tested in this study.

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Messenger RNA expression of selected pro-inflammatory factors and mucins at 5 different endometrial sites of cows depending on uterine health status

Messenger-RNA-Expression ausgewählter pro-inflammatorischer Faktoren und Mucine an 5 verschiedenen endometrialen Regionen von Kühen abhängig vom Gesundheitsstatus des Uterus

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Recent studies have elucidated the role of several pro-inflammatory factors as mediators in inflammatory processes of the bovine endometrium. The objective of this study was to assess the mRNA expression of selected pro-inflammatory factors and mucins at 5 pre-defined endometrial sites depending on the uterine health status. Therefore, samples were collected by the cytobrush-technique from cows with clinical endometritis (CE, vaginal discharge, n = 4), subclinical endometritis (SE, ≥ 5% polymorphonuclear cells [PMN] in cytobrush smear, n = 7), and healthy cows (HE, no vaginal discharge, < 5% PMN, n = 7) between 28 and 34 days in milk. Endometrial samples were obtained from each cow at the following sites: corpus uteri (CU), left horn base, right horn base, left horn tip, and right horn tip. The mRNA expression of cluster of differentiation (CD) *CD45* and *CD66*, cyclooxygenase 2, interleukin (IL) *1A, IL1B*, and *IL8*, and mucins (MUC) *MUC4* and *MUC16* were quantified by RT-qPCR. Messenger RNA expression of *IL1A, IL1B* and *IL8* was significantly higher in SE and CE cows compared with HE cows, whereas *MUC16* was more abundant in SE cows compared with CE and HE. Comparing the mRNA expression obtained from different sites in SE cows, the site CU showed the greatest sensitivity and specificity. In conclusion, *IL1A, IL1B*, and *IL8* may represent suitable potential markers for the detection of SE on a molecular biological basis and the accuracy is highest when samples were collected at the CU site.

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Selecting conditions for droplet vitrification of stallion sperm

Auswahl der Bedingungen für die Tröpfchenvitrifikation von Hengstspermien

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Vitrification of cells and tissues requires high concentrations of cryoprotective agents (CPAs), and high cooling rates. On the other hand, too high CPA concentrations are toxic. In order to define proper vitrification conditions for stallion sperm, the ability of various media to vitrify was investigated. The minimum concentration needed for vitrification was determined for various CPAs, and effects of droplet size and carrier solution were identified. To obtain insights in CPA toxicity, sperm viability was investigated as a function of the CPA concentration and exposure time. Droplet vitrification was done by pipetting droplets on a metal block cooled in liquid nitrogen. Non-successful vitrification (i.e. ice formation) was evident as the appearance of an opaque state. With 10 µL droplets, half of the droplets vitrified at 42 to 46% CPA. At a droplet size of 5 µL, the glycerol concentration needed for vitrification could be reduced from 42 to 38%, whereas for ethylene glycol, a shift from 46 to 44% was observed. Sperm membrane intactness was found to decrease in a concentration and time dependent manner. Sperm appeared to tolerate high concentrations of dimethyl sulfoxide and propylene glycol better as ethylene glycol and glycerol. Only minor differences in sperm viability were found when CPAs were diluted in saline with choline instead of sodium chloride. Five min exposure to a 40% CPA solution already resulted in a drastic decrease in membrane intactness (i.e. pre-freeze; from 69% to 22–54%). In conclusion, sperm droplet vitrification requires establishing the narrow balance between droplet size, CPA type and concentration, and exposure time.

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In vitro measurements of beta-blocker and their influence on placental function

In-vitro-Messungen von Betablockern und deren Einfluss auf die Plazentafunktion

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Introduction Hypertension diseases represent a complex and ongoing health threat, especially during pregnancy. Today the therapies of choice are pharmaceuticals (beta-receptor blocker). However, these blockers are also known for their negative side-effects, such as cytotoxicity and decreasing vitality. Hence, in the present experimental study we want to investigate the influence of the beta-blocker Propranolol and Metoprolol on the placental function.

Methods Cytotrophoblast cells were isolated from term human placentae according to Kliman et al., with slight modifications [Kliman, et al. J Endo 1986; 118: 1567–82]. The influence of Propranolol (0.0375 mg/ml–2.4 mg/ml) and Metoprolol (0.0312 mg/ml–2.0 mg/ml), respectively, was measured with respect to cell viability (MTT; Roche Diagnostics), cytotoxicity (LDH detection, Roche Diagnostics) and hormone levels (17-beta-estradiol and beta-hCG).

Results Both Propranolol and Metoprolol showed dose-dependent inhibition of estradiol synthesis and cytotoxicity. High dosage of Metoprolol led to diminished vitality, whereas Propranolol showed a decrease in all used concentrations. Both drugs had no influence on hCG synthesis.

Conclusion Here we tested the effects of the pharmaceuticals Propranolol and Metoprolol on the production of estradiol and hCG. It was shown that the effects of Propranolol and Metoprolol are dose-dependent and could have negative influence on the nutrition transfer towards the fetus.

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Expert survey on the treatment options for ovarian cysts in the bitch

Expertenbefragung zu den Behandlungsmöglichkeiten von Eierstockzysten bei der Hündin

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When we see rare cases in daily practice, it may be difficult to base clinical decisions on reliable research data [Arlt et al. RDA 2014; 49 (Suppl 3): 11–5]. Therefore, the database project REPROCASES (www.evssar.org/reprocases/) aims to collect cases and information on rare diseases in small animal reproduction so that in future new conclusions from case series may be possible. The first disease we have included exemplarily into REPROCASES is cystic ovarian disease (COD) in dogs. Various treatment options have been suggested for COD, but no reliable research data on efficacy, potential side effects and fertility prognosis is available in literature [Arlt et al. RDA 2016; 51 (Suppl 1): 3–11]. To prepare a case report form for the database including treatment suggestions a survey among experts using an online tool (https://www.questback.com/) was performed beforehand. Experts were contacted via the EVSSAR newsletter, a mailing list (cafereprod-l@list.cornell.edu) and during the EVSSAR Congress 2019. Participation was anonymous and voluntary. When asked which therapy the experts recommended for initial treatment, 15 of the 23 participants suggested a hormonal therapy. Five colleagues recommended surgical therapy, two opted for different approaches, and one participant stated that no therapy is promising. Of the vets who preferred hormonal therapy, eleven suggested a treatment with hCG and/or GnRH analogues. However, treatment regimens and dosages differed substantially so that no overall suggestions could be deduced from the answers. It therefore seems useful that in fields such as COD in dogs, in which single-centre or even network research is difficult or not possible in reasonable time, international case collections may help to learn more about treatment outcomes, side effects and future fertility.

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Viability and proliferation of mesenchymal stem cells after autologous transplantation into uterine cervix in pig

Die Lebensfähigkeit und die Proliferation von mesenchymalen Stammzellen nach autologer Implantation in den Gebärmutterhals beim Schwein

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To achieve better results in in vivo studies we established the protocol for generation of porcine bone marrow mesenchymal stem cells (BM-MSC). The aim of this study was to evaluate the influence of two types of cell-labelling solution on MSC viability and proliferation at the places of autologous transplantation. The gathered MSC were isolated and cultivated in vitro as well as labelled with PKH26 and DID. Thereafter, MSC suspensions were transplanted into the muscle layer of the cervix. Four weeks later, after hysterectomy, 10-µm thick slices were labelled with primary (anti-Ki67) and fluorescent secondary antibodies linked with AF405 and the nuclei with NG. MSC were imaged and quantified. Living MSC were found in all translantation places. PKH26 and DID stained cells were counted (mean% ± SEM) and no significant differences between both labelling types of MSC could be observed. We confirmed that PKH26/DID-labelled MSC underwent in vivo multiplication in transplantation places. The Ki67 expression was significantly higher in PKH26 (22.3 ± 3.1 Ki67$^+$cells of the PKH26$^+$cells) and DID (28.9 ± 9.2 Ki67$^+$cells of the DID$^+$cells) labelled MSC with respect to PKH26/DID$^-$ cells (13.2 ± 1.9) of control tissue. There were no significant differences between Ki67 expression in both labelling types of MSC. The type of cell-labelling solution has no significant influence on MSC viability and proliferation after the transplantation into the muscle layer of porcine uterine cervix. The Ki67 expression of unlabelled cells close to labelled MSC was also significantly higher in both labelling types with respect to control, so we now focus on the paracrine signalling pathway through growth factor expression by MSC.

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Reduced circumfluent oxygen concentration increases cell functionality of bovine endometrial tissue explants *in vitro*

Reduzierter Umgebungs-Sauerstoffgehalt erhöht die Zellfunktionalität boviner Endometriums-Explants *in vitro*

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Post-partum diseases such as subclinical endometritis have an impact on animal welfare and cause significant economic losses in dairy farming. To examine underlying pathophysiological mechanisms of infectious diseases an endometrial tissue explant model was established. To approximate *in vitro* conditions to the situation *in vivo*, culture conditions must be optimized as far as possible. Differing oxygen concentrations have been reported to impact cell viability in several cell culture models, but details concerning explant handling remain unknown. Aim of the present study was to evaluate the effect of two different O_2 concentrations on metabolic cell activity of bovine endometrial explants *in vitro*. Endometrial tissue explants (n = 144; 4 uteri) were collected at the local abattoir with a 5 mm biopsy punch. Incubation of explants (9 per animal) was performed at either 5.5% O_2 (reported physiological tissue concentration) or 16.0% O_2 (common culture conditions) for 3 h or 24 h. To determine metabolic cell activity, water-soluble tetrazolium salt-8 (WST-8) was added 3 h before the end of incubation. Optical densities (OD) of explant supernatants were photometrically determined. Explants incubated for 24 h at 5.5% O_2 showed significantly higher ODs compared to explants incubated at 16.0% O_2 (5.5% O_2: OD 1.74 ± 0.67 vs 16.0% O_2: OD 0.89 ± 0.35; $P < 0.001$). In conclusion, a reduced O_2 concentration of 5.5% increases cell functionality of bovine endometrial tissue and might have an impact on the pathogen-specific immune response.

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Does oral uptake of estradiol-17β affect gene expression in porcine embryos and the endometrial luminal epithelium during pre-implantation development?

Beeinflusst die orale Estradiol-17β-Aufnahme die Genexpression im Preimplantations-Embryo und im luminalen Epithel des Endometriums beim Schwein?

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Endocrine disrupting chemicals (EDC) are exogenic substances that interfere with the endocrine system by interacting with endogenous hormone receptors. Humans and livestock are exposed to estrogenic EDC released to the environment at various doses e.g. via the placenta during prenatal development. Early embryonic development is especially susceptible to endocrine stimuli affecting the epigenome due to epigenetic reorganization at this developmental phase. To investigate the effect of estradiol-17β (E2) in preimplantation development and uterine environment gilts were orally exposed to E2 in environmental and pharmacological doses during early pregnancy. After artificial insemination, animals were randomly distributed in four groups receiving different doses of E2 semi-daily (0.05, 10 and 1000 µg/kg body weight/day, and a solvent control) from day 1 to day 10 of gestation. Embryos were flushed and separated manually into trophoblast and embryoblast. The distribution of the orally uptaken E2 in the maternal organism was analysed by enzyme immunoassay. The E2 treatment neither had an impact on the morphology of the embryos, the pregnancy rates nor on the number of embryos that were recovered per mother. Total estrogen and E2 concentrations were significantly higher in bile from gilts receiving the pharmacological high doses of E2. In the future, whole genome bisulfite sequencing and transcriptome analysis will be applied in cells of the luminal epithelium (isolated by laser capture microdissection), trophoblast and the embryoblast. In the present study, we assess and compare the physiological, molecular and epigenetic consequences of E2 exposure during early pregnancy in an *in vivo* approach.

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Field study on the implementation of a controlled method of selective dry cow treatment in Bavarian dairy farms

Feldstudie zur Implementierung eines kontrollierten Verfahrens zum selektiven Trockenstellen in bayerischen Milchviehbetrieben

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Since the increasing discussion on the use of antibiotics, it is necessary to establish possibilities to reduce the use, like selective dry cow treatment (SDCT). The main objective of the present study was to investigate the implementation of a controlled method for SDCT in Bavarian dairy farms which were using blanket dry cow treatment (DCT) until then. Overall, data (e.g. milk yield recording

data and results of quarter foremilk samples) from 18 Bavarian dairy farms that implemented SDCT were used for analysis. Based on a decision tree the cows were either dried non-antibiotic or the animals received an antibiotic dry cow preparation. At cow level the results of the different treatment groups in the dry period and p.p. were compared to each other. At farm level, various parameters of the udder health were collected for the first period (one year before the start of the trial: DCT) and for the second period (first year in the trial: SDCT) and compared between those periods. Overall, 60.94% (n = 468) of the cows were treated with DCT on the day of dry-off and 39.06% (n = 300) were dried off without DCT. There was a big variance between the herds in terms of the percentage of animals without DCT (minimum: 23.26%; maximum: 62.22%). Especially, considering the dry period and time p.p., cows of the group 'without DCT' had mainly no poorer udder health than cows of the group 'with DCT'. At farm level, it could be demonstrated, that the udder health (e.g. number of cows with a treated clinical mastitis, herd somatic cell count, rate of new intramammary infections and cure rate) was not significantly different between the two considered periods.

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The number of factors which prove to be involved in the modulation of bovine corpus luteum activity increases

Die Anzahl der Faktoren, welche sich als Modulatoren der Aktivität boviner Corpora lutea erweisen, steigt

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The luteal capacity of bovine ovulatory follicles (OF), as reflected by the later corpus luteum's (CL) progesterone (P4) output, is modulated by a variety of pre- and peri-estrus conditions whose interplay is still poorly understood. This study's aims were to check an assumed effect of mating on the ensuing CL activity and to test the hypothesis that the luteal capacity of potential future OFs is shaped during the selection of 1 dominant follicle (DF) out of a cohort of competitors. Between d-21 and d21 (d1 = estrus), the ovarian function of 135 cycling unmated and 139 mated Swiss Brown dairy cows was monitored by transrectal palpation at intervals of 1-2 days and daily P4 radioimmunoassay in the peripheral blood. In 104 animals DF waves were interrupted at various instants between d-19 and d-1 by manual follicle destruction. Mating, irrespective of its outcome, had a blurring effect on correlations between the pre- and post-estrus P4 secretion which are obvious in unmated animals. After the DF destructions, either directly the next OF (n = 65; group A) or first an intermediate anovulatory DF (n = 39; group B) arose. In both groups and also in untreated animals, the P4 secretion before the rise of the later OF was positively correlated with the ensuing CL's activity. In group B, which showed higher median circulating P4 than the other animals during the DF selection, this correlation was most obvious. As shown by this study, (1) mating affects correlations between the pre- and post-estrus P4 secretion, (2) the luteal capacity of potential later OFs is shaped while they develop into solitary DFs, and (3) P4 modulates this process.

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Efficacy of antimicrobial peptides (AMPs) against major bovine mastitis pathogens

Wirksamkeit antimikrobieller Peptide (AMPs) gegenüber häufigen bovinen Mastitiserregern

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Mastitis accounts for the highest antimicrobial usage in dairy farming. During intramammary infections, endogenous AMPs are highly upregulated in mammary tissues, however little is known about their efficacy against mastitis pathogens. The aim of the study was to analyse the influence of relevant recombinant AMPs (S100A8/A9, S100A9, LAP, CCL20, SLPI) on bacterial growth of major mastitis pathogen isolates *in vitro*. Within a time kill assay, *Escherichia coli, Staphylococcus aureus* and *Streptococcus uberis* were incubated in the presence and absence of different concentrations of AMPs. Bacterial growth was detected photometrically (OD 600 nm) every 30 min over a time period of 6 h. The AMP S100A9 and the antimicrobial chemokine CCL20 significantly ($P < 0.05$) inhibited growth of *S. aureus* and *Sc. uberis,* whereas the heterodimer S100A8/A9 only inhibited *E. coli* growth ($P < 0.05$) and partly *Sc. uberis* growth at comparably high concentrations. In contrast to earlier reported data on recombinant bovine LAP, recombinant bubaline LAP had no inhibitory effect on all tested pathogens. The same accounted for SLPI. However, high concentrations of CCL20, LAP and SLPI enhanced *E. coli* growth *in vitro*. In conclusion, AMPs show antimicrobial activity against major mastitis pathogens, however, effects are pathogen-specific and depend on concentration and the recombinant peptide target species. Triggering the expression of endogenous AMP may be a future approach to more effectively battle intramammary infections and to reduce the amount of antimicrobial drugs in the dairy industry.

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Determination of sex steroids in shed skin – a method for sexing in the Gila monster *(Heloderma suspectum)*?

Ermöglicht die Bestimmung von Sexsteroiden in abgestoßener Haut eine Geschlechtsbestimmung bei der Gila-Krustenechse *(Heloderma suspectum)*?

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The measurement of steroids in skin appendages such as hair or claws is used for their non-invasive determination in mammals and allows characterizing their presence over longer periods of time. Our aim was to test whether the measurement of sex steroids in shed skin can be used for sexing in reptiles which do not exhibit a clear sexual dimorphism. This would be particularly advantageous for species without genetic sex determination and for dangerous species. For this study shed skins of 11 female (F) and 7 male (M) adult Gila monsters, a venomous lizard, were available. Large skin pieces were first chopped with scissors, finely ground under liquid nitrogen and the samples were finally extracted with organic solvents. The following parameters were radioimmunologically determined in the dried and redissolved extracts: progesterone (P4), estradiol-17β (E2), testosterone (T), free total estrogens (fTE), free plus conjugated total estrogens (TE). For P4 ($p = 0.007$), E2 ($p = 0.011$) and T ($p = 0.030$) significant sex differences were found with higher concentrations in F compared to M. T concentrations were only slightly above the detection limit. Concentrations for fTE and TE were practically identical, with higher mean values measured for F than for M. However, this difference was not statistically significant. Even though the methods applied in this pilot study did not allow reliable sexing in single animals either alone or in combination due to overlapping between the sexes, the results suggest that the measurement of sex steroids in shed skins could basically be a useful method for non-invasive sexing in reptiles.

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Suitability analysis of a laboratory-independent test to evaluate the motility of canine sperm

Überprüfung eines laborunabhängigen Tests zur Bewertung der Motilität von Rüdensperma

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The use of good quality sperm is essential for achieving high pregnancy rates and litter sizes in dogs. To evaluate the sperm motility in dogs, a new laboratory-independent commercial test (PetCount®, MotilityCount ApS, Denmark) was developed. This test may provide faster results under field conditions according to conventional methods. The reliability of this new test, compared to conventional methods, has not been investigated sufficiently yet. Therefore, the aim of this study was to determine the sperm motility of the dog by comparing this rapid test with the results of two other established methods. In the study, native sperm samples of 15 dogs from different breeds and ages were used. The evaluation of motility (total motility, million/ml) in each semen sample was done with the counting chamber, CASA (Andro Vision®) and the PetCount® test. Based on the ordinal scale level of the result of the PetCount® test, the relationship to the results of the counting chamber and CASA were statistically tested using the Spearman rank correlation coefficient. To compare the values of the quantitative methods with the levels of the PetCount® test, they were presented in the form of the box-and-whisker plot. Statistical analysis showed that the tested PetCount® procedure does not allow a reliable assignment compared to the classical methods (correlation coefficient of spearman rank of $r_s = 0.445$, $p = 0.097$).

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Can dietary lipids influence bovine oocyte competence and granulosa cell functions?

Können diätetische Lipide die Eizellenkompetenz und die Funktionen von Granulosazellen beeinflussen?

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The aim of the study was to assess the effect of dietary essential fatty acids on the developmental competence of oocytes in cows and on the functionality of follicular granulosa cells (GC). Lactating German Holstein cows were supplemented with different lipids from week 9 ante partum (ap) until week 8 post-partum (pp) in four groups designed as (i) control (CTRL [n = 7]: coconut oil), (ii) essential fatty acid (EFA [n = 7]: linseed and safflower oil), (iii) conjugated linoleic acid (CLA [n = 8]: Lutalin®), and (iv) EFA+CLA [n = 8] (mixture of linseed oil, safflower oil and Lutalin®). For *in vitro* embryo production, cows in wk 9 pp were slaughtered to obtain the ovaries and oocytes were collected by slicing method. EFA (n = 64), CLA (n = 65) or EFA+CLA (n = 70) diet groups had no effect on cleavage or blastocyst rates as compared to CTRL (n = 61). Gas chromatography analysis showed a higher proportion of α-linolenic acid (ALA) in the follicular fluid (FF) of EFA and EFA+CLA diet group cows and a higher proportion of *cis-9, trans-11* CLA isomer in the FF of CLA and EFA+CLA diet group as compared to CTRL group. Further, we analysed the effects of increased concentrations of ALA and *cis-9, trans-11* CLA in our GC culture model. Both ALA and *cis-9, trans-11* CLA up-regulated *CD36* and down-regulated the expression of *FOXL2*. Also, ALA and *cis-9, trans-11* CLA significantly down-regulated the expression of *STAR, CYP19A1, FSHR, LHCGR* and decreased the 17β-Estradiol production. Thus, dietary lipids did not improve *in vitro* embryo production, whereas higher proportions of ALA and *cis-9, trans-11* CLA in the follicular fluid could affect GC functionality thus suggestively compromising follicle development and ovarian cyclicity in dairy cows.

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Local immune cells in the prepuce and urethra of sheep lambs

Lokale Immunzellen in Präputium und Urethra bei Schaflämmern

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Despite its exposed location, the urinary tract is supposed to be sterile due to its effective local mucosa associated lymphatic immune system (MALT) [Acosta-Dibarrat et al. PVB 2014; 34: 270–6]. The relevance of MALT within the urogenital tract has been shown for human and veterinary medicine [Zasloff, JASN 2007; 18: 2810–6]. The influence of castration or rather puberty on the local immune system is still unclear. Therefore, preputial and urethral tissue samples from 6-months-old sheep lambs (n = 33; 11 intact, 11 early castrated and 11 late castrated) and of rams with urolithiasis were harvested and prepared for immunohistochemistry (IHC). Tissue samples were taken from the prepuce, glans penis, penile urethra, distal and proximal sigmoid flexure and pelvic flexure of the penis. IHC was performed for CD-3 (T-cells), CD-79α (B-cells) and MAC 387 (macrophages). Staining results were obtained via a light microscope with adapted digital camera (Leica Microsystems, Germany). The IR-positive-cell count per optical field was evaluated by Adobe Photoshop®. Only CD-3-IR-pos. cells could be consistently shown within the tissue samples. No statistically significant group difference but a significant difference between the localizations ($P = 0.002$) could be shown. The distribution pattern of CD-3-IR-pos. T-cells differs between castrated and intact lambs ($P = 0.004$). In rams with urolithiasis, there is a lack of T-cells within the penile part of the urethra. Cell count within the prepuce was higher than within the urethra, but the group comparison failed to be significant. The study suggests an existing influence of castration on the distribution pattern of CD-3-pos. T-cells within the urethra of sheep lambs.

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In vitro culture models to study bovine mammary gland extracellular vesicles

In-vitro-Kulturmodelle zur Untersuchung von extrazellulären Vesikeln der Rindermilchdrüse

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Extracellular Vesicles (EV) are cell-derived structures carrying proteins, lipids and nucleic acids. They are found in all biofluids and mediate the communication between donor and receiving cells. In cattle, mammary gland epithelial cells (MEC) release EV in milk, and their content changes upon pathological conditions, e.g. mastitis, the inflammatory response of the mammary gland against microorganisms. To study the EV communication of MEC, we aimed at establishing an Air-Liquid-Interface (ALI) culture to model the mammary gland alveolus. Therefore, first we set up a two-dimension (2D) culture of primary functional MEC in fetal bovine serum (FBS)-free conditions. FBS is a common supplement in cell culture that naturally contains EV that can interfere with downstream analysis. In order to support epithelial cell growth without FBS, we coated the culture plates with different concentrations of collagen I or laminin. Transmission electron microscopy and tuneable resistive pulse sensing did not show any vesicles in our FBS-free medium, but it did in the presence of cultured MEC. FBS-free medium supported cell growth, but to a lesser extent compared to FBS-containing medium, regardless of the coating of the plates. However, we observed a higher enrichment of epithelial cells in all FBS-free conditions. This observation was confirmed by gene expression analysis, which showed that the ratio of Keratin 18/Vimentin (epithelial and fibroblastic marker, respectively) was higher than in FBS containing medium. As our FBS-free medium did not contain interfering EV and enriched the culture in epithelial cells, it will be kept to expand MEC before culturing them in an ALI culture.

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Using CRISPR/Cas9 in bovine somatic cells to decipher regulation of bovine preimplantation development

CRISPR/Cas9 in bovinen somatischen Zellen zur Entschlüsselung der präimplantativen Entwicklung von Rinderembryonen

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Major differences in the regulation of the first differentiation events between mammals require the investigation of other model organisms than mouse. Similarities to human development and well established *in vitro* reproduction techniques designate bovine embryos as an optimal alternative. Using a reverse genetics approach, we aim to study the function of key regulatory genes and with *NANOG* maintaining pluripotency during the second lineage differentiation, it is of special interest. To delete the function of *NANOG*, we induced a frameshift mutation in a non-homologous end joining approach by transiently transfecting a single plasmid expressing the gRNA, Cas9 and Puromycin resistance to bovine fibroblasts targeting exon 2 of *NANOG*. As a negative control, a gRNA with no target in the bovine genome was transfected. After selection with Puromycin, the mixed cell population was analysed by Sanger sequencing and single cell clones were produced. We tested 3 different gRNAs using sequence trace decomposition (TIDE web tool), which showed mutation rates of 13.3%, 16.4% and 32.9%, respectively. Next, we compared the most efficient gRNA to a manual analysis of single cell clones. Similar results between manual and TIDE analysis (26.1% vs 32.9%) recommend TIDE as a helpful tool to preliminarily analyse the mutation rate. Using the same gRNA with different cell lines, mutation rate achieved up to 54.4% and 71.4% in a manual analysis. Cell clones with a homozygous mutation will serve as donor cells in somatic cell nuclear transfer to produce embryos without functional *NANOG* and to study the role of *NANOG* during preimplantation development.

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Timing of artificial insemination using fresh or frozen semen after automated activity monitoring of estrus in lactating dairy cows

Untersuchung zum optimalen Besamungszeitpunkt unter Verwendung von tiefgefrorenem und flüssig konserviertem Sperma auf Milchkuhbetrieben mit automatischer Brunsterkennung

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The objective of this observational experiment was to determine the association between the interval from reaching an activity threshold (AT) using an automated activity monitoring system (AAM) and the time of artificial insemination on pregnancy per AI (P/AI) for lactating Holstein cows inseminated with either fresh or frozen semen. Lactating Holstein cows (n = 5,200) from 8 commercial dairy farms were inseminated based on visual heat detection and/or based on the alert of an accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel). Data were analysed using the GENLINMIXED procedure in SPSS. Onset of estrus was defined as the time when the AT (index value ≥ 35) was reached. The mean (± standard deviation) interval from onset of estrus to AI was 13.8 ± 8.9 hours. Pregnancy per AI was affected by parity (P = 0.01), THI one week before AI (P = 0.01), estrus intensity (P = 0.01), AI number (P = 0.01), milk yield one week before AI (P = 0.03), type of semen (P = 0.05), and the interval from reaching AT to AI (P = 0.01). The interaction of type of semen with interval from reaching AT to AI (P = 0.47) had no effect on P/AI. There was a quadratic effect of the interval from reaching AT and AI on probabilities of pregnancy. Pregnancy per AI at 35 d after AI was highest for cows inseminated between 7 to 24 hours after onset of estrus (–24–0 hours = 32.0%, 1–6 hours = 32.6%, 7–12 hours = 42.2%, 13–18 hours = 45.0%, 19–24 hours = 43.7%, 25–48 hours = 36.0%). In conclusion, inseminating cows 7–24 hours after passing the AT yielded the highest P/AI irrespective of type of semen. Inseminating cows before or 6 h after reaching AT was detrimental on P/AI.

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Comparison of isolation methods for placenta-specific exosomes from rabbit and human serum samples

Etablierung eines Isolationsprotokolls für plazenta-spezifische Exosomen aus Serumproben des Menschen und des Kaninchens

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The putative role of extracellular vesicles such as exosomes ranges from intracellular signals to biomarkers of disease. Deviating exosomes are discussed intensively as early diagnostic markers for pregnancy complications. A crucial factor in exosome biology is their quantitative and qualitative isolation based on particle density and size. In the current study we compared two isolation methods of placenta-specific exosomes, ultracentrifugation and membrane affinity spin columns (exoEasy Maxi Kit, Qiagen) and analysed serum samples from women at 26 weeks of gestation and rabbits on day 6 and 14 *post coitum*. To validate the quality of both isolation approaches flow cytometry analysis for the exosome marker CD63 was performed. Further, the protein amounts of placental alkaline phosphatase (PLAP, positive marker), the membrane-bound protein, ezrin; the endoplasmatic reticulum proteins, calnexin; and the cytoplasmatic protein, heat-shock protein (HSP) 70 (all negative markers) were quantified by Western Blot analysis. With both experimental approaches CD63-positive exosomes were isolated, whereby the amount was higher for membrane affinity spin columns. In human exosome samples PLAP was detectable, confirming the placental origin of these exosomes. None of the negative marker proteins were detectable in the isolated samples, demonstrating the specificity of the isolation methods. In conclusion, both methods are suitable for placental exosome isolation with a higher efficiency for the membrane affinity spin columns system compared to ultracentrifugation.

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Functional alterations of hypoxia-inducible factor (HIF1α) impair maturation of cumulus oocyte complexes (COCs) and suppress blastocyst development rates in cattle

Die funktionale Beeinträchtigung des Hypoxie-induzierbaren Faktors (HIF1α) hat einen negativen Einfluss auf die Reifung von Kumulus-Oozyten-Komplexen und unterdrückt die Blastozystenentwicklungsraten beim Rind

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Hypoxia is essential for follicular development and HIF1α is one of the most important regulators of cellular responses to hypoxia. Yet the function of HIF1α in regulating maturation of bovine COCs remains to be elucidated. In particular, the effects of HIF1α on the steroidogenic activity of cumulus cells, their expansion, metabolic activity and cell-to-cell communication are of particular interest. Therefore, COCs were isolated from ovaries collected at a local abattoir and were divided into four experimental groups (n = 10 experiments, 30 COCs/well/experiment): immature, in vitro maturation (IVM) control (standard protocol, 24h) and IVM/treated (IVM/T) either with echinomycin (functional blocker of HIF1α) or with PX478 (suppressor of HIF1α expression). In both IVM/T groups, cumulus expansion was reduced. The expression of several factors was investigated in cumulus cells following IVM showing, i.a., strong inhibition of both mRNA and protein expression of STAR, resulting in lower steroidogenic output from treated COCs. Similarly, the expression of Cx43 (involved in intercellular communication), as well as of HAS2 and TNFAIP6 (involved in the synthesis of hyaluronic acid), was strongly suppressed in treated cumulus cells. The metabolic impact of HIF1α was mirrored in diminished GLUT1 levels. Functionally, lower oocyte maturation and blastocyst rates were obtained from treated COCs. In summary, suppression of HIF1 function and/or expression multidirectionally alters COCs, functionality by suppressing their intercellular communication and maturation, steroidogenic activity and oocyte development rates.

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Bovine cumulus oocyte complexes secrete complement C3 during maturation

Complement C3 wird während der Maturation von bovinen Kumulus-Oozyten-Komplexen sezerniert

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Complement C3 is a central player in the complement and coagulation cascades, which was already characterized as constituent of follicular fluid. Overrepresentation of C3 in cumulus of *in vivo* successfully matured oocytes compared to *in vitro* successfully matured was detected in a previous proteomics study. This raised the hypothesis of an active secretion of C3 by cumulus cells. Therefore, a commercially available enzyme-linked immunosorbent assay kit against complement C3 (ELISA KIT SEA861Bo, Cloud-Clone Corp., Katy, Tx, USA) was used to analyse maturation medium (MM) as well as follicular fluid (FF). For MM samples, pools of 35 COCs were matured in 400 µl drops. Samples were collected after 12 h (n = 4) and 24 h (n = 10). MM without cell contact and incubation (n = 3) and MM in contact with COCs for 1 hour but incubation without COCs for 24 hours (n = 4) served as negative controls. FF samples were collected from ovaries of slaughtered animals (n = 6). After 12 h and 24 h of maturation the mean concentration of C3 in MM was 48.4 ± 1.3 ng/ml resp. 68 ± 0.6 ng/ml. In all negative control samples C3 was not detectable. In FF a concentration > 100× higher was detected with a mean concentration of 9433 ± 330 ng/ml. The Mann-Whitney-Test revealed significant differences between all groups ($p \leq 0.001$). These results document the active secretion of C3 to MM. There seems to be a major lack of C3 during maturation under *in vitro* conditions, which was already detected in the proteome analysis. The available literature gives already a hint for a positive influence of C3 on the developmental competence of the oocyte, as well as for a role in sperm-oocyte binding. Still, the specific role of the complement cascade, during bovine *in vitro* maturation needs further investigation.

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Activity of glycosidases in bovine placenta during pregnancy and at term

Aktivität von Glykosidasen in der Rinderplazenta während der Trächtigkeit und der Geburt

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β-galactosidase, α-l-fucosidase, β-N-acetyl-hexosaminidase and sialidase are the enzymes responsible for the hydrolysis of O-glycosidic bonds. They act on different glycans, glycoproteins and glycosphingolipids. Enzymatic removal of sugar moiety from conjugated proteins may influence their structure and their biological function. The aim of our study was to describe the ability of bovine placental tissues to break down O-glycosidic bonds in different glycoproteins by the determination of activity of selected glycosidases in early-mid pregnancy as well as in released and retained placenta. Moreover, the presence of substrates for these enzymes in placental tissues was detected. Placental samples were collected from healthy pregnant HF cows (n = 8) at abbatoir and at parturition and divided into not retained (NRF n = 8) and retained placenta (RF n = 8). Enzyme activity was measured in placental homogenates by spectrofluorimetry and spectrophotometry. Samples were separated by use of 1D PAGE and gels were stained with Alcian Blue and Fuchsine. Statistical analysis revealed significant differences ($p < 0.05$) between samples collected at pregnancy and parturition NRF in activity of N-acetyl-hexosamindase in fetal part and alpha fucosidase in both examined parts of placenta. Significant differences ($p < 0.05$) were detected also between samples collected at pregnancy and at parturition RF. Gel analysis showed significant differences within glycoprotein pattern stained with Fuchsine and Alcian Blue between early pregnancy and parturition, as well as between NRF and RF. In conclusion, glycoprotein metabolism during pregnancy and parturition exerts dynamic changes and may be altered during the retention of placenta in cows.

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Comparative analysis of the uterine transcriptome and bacterial microbiome in fertile and subfertile mares

Vergleichende Analyse des uterinen Transkriptoms und bakteriellen Mikrobioms von fertilen und subfertilen Stuten

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Subfertility is a major problem in modern horse breeding. To obtain deeper insights into causes of fertility problems, the uterine transcriptome and microbiome of fertile and subfertile mares were compared. Uterine cytobrush and swab samples were taken in estrus and used for DNA and RNA extraction and for microbiological plating analysis, respectively, the latter to exclude mares with culti-

vable pathogenic bacteria. The isolated RNA of 22 selected samples was used for RNA-sequencing to identify differentially expressed (DE) mRNAs between fertile and subfertile mares. The DNA samples of the two different groups were pooled and used for PacBio SMRT 16S rRNA gene full-length amplicon sequencing. 114 genes were found as DE in fertile vs subfertile mares (FDR = 10%). The downregulated genes in subfertile mares were related to 'Extracellular matrix (ECM)', 'ECM-receptor interaction', 'focal adhesion', 'vasculature development', 'guanyl exchange factors activity', 'hydrolase activity' and 'adaptive and innate immune response'. DEGs upregulated in subfertile mares were related to 'transmembrane transport activity'. The results suggest an important role of these categories in fertility. The equine uterine bacterial microbiome was characterized by four main phyla: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. The relative abundance of Firmicutes and Actinobacteria was higher in subfertile mares than in fertile ones. Fertile ones showed higher abundance of Bacteroidetes and unclassified OTUs. Further, subfertile mares showed lower uterine bacterial diversity than fertile mares. Our results revealed differences between fertile and subfertile mares in the uterine transcriptome and bacterial microbiome and provide leads for further research on fertility.

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Evaluation of a rapid semi-quantitative immunoglobulin test for neonatal calves

Überprüfung eines Schnelltestes zur semiquantitativen Überprüfung der Immunglobulinversorgung des neonatalen Kalbes

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Measuring the serum IgG concentration is important for controlling and monitoring the colostrum supply to neonatal calves. In the present study, we compared the sensitivity and specificity of a semi-quantitative rapid ELISA (Fassisi® Bovine IgG Test) for the detection of failure of passive transfer compared with that of the gold standard (sandwich IgG ELISA). The influence of health status and age on the test results was also assessed. Serum samples obtained from 277 calves were tested from immediately at birth to 10 days. Calves were classified as healthy or sick based on clinical examination. The IgG concentration in all samples was determined by both sandwich ELISA using laboratory-bound equipment and by the Fassisi Bovine IgG immunoassay. The IgG values of the rapid test results were highly correlated with those of the sandwich ELISA test ($p < 0.0001$), but the correlation coefficient was low ($r_s = –0.36$). The sensitivity for the detection of failure of passive transfer was 61.1% (n = 113), and the specificity was 58.7% (n = 54). Age significantly influenced the results ($p < 0.0001$). Health status, however, had no statistically significant effect on the test results ($p = 0.98$). Previously established indirect rapid test methods provide remarkably more accurate results for detecting failure of passive transfer. Therefore, the Fassisi Bovine IgG rapid test is not an adequate method for assessing passive immunization in newborn calves.

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Functionally relevant connective tissue septa of the epididymis already exist before the first sperm reach the epididymis

Funktionsrelevante Bindegewebs-Septen des Nebenhodens existieren bereits, bevor die ersten Spermien den Nebenhodengang erreichen

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The epididymis is anatomically divided in caput, corpus and cauda. Furthermore, connective tissue septa (CTS) subdivide the epididymis into different segments. In adults, these CTS are involved in creating a specific segmental interstitial milieu, which is important for the different steps during sperm maturation within the epididymal duct. In the first days after birth individual septa begin to develop and epithelial cells start differentiation (at postnatal day 14 in the rat). However, data about structural integrity and function of CTS as well as a segmental interstitial milieu before adulthood are missing. The two- and three-dimensional structure of the epididymal CTS of 6-day-old – 40-day-old rats were visualized morphologically (serial sections, clarity). Interstitial and luminal perfusions with colour tracer were used to characterize barrier functions. The presence of CTS could be revealed at all investigated days in the postnatal rat epididymis. The known 19 segments in adult rats matched with the clearly visible 19 segments in postnatal rats. Infusion into the interstitial tissue resulted in a tracer distribution of one single segment restricted by sharp boundaries to the next non-infused segment. Luminal perfusion showed no restriction. Our data showed CTS in the postnatal epididymis. These CTS created an interstitial segmental milieu already before the first sperm reaches the epididymis or the differentiation of epithelial cells begins. This suggests an additional yet unknown function of the CTS during postnatal development.

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Isolation of lactic acid bacteria from the bovine male genital tract and their influence on endometrial epithelial cells and spermatozoa *in vitro*

Isolierung von Milchsäurebakterien aus dem bovinen männlichen Genitaltrakt und deren Einfluss auf endometriale Epithelzellen und Spermien *in vitro*

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Pathogenic bacteria in the bovine genital tract have been investigated intensively but knowledge about commensal bacteria is rare. Aim of the study was to isolate commensal bacteria from the bovine male genital tract and to evaluate their influence on endometrial epithelial cells. Penis and preputial cave of 12 slaughtered bulls were sampled by swabbing. Obtained colonies were characterized by sequencing. Endometrial epithelial cells were co-cultured with isolated bacteria for up to 72h for viability assay and up to 6h for mRNA expression analysis. mRNA expression of selected pro-inflammatory factors was detected by RT-qPCR. *Lactobacillales* isolated from the bovine seminal plasma and bovine uterus were incubated with bovine spermatozoa for 6h. Spermatozoa were monitored by computer-assisted-sperm-analysis. *Lactobacillales* were isolated from all animals: *Pediococcus acidilactici, P. pentosaceus, Weissella hellenica, W. thailandensis* and *Lactococcus garviae*. Most of the investigated bacteria led to death of epithelial cells after 72h in a high concentration whereas lower concentrations of bacteria did not lead to cell death.

RT-qPCR revealed an overall increase of *CXCL1/2, -3, -5, IL6, IL8* and *PTGS2* mRNA expression in individually different manners. Isolates of *Lactobacillus mucosae, L. buchneri* and *Leuconostoc mesenteroides* were co-cultured with bovine spermatozoa and these isolates did not have an influence on the progressive motility of spermatozoa. In conclusion, the influence of *Lactobacillales* on endometrial epithelial cells and spermatozoa *in vitro* differs in a strain-specific manner.

Grants: The study was supported by the FBF.

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Higher mRNA expression of selected pro-inflammatory factors on day 1 after artificial insemination in cows which conceived

Höhere mRNA-Expression ausgewählter pro-inflammatorischer Faktoren am Tag 1 nach künstlicher Besamung in Kühen, die trächtig wurden

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Subfertility of cows represents a substantial problem for dairy cattle farmers and is the main reason for culling, which contributes to enormous financial losses. The hypothesis of this study was that the extension of the voluntary waiting period to 120 days postpartum (pp) improves fertility. Therefore, the objective of this study was to assess the mRNA expression of selected pro-inflammatory factors obtained from the endometrium by cytobrush technique from cows at day 35–41 pp, 115–120 pp and 1 day after artificial insemination (AI), respectively. Twenty dairy cows were classified in each group: pregnancy positive (PREG-POS) and pregnancy negative (PREG-NEG). Pregnancy diagnosis was performed 32 days after AI by ultrasound. Extracted total RNA from the endometrial samples was subjected to RT-qPCR for selected pro-inflammatory factors interleukin *(IL) 1A, IL1B, IL8*, chemokine CXL ligand *(CXCL)* 1/2, -3 and -5. A higher mRNA expression of *IL1A* (P = 0.01), *CXCL1/2* (P = 0.06) and *IL8* (P = 0.07) was observed in the PREG-POS group compared with the PREG-NEG group at day 1 after AI. Furthermore, an increase of the mRNA expression of *IL1A, IL1B, IL8* and *CXCL5* was observed on day 1 after AI compared with days 115–120 pp in the PREG-POS group. In contrast, mRNA expression of *CXCL3* decreased. Such effects were not observed in the PREG-NEG group. No differences were found between groups at day 35–41 and 115–120 pp for the selected factors. The findings of this study showed that a weak up-regulation of the mRNA expression of pro-inflammatory factors seems to be important for conceiving.

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Vascular remodelling in the course of endometriosis in mares measured by the Masson's Trichrome (MT) staining

Vaskuläre Veränderungen im Verlauf von Endometriose bei Stuten anhand von Messung nach Masson's-Trichrome- (MT-) Färbung

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Endometriosis is a common disease occurring mostly in older mares. Some scientists suspect a haemodynamic role in pathogenesis of it. Yet it is still unknown which is first, degenerative changes in blood vessels, or endometrium itself. The aim was to compare properties of blood vessels in different stages of endometriosis, depending on the Kenney and Doig classification. Full-thickness sections of corpus uteri were taken in the luteal phase (n = 24), fixed, cut and stained with hematoxylin-eosin (HE) and Masson's Trichrome (MT). Samples were classified into categories I, IIA, IIB and III on HE. MT-stained slides were scanned using semiautomatic analysis of slides on brightfield system (TissueFaxsPlus) and analysed with HistoQuest software. Areas (µm²) of all blood vessels with a diameter > 40 µm were marked and measured. Results (mean ± SD) were compared between groups with Kruskal-Wallis and Dunn's multiple comparisons tests. The lumen of vessels was significantly (P < 0.01) larger in category III (2623 ± 580 µm²) than in IIA (956 ± 127 µm²) and IIB (869 ± 122 µm²). Wall area was also significantly larger in category III (2301 ± 592 µm²) than in IIA (715 ± 152 µm²) and IIB (739±124 µm²) as well as fibrosis area in III (3981 ± 838 µm²) than in IIA (984 ± 221 µm²) and IIB (1059 ± 199 µm²). We have found that changes in the course of endometriosis involves wall of vessels, causing its thickening along with the degree of endometrial degeneration. This information followed by increase of lumen of blood vessels may indicate that along with the increase of severity of endometriosis blood vessels may become less functional and possibly blood stasis is occurring.

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–CC–chemokines modulate porcine endometrial endothelial cell functions

–CC–Chemokine modulieren die Funktion porziner endometrialer Endothelzellen

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Endometrium undergoes intensive changes in its structure during the estrous cycle and pregnancy. Extensive vascular remodelling is associated with increased proliferation and migration of endothelial cells as well as enhanced cell-cell communication. It was previously determined that porcine endometrium is a great source of chemokines – small proteins with the affinity to G protein-coupled receptors and having broad range of functions in various cell types. The aim of the study was to screen the effect of –CC–chemokines (CCL2, CCL4, CCL5, and CCL8) on endometrial endothelial cell functions. Immunofluorescence staining allowed to localize receptors CCR1, CCR2, CCR3 and CCR5 in cultured endothelial cells. All receptors were observed in cell cytoplasm, whereas CCR2 and CCR5 were found also in cell nuclei. Both CCR3 and CCR5 were additionally localized in cell membranes. Further experiments, in which ligands for those receptors were used, indicated increased proliferative and migration potential of endothelial cells as response on CCL4 and CCL8 stimulation. Capillary-like tubes formation assay was performed on growth factor reduced matrigel and five parameters were taken into consideration after incubation with single chemokines: number of nodes, junctions, segments, branches and meshes. Analysis of obtained results did not reveal significant effect of chemokines used in low physiological concentration on structure formation. In conclusion, among all examined chemokines, CCL4 and CCL8 are suggested to participate in process of angiogenesis in porcine endometrium by stimulating endothelial cell proliferation and migration.

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