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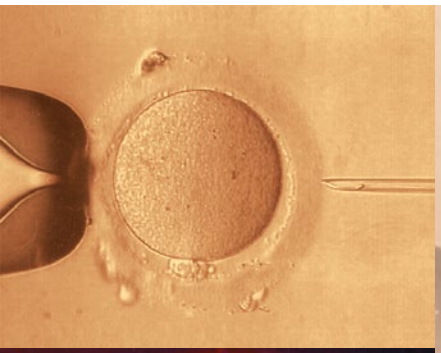

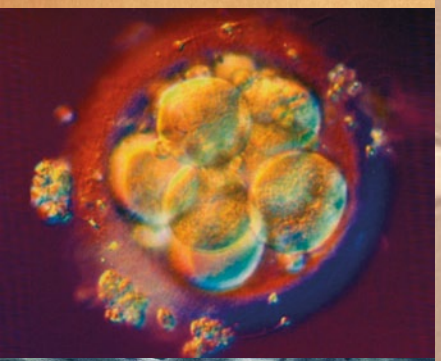

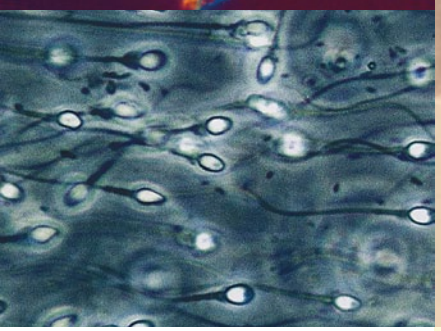

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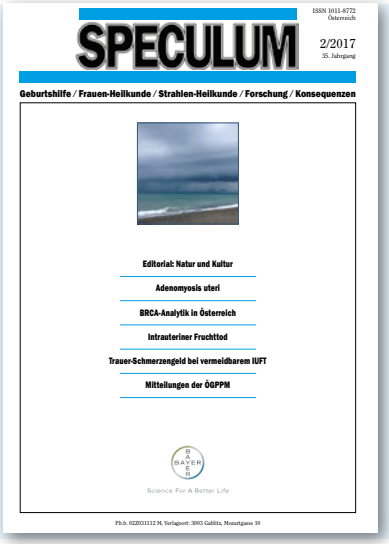

Speculum

Journal für Reproduktionsmedizin und Endokrinologie

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Abstracts*

01

New Serum Parameters for Detection of Uterine Puerperal Disturbances in Dairy Cows

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Impairment of puerperal fertility may be a severe problem in some dairy herds. Within the last years, several diagnostic possibilities were developed and validated. The aim of this study was to evaluate the applicability of blood serum parameters substance P (SP) and vasoactive intestinal polypeptide (VIP) for their use in early diagnostics of uterine involution disturbances. Blood serum samples of 86 dairy cows taken within the first 20 days post partum were examined using commercially available ELISA test kits. Animals were divided into two groups (healthy or diseased) depending on the results of clinical and gynecological examination. Statistical analysis consisted of the timely changes in blood serum levels as well as the group comparison of healthy cows and cows with uterine disease. Blood serum concentrations for SP increased statistically significant within the first 20 days after calving ($P < 0.04$). There was no statistically significant difference between the groups. Concerning VIP, neither for the timely course nor for the group comparison a significant difference could be shown. SP represents an accredited biomarker for pain in cattle. An increase of substance P within the first 20 days post partum suggests the presence of persisting pain presumably due to uterine involution processes. Nevertheless, both serum parameters do not seem suitable as indicators for the presence of uterine involution disorders.

02

Fetotomy in Dromedary Camels: an Evaluation of 50 Cases

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The objectives of this study were to investigate the cases of camel dystocia in which fetotomy was indicated, the related complications and the associated risks for maternal mortality and fertility. Fifty female dromedary camels were handled with fetotomy due to uncorrectable dystocia. The complications during and after fetotomy and post-operative fertility rates were recorded. Logistic regression was performed to identify risk factors for the dependent variables of maternal mortality and fertility after fetotomy. The independent variables were parity, duration of dystocia, presence of foetal emphysema, number of cuts, duration of fetotomy and occurrence of complications during or after fetotomy. Common indications of fetotomy were head and neck deviations alone or with carpal or shoulder flexions (54%) and breech presentation (14%). Complications included tearing of the soft tissue of the birth canal (24%), uterine prolapse (6%) and retained placenta (4%). Maternal mortality occurred in 26% of the cases. A significant association was detected between maternal deaths and duration of dystocia (odds ratio = 4.67, $P = 0.03$), presence of foetal emphysema (odds ratio = 3.93, $P = 0.04$) and occurrence of complications during or after fetotomy (odds ratio = 8.9, $P = 0.003$). Post-operative fertility was 62.2%. None of the estimated factors showed a significant relationship with post-operative fertility. In conclusion, postural abnormalities constituted the most common indication of fetotomy in dromedary camels. The duration of dystocia, presence of foetal emphysema and occurrence of complications during or after fetotomy represented risks for maternal recovery. The fertility rate after fetotomy was generally encouraging.

03

Effect of the Ovarian Superstimulation and Ovum Pick-up Procedure on the Fertility of Donor Heifers

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The aim of this study was to assess the effect of ovarian superstimulation and ovum pick-up on the fertility of donor heifers. Twenty Frisian heifers (12 treated and 10 control) were used in this study. The treated group received 2000 IU eCG on day 10 and 25 mg PGF2α on day 12. All follicles > 9 mm were transvaginally aspirated on day 14. One week after follicle aspiration, these heifers received 25 mg PGF2α. Two days later, they were artificially inseminated. Heifers of the control group were artificially inseminated on a PGF2α-induced estrus. Pregnancy diagnosis was performed using ultrasonography one month after insemination. Non-conceived animals were re-inseminated. The results showed that the total number of follicles > 9 mm in diameter existed on day 14 were 143 (14.3 follicles/animal). The number of aspirated follicles were 122 (12.2 aspirated follicles/animal). The number of the recovered oocytes was 36 (3.6 oocytes/animal). The conception rates after the first, second and third insemination were 60%, 80%, and 90% in the treated group. The corresponding rates in the control group were 70% and 100% after the first and second insemination. The difference between groups was not significant. In conclusion, ovarian superstimulation and ovum pick-up procedure did not negatively affect the fertility of donor heifers.

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04

Vaginal and Cervical Tumors in Dromedary Camels

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Female dromedary camels (n = 1621) were examined for failure of conception. The reproductive system was evaluated using transrectal palpation, ultrasonography and exploration of the vagina. Tissue overgrowths, which partially or completely surrounding the vaginal lumen or the cervix were detected in 8 cases (incidence, 0.005%). A common history of post-mating vaginal bleeding of these females was noticed. The overgrown tissue masses bled easily upon palpation. All females were multipara and aged between 9 and 13 years. Vaginal specimens were taken for histopathology. Blood samples were obtained for hematology and biochemistry. Microscopically, vaginal adenocarcinoma (n = 5), vaginal leiomyoma (n = 2) and cervical adenocarcinoma (n = 1) were identified. By ultrasound, these tumors were homogenous and echogenic, but sometimes with multiple hypo-echogenic cavities. In one case, metastasis was observed in the regional and mesenteric lymph nodes and liver. Compared to healthy controls (n = 15), camels with tumors showed significant increases of lymphocytes and monocytes and decreases in erythrocytes, hemoglobin and packed cell volume. Blood chemistry of camels with tumors revealed significant decreases in the total protein, albumin, calcium, phosphorus and magnesium and increases in globulin and alkaline phosphatase. The serum activity of creatine kinase, aspertate aminotransferase and gamma glutamyl transferase did not differ significantly compared to controls. In conclusion, this primary report described the prevalence, types, gross and microscopic appearances and changes in the hemogram and blood chemistry of female dromedary camels affected with vaginal and cervical tumors. Further investigations are needed to identify the associated risk factors.

05

Expression of Cocaine and Amphetamine Regulated Transcript (CART) in Eutopic and Ectopic Endometrium in Mice

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CART is a recently described neuropeptide with anorectic activity, which appears to play an important role in the appetite regulation and energy homeostasis. There is growing evidence that energy homeostasis factors such as leptin and ghrelin may play a role in reproduction and may be involved in pathogenesis of some reproductive disorders such as endometriosis. Accordingly, the present study was aimed at investigation whether CART may be expressed in uterine tissues and whether this expression may be present in heterotopic endometrial tissues in the murine model of endometriosis. The study was performed on 8-week-old inbred C57BL/6 female mice. Uterine fragments were transplanted into abdominal wall of syngeneic recipients under general anesthesia. Following 2, 4 and 8 weeks of observation the ectopic endometrial foci were excised and processed for immunohistochemical examinations. Tissues were fixed by immersion in 4% buffered formaldehyde, and, following dehydratation, were embedded in parafin wax. The specificity test performed for the CART antibody included: negative control, where the antibodies were replaced by normal rabbit serum (Vector Laboratories; Burlingame, CA, USA) at the respective dilution. Positive control was carried out for the specific tissue, as recommended by the producer (for our research we used mice stomach). Immunoperoxidase staining with CART-specific antibodies showed that this peptide is constitutively expressed in endometrial epithelial cells in normal eutopic uterus and heterotopic endometrioid cysts. This suggests that CART may play a part in regulation of murine reproductive system, however, the possible role of this neuropeptide in the reproduction remains to be elucidated.

06

Two-Pore Channel Protein 1 contributes to NAADP triggered Acrosome Reaction in Spermatozoa

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The sperm acrosome reaction, an all-or-none secretion process, mainly follows the principles of the conserved process of Calcium-regulated exocytosis. However, the functional relationship between the formation of hundreds of fusion and the mobilization of calcium from the acrosomal organelle has only been partially defined. Hence, the second messenger NAADP, promoting efflux of calcium from lysosome-like compartments, and one of its potential targets, the two-pore channel TPC1, were analyzed for its functional involvement in triggering acrosome reaction using a TPCN1-gen deficient mouse strain. The present manuscript documents that TPC1 and NAADP-binding site show a co-localization at the acrosomal region, and that treatment of spermatozoa with NAADP resulted in a loss of the acrosomal vesicle. Furthermore, it was found that the NAADP antagonist trans-Ned-19 significantly reduces acrosome reaction events in response to the natural ligand Zona pellucida. Importantly, applying a broad concentration range of NAADP two narrow bell-shaped dose-response-curves with maxima in the nanomolar and low micromolar concentration range were registered. Moreover, quantifying loss of the acrosomal vesicle in TPC1 null sperm upon application of different NAADP concentrations, responsiveness to low micromolar NAADP concentrations was abolished. Our study shows that NAADP mediates acrosomal secretion in mouse spermatozoa without additional extracellular Ca^{2+}, and that TPC1 is expressed in spermatozoa from mice and humans. The finding that two convergent NAADP-dependent pathways with non-overlapping activation and self-inactivation profiles for distinct NAADP concentrations operate in driving acrosomal exocytosis supports the concept that both NAADP-gated cascades match local NAADP concentrations with the efflux of acrosomal calcium.

07

Microscopical Investigations on the Early Development of Binucleate Trophoblast Giant Cells in the Bovine Placenta

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Bovine binucleate trophoblast giant cells (TGCs) play an important role for the transport of fetal mediators into the maternal tissue. Functional and morphological characteristics of the TGCs are quite well known, but their origin is yet still discussed. Different authors hypothesized whether TGCs develop from stem cells (SCs) within the trophectoderm or whether they can arise from any uninucleate trophoblast cell (UTC). Within the latter, generally accepted theory, a basally located, mononuclear cell without contact to the feto-maternal interface would represent a transient cell (TC) between UTC and TGC. So far no evidentiary images either for the existence of such TCs or for the presence of SCs have been shown. The aim of the presented study is to illustrate basally located, potential progenitor cells microscopically and to define the sequence of their development to mature TGCs more detailed. Placentomal tissue of 6 pregnant cows in different gestational stages has been examined for basally located, mononuclear cells and TGCs either in serial sections (light and transmission electron microscopy (TEM), n = 3), in single sections (TEM, n = 2) or in automatic serial sectioning (serial block face scanning electron microscopy (SBF-SEM, n = 1). These investigations revealed the occurrence of basally located, mononuclear cells without apical contact to the feto-maternal interface. To differentiate whether these cells are SCs or TCs, further investigations will be needed. Additionally, for

the first time TGCs with contact to the basement membrane could be shown by TEM. Further morphological investigations will be done to approach the roles of these newly revealed trophectodermal cell stages.

08

Necroptosis – an Unexplored Form of Cell Death in the Ovary

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A previous study in human IVF-derived granulosa cells 1 (GCs) [Blohberger et al., Cell Death Dis 2015] led to the discovery of a form of cell death, termed necroptosis (programmed necrosis) in the ovary. This type of cell death involves RIP1/3 and MLKL and can be prevented by necrostatin-1 and necrosulfonamid. In cultured human primary GCs it occurs spontaneously and in the ovary, it may be involved, among others, in the demise of large follicles. While it was linked to a splice-variant of AChE (AChE-R), the mechanism and the regulation of necroptosis of GCs are, however, not known. Human IVF-derived GCs are notoriously heterogeneous. To be able to study the mechanisms involved in induction and execution of GC-necroptosis, we explored the suitability of a human GC tumor cell-line, KGN. It is thought that these cells stem from follicular GCs, to which they are closely related. Indeed as shown by PCR, KGN express typical GC markers, including FSH-receptors and aromatase. Furthermore, life cell imaging showed that without renewal of medium, KGN die within 72–120 h. Morphological signs of the cell death suggested necrotic cell death, including a ballooning and cell burst. A blocker of apoptosis (zVAD-fmk) did not prevent this process, but necrostatin-1 effectively did. Further evidence for necroptosis as the form of cell death is derived from immunoblotting. Results show that phosphorylation of MLKL at Ser358 occurs when KGN die. Thus KGN can undergo necroptosis and are a suitable model to identify the mechanism of GCs-necroptosis. We conclude that further studies in KGN may lead to insights into the role of necroptosis of GCs in health and disease.

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09

Are Leptin and Corticosterone responsible for Metabolic and Reproductive Modifications in the Syndecan-1 Knock out Mouse?

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Introduction Syndecan-1 (Sdc-1) belongs to a family of transmembrane heparansulfate or chondroitinsulfate binding proteoglycans. Sdcs have been linked to diverse functions in human and mice, e.g. wound healing, immune response, angiogenesis, cell proliferation and migration. Regarding the preparation of implantation of an embryo, an upregulation of Sdc-1 during secretory phase was found in human endometrium. Furthermore, a decreased expression in 1st trimester pregnancies was shown to be a possible prognostic factor for low birth weight and preterm birth as well as low placental levels of Sdc-1 were expressed in preeclamptic females. Due to legal restrictions concerning human pregnancy, we performed a detailed analysis of metabolic and reproductive factors in Sdc-1 knock out (ko) mice.

Material and Methods Phenotypically, female and male Sdc-1 (ko) mice seemed to be smaller compared to C57BL/6 wildtype (wt) mice. We could prove this lower body weight from birth to adolescence in more than 100 offsprings of each line. Lower body weight might be based upon deviated metabolic features. Therefore, corticosterone, insulin and leptin levels were determined in 6 weeks and 6 months old animals. Corticosterone was reduced in 6 months old female and male Sdc-1 ko mice compared to wt. On the contrary, leptin was increased in adult ko mice of both sexes. Regarding pregnancy parameters, we found more implantation sites in ko females on day 6 post mating, but a significantly higher number of dead pups during the first week after birth.

Conclusion In summary, Sdc-1 ko mice show a striking reproductive influence according to the absence of this surface receptor that might also be associated with metabolic changes and therewith possibly lower appetite.

10

Transcriptome Analysis demonstrates a Luteinization-like Differentiation of Bovine Granulosa Cells Cultured at High Plating Density

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During folliculogenesis the pre-ovulatory LH surge leads to dramatic changes of the follicle including alterations of the gene expression profile. To identify underlying mechanisms cell culture models are essential tools. In previous studies it has been shown that bovine granulosa cells (GC) mimic a process of early luteinization if the cells were cultured at a high plating density. Therefore, GC were cultured serum-free with FSH, IGF-1 and androstenedione at normal and high density i.e. 1.0×10^5 and 10.0×10^5 cells per well, respectively. GC cultured at high density switches from estradiol- to progesterone-producing cells and displayed a change of the expression of selected marker genes, like CYP19A1, FSHR, RGS2 and VNN2. The present study aims in identifying the mechanisms of the density derived effects performing a genome-wide mRNA microarray transcriptome analysis. Data revealed that the expression of 1,510 annotated genes (represented by 1,575 transcript clusters) were differentially regulated comparing both culture conditions (fold change > 1.5, $p < 0.05$, FDR < 0.05). Of these, nearly two-thirds were up- and one-third down-regulated. Among the top up-regulated genes VNN2 and RGS2 could be identified, as well as HBA or LOXL2. Besides important key genes of folliculogenesis (e.g. CYP19A1 and FSHR) we could newly identify TXNIP or XDH as tremendously down-regulated. Ingenuity pathway analysis identified "AMPK signaling" and "cAMP-mediated signaling" as some of the most affected pathways. Main putative upstream regulators were TGFB1 and VEGF, both factors involved in angiogenic processes. From this data we hypothesize that specific cell-cell interactions induce an early post-LH stage in GC cultured at high density including transformations necessary to promote angiogenesis.

11

Inflammatory Changes in Dogs with Azoospermia – a Normal Finding?

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Azoospermia is a common, yet not well-understood problem in male dogs with a generally poor prognosis. Testicular biopsies could provide deeper insights necessary for understanding of the etiology. Ten dogs with confirmed azoospermia were included; blood samples for hormone analysis (LH, testosterone, T, and estradiol, E2) and karyotyping, semen samples for alkaline phosphatase in seminal plasma and bacteriology, and testicular biopsies for histology were collected. To assess the stage of spermatogenesis, the most developed germ cells observed were evaluated in 50 round tubules. Additionally, 6 testicular inflammatory parameters (thickness of basal membrane, presence of fibrosis, immune cells, shrinkage of lumen, abnormal mitotic patterns, vacuoles) were assessed. To identify the percentage distribution of the three compartments (seminiferous tubules, STC, lymph and blood vessels, VC, and interstitial tissue, IC), a morphometric evaluation of the testicular tissue was performed individually for each left and right testis and compared to normospermic control dogs (n = 5). 7/10 dogs sired successfully before with a mean of 2.3 ± 1.5 years since the last successful mating. Testicles were smaller (n = 5) or in the lower reference range (n = 4) in andrological examination. LH, T and E2 were within the reference ranges. Inflammatory changes were present in 9/10 dogs with generalized autoimmune orchitis in 6 dogs – 4 with early arrest (Sertoli-cell only or spermatogonia) and

2 with late arrest of spermatogenesis. 3 other dogs with late arrest had focal inflammatory changes. IC and VC were increased, STC decreased compared to normospermic dogs; no difference was found between sides. Our results show that inflammatory changes are common in dogs with acquired azoospermia.

12

PTPIP51 – Localisation, Expression and Interactions in Relation to Sperm Motility

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Protein tyrosine-phosphatase-interacting protein (PTPIP51) was identified as a substrate of PTP1B and TcPTP. The human protein is coded at chromosome 15 (15q15.1). In its structure there are several binding domains to generate multi-enzyme complexes. Many interaction partners of PTPIP51 were found – enzymes, receptors, proteins of mitosis, small GTPases and motor proteins. Due to these interactions signaling pathways are regulated and modulated. Hereby cell motility is influenced. The interaction of PTPIP51 is controlled by phosphorylation state of its tyrosine or serine residues. PTPIP51 is connected to MAP kinase pathway by Raf1 directly and by 14-3-3 proteins indirectly. Because of this Erk is activated and so cell motility is increased. Erk1/2 stimulates sperm motility. Newer studies prove the interaction of PTPIP51 and axonemal dynein. In sperm flagella dynein is associated with microtubules and it is considered as effector molecule of motility. Sperm motility is induced by variation of calcium homeostasis and modified by PKA activity. As an interaction partner of PKA PTPIP51 is involved in the regulation of calcium level. That is why we ask which role does PTPIP51 play in human sperm in terms of motility. For this purpose immunohistochemical analyses of localization and expression profiles of PTPIP51 and its interacting partners for sperm motility were realized. Therefor we tested both, ejaculates with normozoospermia and such which were classified as dysfunctional by spermiogram. Additionally the effect of one specific modulator of PTPIP51 was assayed concerning sperm motility. This pharmaceutical developed by our cooperation partners changes the interaction profile of PTPIP51 and accelerates sperm motility.

13

In vitro maturation of Roe Deer Oocytes

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Assisted reproductive technologies (ARTs) application in wild animals is a big challenge due to the variability among species and the limited research access. The roe deer (*Capreolus capreolus*) is an interesting animal model due to its embryonic dormancy phenomenon. Here, we evaluated the effects of culture time on in vitro oocyte maturation (IVM) in the roe deer. Ovaries were collected from hunted animals between September and October. A total of 237 oocytes were obtained by slicing from 7 animals. Retrieved oocytes were classified morphologically according to 5 categories: I (> 4 layers of compact cumulus cells [CC], clear and even cytoplasm), II (< 4 layers CC, cytoplasm with coarser appearance), III (1–2 layers CC, irregular cytoplasm), IV (denuded) and V (expanded or degenerated). After either 20 h or 24 h IVM (oocyte qualities I–III), oocytes were denuded, fixed and stained for nuclear evaluation. Fisher's exact test was used for meiotic status comparison ($p < 0.05$). The total number of oocytes/animal was 33.9 ± 3.5. Oocyte qualities I and II were scarce (0.3 ± 0.2 and 1.9 ± 0.6/animal). Qualities III and IV were more abundant (12.6 ± 1.5 and 18.1 ± 2.9/animal). Similar number of oocytes reached the MII stage after 20 h (33.3% [15/45]) and 24 h (43.1% [25/58]). Several oocytes remained between the MI and TI stage after 20 h (37.7% [17/45] or 24 h (31.0% [18/58]). Some oocytes did not resume meiosis after 20 h (8.9% [4/45] or 24 h 5.1% [3/58]). The results show that roe deer oocytes as their counterparts from domestic ruminants are able to reach the metaphase II *in vitro*. However, a considerable number of oocytes remain in earlier stages after standard IVM (BO-HEPES-IVM, IVF Bioscience). These preliminary studies represent a promising start for the use of ARTs in this species.

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Testicular Microvasculature and Leydig cell function in the ApoE-/-/LDL Receptor-/-Arteriosclerosis Mouse Model

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Introduction Arteriosclerosis affects many vascular beds. However, there is virtually no information on arteriosclerosis-based changes in testicular vasculature and in how far disturbances of the local vascular system of the testis may result in testicular malfunction. We used the Apolipoprotein E (ApoE)/Low Density Lipoprotein (LDL) receptor deficient mouse model (KO), in which disturbed spermatogenesis was previously described by us. This could be due to a reduction of the capillary network, an impaired testosterone synthesis, a reduced Leydig cell number or an insufficient transport between Leydig cells and the Inter-Leydig cell capillaries. Against this background, we analysed to what extent potential changes of testicular microvasculature are associated with changes of Leydig cell function.

Methods Testicular volume was reduced correspondingly to the vascular volume as assessed by micro-CT. Stereology was used to assess the smaller vessels which are not accessible by micro-CT and revealed the reduction of capillary length, volume and surface area. Stereological investigation of Leydig cell number in testes was similar in WT and KO and remained unaffected by age. However, Leydig cell size was significantly lower in KOs in all age groups. In line with this finding, serum testosterone levels were significantly lower in KOs, whereas intratesticular testosterone was unchanged between WT and KO.

Results Our data suggest a link between reduced microvascular density, Leydig cells, testosterone reduction and male infertility. This study calls for specific treatment of male infertility induced by microvascular damage through hypercholesterolemia and arteriosclerosis.

15

Membrane Bound Progesterone Receptors in the Bovine Uterine Wall and Placentome

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As a hormone of pregnancy, progesterone (P4) is known to interact with its well-investigated nuclear receptors (PGR). However, it can also exert its effects by binding to membrane-bound P4 receptors, which mediate rapid, non-genomic actions. Two of these membrane-bound receptors are the progesterone receptor membrane component-(PGRMC-) 1 and -2. The distribution patterns of these 2 receptors have been examined in different female reproductive tissues, but little is known about their presence in the bovine uterine wall and placentome during pregnancy, especially in mid and late stages. Therefore, the aim of this study was, to demonstrate the cellular localisation, as well as mRNA and protein expression of PGRMC-1 and -2 in the bovine uterine wall and placentomes during the entire period of pregnancy at the protein and mRNA level, by immunohistochemistry and qPCR, respectively. Tissues from 45 cows (n = 5 per each month of pregnancy) were used. The strongest positive reaction in the uterine wall was found in the luminal epithelial cells, the glandular epithelial cells, and also in the endothelium of blood vessels. The staining was less pronounced in the smooth muscular and stromal cells. In the placentomes, both proteins were detected in the maternal part (stroma, epithelial and endothelial cells) as well as in the endothelium of fetal blood vessels. PGRMC-2 was additionally expressed in the fetal chorionic epithelium. Staining intensities and mRNA expression levels did not vary greatly for both receptors throughout gestation. The

expression and distribution patterns of signals, however, suggest that PGRMCs may be involved in the non-genomic action of P4 in bovine uterine wall and placentomes during pregnancy.

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Effect of Intravaginal Prostaglandin E2 Application in Farrowing Sows During Parturition

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The duration of birth is an important parameter linked to piglets' survival and sow's health in the context of parturition. Therefore, prolongations are frequently treated with oxytocin – a potent uterotonic agent – although several undesirable side effects such as higher incidence of umbilical cord lesions, meconium staining and weak piglets have been described. In human medicine, Prostaglandin E2 (PGE2) is used instead, because less side effects have been observed. The aim of this pilot study was to test, whether 2 mg PGE2 applied as intravaginal gel after the birth of the fourth piglet influences the birth process. Overall, 3 randomly selected sows in a pig herd were treated with PGE2-Gel (group T) and 3 other sows were treated with a placebo gel (group C). The total duration of birth (time between first piglet and last placenta) and the piglet interval were recorded, and each piglet was scored for meconium staining and vitality. In group T the duration of birth was 386 min (average of 20.0 piglets per litter) compared to 439 min in group C (average of 14.3 piglets per litter). The piglet to piglet interval was 10.2 min in group T compared to 14.3 min in group C. No or only slight meconium staining was observed in 53% and 35% of piglets in group T, and in 70% and 30% of the piglets in group C. Severe meconium staining was only found in 12% of piglets in group T. Moreover, 10% of piglets in group T showed an oedematous and haemorrhagic umbilical cord, lethargy and anoxia. This study describes the impact of PGE2 gel on the birth process in sows. The duration of birth and the piglet interval tended to decrease, whereas umbilical cord lesions, fetal distress and anoxia increased. Further investigations should focus on the optimal dosage of PGE2 in order to evaluate the use of this drug in farrowing sows.

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High Glucose-6-phosphat-Dehydrogenase Activity in Oocytes is Related to low Embryo Developmental Rates after In vitro fertilization with Oxidatively Stressed Sperm

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Introduction Oxidative stress in sperm is inducing DNA damage. Oocytes can repair paternal damaged DNA to a certain extent. The ability of the oocyte to repair compromised paternal DNA is probably dependent from different factors. Brilliant Cresyl Blue (BCB) stain indicates the activity of the glucose-6-phosphate dehydrogenase (G6PDH) and has been used as marker of oocyte quality. We hypothesized that oocytes with a high G6PDH activity (BCB-) fail to repair damaged sperm and consequently have a lower competence to support embryo development after fertilization with oxidatively stressed sperm.

Methods To investigate this, bovine sperm were incubated with or without 100 µM H_2O_2 for 1 h at 38° C and were used to fertilize *in vitro* matured bovine oocytes which were selected using BCB stain. Oocytes incubated in PBS served as control. Presumptive zygotes were cultured in vitro and blastocyst rate on day 6 and 7 was determined in 7 IVF cycles. Fertilization of control oocytes with control sperm yielded in significant higher blastocyst rates (d6: 12 blastocysts/120 oocytes, d7: 34 blastocysts/135 oocytes) than fertilization of BCB-oocytes with control sperm (d6: 10 blastocysts/167 oocytes, d7: 22 blastocysts/180 oocytes). After fertilization of control oocytes with H_2O_2 exposed sperm, the blastocyst rates on d6 were reduced by 32.6 ± 31.8% (10 blastocysts/204 oocytes) and on d7 by 58.7 ± 21.1% (23 blastocysts/233 oocytes), in comparison to fertilization with control sperm. When BCB-oocytes were fertilized with H_2O_2 exposed sperm, blastocyst rate was significantly lower by 94.8 ± 13.8% on d6 (1 blastocyst/299 oocytes) and 87.1 ± 19.36% on d7 (7 blastocysts/352 oocytes) than after fertilization with control sperm.

Results In conclusion BCB-oocytes have a lower competence to support embryo development after *in vitro* fertilization with H_2O_2 exposed sperm, which indicates a possible involvement of G6PDH activity in oocyte competence to repair damaged sperm.

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Liquid Preservation of Bovine Embryos as an Alternative to Cryopreservation

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At present, the only reliable possibility to store biopsied embryos is cryopreservation with lower pregnancy after thawing. The aim of the present study is to develop an efficient procedure that is able to preserve biopsied, in vitro produced bovine embryos for up to seven days under hypothermic conditions.

Bovine IVP-derived day 6 morulae were biopsied, followed by liquid preservation (LP) in TCM plus either BSA (1 mg/ml and 10 mg/ml), FBS (25% and 50%) or BSA with FBS (25% FBS and 50% FBS with 1 mg/ml BSA each) for seven days at 0–4°C. A control group of non-biopsied embryos was stored under the same conditions. The gross morphological quality was determined before and after LP and further assessed with live-dead staining. Embryos were again cultured for 48h to determine re-expansion and hatching rates. The live dead cell ratio was significantly lower in embryos out of the group with BSA compared to those out of the group with FBS and FBS with 1mg/ml BSA (BSA:FBS $P < 0.05$; BSA:FBS with 1 mg/ml BSA $P < 0.05$). Embryos from the group 1 mg/ml BSA (biopsied and non-biopsied) have similar re-expansion rates (50.0% vs 66.7%), but different hatching rates (12.5% vs 33.3%), as well as embryos out of group with 10 mg/ml BSA (biopsied and non-biopsied; re-expansion rates: 57.1% vs 46.7%; hatching rates: 14.3% vs 26.7%). Biopsied embryos out of group with FBS (25% and 50%) show similar re-expansion rates (100.0% vs 80.0%) and hatching rates (88.9% vs 70.0%). Similar results were obtained with non-biopsied embryos out of group with FBS (25% and 50%; re-expansion rates: 70.0% vs 80.0%; hatching rates: 50.0% vs 50.0%). Results show for the first time that LP could be an alternative to cryopreservation.

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Expression of MIF, DDT and Zinc Transporters in the Stallion's Reproductive Tract

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Zinc is a trace element that has received particular attention for its role in male fertility. Within sperm cells, zinc is localized primarily to the tail where it regulates the oxidation status of the sulfhydryl groups located in the outer dense fibers, and thereby indirectly modulates sperm motility. During epididymal transit, the zinc content of the sperm tail is reduced by a mechanism involving macrophage migration inhibitory factor (MIF), secreted by the epididymal epithelium. Using real-time RT-PCR and immunohistochemical staining the expression of MIF, D-dopachrome tautomerase (DDT; a functional homolog of MIF) and selected zinc transporters was examined in the equine testis and epididymis. The effect of zinc on sperm motility was also assessed. MIF expression was highest in the head of the epididymis, where an accumulation of intensely stained material could be seen engulfing the sperm cells. DDT staining intensity and pattern was similar across the different regions of the epididymis; immunoreactive material was visible in the lumen of the epididymis, with the largest amount associated with the tail region of spermatozoa. Among the zinc transporters, SLC39A5 displayed a distinctive expression pattern with significantly higher abundance in the caput epididymis than any other tissue investigated. Both addition of free, extracellular zinc to, and intracellular chelation of zinc in, ejaculated sperm significantly reduced sperm mo-

tility in a dose and time dependent manner, whereas viability was unaffected. We conclude that zinc plays a role in regulating motility of stallion sperm, while the MIF expression pattern in the epididymis suggests a central role for MIF in depleting zinc from the sperm tail during epididymal maturation.

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Method for the Indirect Detection of Interferon-τ by Using an In vitro Leukocyte stimulation test

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Interferon-τ (IFN-τ) is the signal of pregnancy recognition produced by the conceptus around day 16 of early pregnancy in cattle. Because circulating concentrations of IFN-τ are extremely low, the measurement of Interferon-stimulated genes (ISG) as response in leukocytes is an alternative measure. The aim was to establish a simple and indirect method to detect IFN-τ by using a leukocyte stimulation test. Leukocytes were isolated from a non-pregnant donor cow. Plasma samples from early pregnant (p) heifers on day 18 (n = 4) and from the animals during a non-pregnant cycle (np, n = 5) were available from a previous project [Meyerholz et al. 2015]. The leukocytes were incubated in vitro in 12-well plates with plasma samples from either pregnant or non-pregnant animals for 2 h and 37°C in Iscove-medium. Plasma from a non-pregnant cow served as negative control and this plasma spiked with 0.06 ng recombinant bovine IFN-τ was used as positive control. After the incubation, mRNA was extracted and the relative expression of ISG (MX1, MX2, ISG15, OASX1) in leukocytes was measured by using quantitative real-time PCR. Due to small number of samples only descriptive statistics was performed. The mRNA expression of ISG in the leukocyte stimulation test with plasma samples of pregnant heifers was numerically higher than the cycle-control samples (mean ± SD; MX1 p = 21.5 ± 10.3 vs np = 11.8 ± 6.4; MX2 p = 33.8 ± 28.7 vs np = 13.1 ± 8.6; ISG15 p = 53.1 ± 49.5 vs np = 35.4 ± 14.7; OASX1 p = 23.2 ± 13.7 vs np = 11.8 ± 5.7). The ISG mRNA expression in leukocytes from samples of pregnant heifers was comparable to the ISG expression of the positive-control. In conclusion, the incubation in vitro of leukocytes with plasma samples from pregnant cows allows the indirect detection of IFN-τ in early pregnancy.

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ROS-Generating NOX Enzymes and H_2O_2 in Human Ovary and Granulosa Cells

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The mammalian ovary generates reactive oxygen species (ROS). However, the roles of ROS and the mechanism of ROS-generation in the ovary remain poorly understood. Physiological levels of ROS modulate signal transduction and hormone signaling, while its accumulation leads to oxidative stress. Human granulosa cells (GC) express two members of NADPH oxidase (NOX) family, which produce ROS, namely NOX4/5. The present study was aimed to further characterize the expression of NOX members in the ovary and in GC, to determine their contribution to ROS generation, and to obtain insights into their regulation. We studied IVF-derived GC and human ovarian sections. RT-PCR studies confirmed the expression of NOX4/5 and further identified DUOX1/2 but not NOX1-3 in GC and human ovarian sections. Immunohistochemistry/immunocytochemistry showed pre-adsorbable NOX4 in isolated GC and in GC of ovarian follicles. Cultured GC produce ROS, especially H_2O_2 as determined by fluorometric assays. Superoxide was not detected. To study the contribution of NOX4 to ROS produced by GC, we employed the NOX4-blocker GKT137831. It significantly reduced ROS/H_2O_2 production by about 50% without altering cell morphology and viability. As isolated/cultured GC stem from large antral follicles, this may indicate that NOX4 is significantly contributing to the ROS environment of the follicle. Furthermore, our qPCR data suggest that the addition of follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) to the GC elevated mRNA levels of NOX5 and DUOX1/2 but not of NOX4. Thus, gonadotropins may be involved in the regulation of ovarian ROS production.

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Effect of Stem Cell Media on the Development of Porcine Parthenogenetic Embryos

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Since induced pluripotent stem cells (iPS) can be artificially produced from farm animals, the generation of animals for disease models and xenotransplantation by chimera formation of iPS cells with embryos is possible. However, embryos and stem cells have totally different requirements concerning the culture media. The aim of this study was to find optimal culture conditions for both cell types by testing the development of porcine parthenogenetic embryos in different culture media. Porcine parthenogenetic embryos were cultured in different media mixtures of the standard embryo culture medium (PZM-3) and stem cell media beginning at day 3 (4–8 cell) or day 4 (early morula) after activation (1.0kV/cm for 45 µs, followed by incubation with 2 mM 6-DMAP for 3 h). If not otherwise indicated, total embryo number used per medium mixture was 74–110, subdivided into 3–4 repeats. It was shown, that the blastocyst rate from day 3 embryos in PZM-3 (n = 12, 305 embryos) was significantly higher (41.0%) than in both stem cell media (ES medium 1.3%, ciPS medium 3.7%). A mixture of PZM-3 with 25% or 50% stem cell medium improved the blastocyst rate (16.2–25.0%). The results for the day 4 embryos were different. Blastocyst rate in PZM-3 (34.7%, n = 10, 251 embryos) was between the rates of ES medium (39.0%) and ciPS medium (34.1%). Developmental rates in media mixtures (40.5–46.4%) were even better than in PZM-3. It can be concluded that the development of parthenogenetic embryos in pure stem cell medium is possible beginning on day 4 after activation, however medium-specific differences have to be considered. For porcine parthenogenetic embryos beginning on day 3, a mixture of PZM-3 and stem cell medium is recommended.

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Investigations of some Life Quality Parameters during early Neonatal Period in Low Environmental Temperature induced born Lambs

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The purpose of this study was to investigation of some life quality parameters during early neonatal period in low environmental temperature induced born lambs. The animal material was consisted of 20 pregnant Morkaraman sheep housed in Research and Application Farm in University of Yuzuncu Yil Van-Turkey. Parturition of 10 sheep (Group I) was induced by corticosteroids at the 141th day of gestation. Ten sheep with spontaneous parturition constituted the control group (Group II). Some blood parameters (blood gases and glucose), body temperatures and birth weights of these lambs who were born in extremely low environmental temperature (day: 3–4°C, night –5 to –1°C) were recorded in the first week of birth. Differences between the life qualities of these lambs were determined by comparing all pa-

rameters. Only blood PO_2 value was different between Group I and Group II in the first 24 hours ($p < 0.05$). However these differences did not lead to any advantage or disadvantage on the life qualities. Another interesting finding is that the birth weights of all lambs did not have any difference. Individual changes in the body temperatures did not lead to unfavourable effects. Results of our study show that lambs whose birth induced by exogenous hormone in 141th day of pregnancy; up to –5˚C low environmental temperatures didnt lead to negative effect in their life quality. Morkaraman lamb's neonatal life quality, gestation period and genetic resistance against the environment need further investigation for a better understanding.

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Mimicking Estrous Cycle Stages *in vitro*: Impact of Estradiol and Progesterone on the Transcriptomic Profile of Porcine Oviduct Epithelial Cells

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Cyclic changes in the oviduct epithelium are known to be predominantly regulated by Estradiol (E2) and Progesterone (P4). Thus, the present study aimed to examine *in vitro*, the transcriptomic impact of these hormones on highly differentiated porcine oviduct epithelial cells cultured at the air-liquid interphase (ALI-POEC). Diestrus (D, 10 days) and estrus (E, 2.5 days) were sequentially simulated in ALI-POEC (5 donor animals) by basolateral application of physiological levels of E2 and/or P4. Three groups were included: E2 group (D: 10 pg/ml E2; E: 50 pg/ml E2), P4 group (D: 35 ng/ml P4; E: 0.5 ng/ml P4) and E2+P4 group (D: 10 pg/ml E2 and 35 ng/ml P4; E: 50 pg/ml E2 and 0.5 ng/ml P4). Cultures were harvested and processed for histology and microarray analysis after each simulated phase. In vivo-like morphological changes could only be observed in the P4 and E2+P4 groups, whose transcriptional profiles were further studied. Gene expression patterns of simulated estrus and diestrus stages were clearly distinct in both groups as demonstrated by principal component analysis. Comparing estrus versus diestrus stage 166 genes were up and 35 were down regulated in the E2+P4 group; 75 genes were up and 18 genes were down regulated in the P4 group. In concordance with in vivo studies top regulated genes were CDC20, CDC20B, OVGP1 and PGR. Comparison of P4 estrous stage with E2+P4 estrous stage showed only 9 up (top regulated NANOG, CDC20, CDC20B) and 8 down regulated genes. P4 and E2+P4 diestrous stages did not exhibit any differentially expressed genes. Our study builds the basis for further investigations on steroid driven mechanisms behind cyclic functional changes in the oviduct epithelium.

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Evaluation of Selected Parameters of Ram Semen, including Motile Sperm Organelle Morphology (MSOME), after Thawing and Stimulation with Pentoxifylline

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Artificial insemination is a widely used method in livestock breeding. In case of sheep, results are insufficient, what is caused by anatomic structure of female reproductive organs, as well as semen conservation problems. It has been proven, that successful insemination is significantly dependent on spermatozoon's functional activity. Among these sperm motility is particularly important. It can be stimulated by some activators, such as pentoxifylline. The aim of the study was to determine the effect of pentoxifylline on selected sperm kinetics parameters and motile sperm organelle morphology (MSOME) of thawed semen from wrzosówka breed rams. In the study, 18 healthy rams were used. For sperm motility assessment, the Sperm Class Analyser CASA system (Microptic S.L, Spain) was used. Furthermore, samples were taken to MSOME (Leica DMi8 DIC inverted microscope) at 6600 magnification. The results showed that pentoxifylline in concentrations of 1 µg/ml, 10 µg/ml, 100 µg/ml have a positive effect on straight-line velocity (VSL); linearity (LIN) and straightness (STR). Pentoxifylline at the concentration of 1 µg/ml showed a major effect, however there were no significant differences between the tested concentrations of pentoxifylline. The same was true for MSOME. In conclusion the positive effect on ram sperm motility and a lack of negative effect on morphology suggested that pentoxifylline can be utilized to improve the effectiveness of artificial insemination in various breeds of the sheep.

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Sensitivity of different Microbial Isolates to Selected Antimicrobials in vitro in Repeat Breeder Cows and Buffalos in Upper Egypt

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The present study aimed to investigate the microbial and mixed infections (bacterial and fungus) associated with repeat breeder buffalos and cows. This work was carried out on 120 cervico-vaginal and uterine swabs collected from 50 buffalos and 70 cows suffering from repeat breeding. Bacteriological examination revealed that 200 microbial agents were identified and isolated from the collected samples. The results showed that 60%, 20%, 12.5% and 7.5% of the isolates were single bacterial, mixed bacterial, single fungal and mixed bacterial and fungal, respectively. The most prevalent aerobic isolates were *E. coli* (11.9%), *Trueperella pyogenes* (9.4%). The most common mixed bacterial isolates were *E. coli* + *Trueperella pyogenes* + *Proteus spp* (22%) and *Staphylococcus aureus* + *Corynebacterium bovis* (20%). The most common fungal isolates were *Aspergillus spp* (24%) and *Candida* (24%). *E. coli* and *Aspergillus spp* (33.3%) were mostly isolated as a mixed bacterial and fungal infection. After Sensitivity test, the most active antibiotics were Enrofluxacin, Oxytetracycline, Gentamicin and Nalidixic acid. Most bacterial isolates were resistant to Neomycin, Erythromycin and Ampicillin. Sensitivity test revealed that most bacterial isolates, in this study, were highly sensitive to Enerofluxacin, Oxytetracycline, Gentamicin and Nalidixic acid and resistant to Neomycin, Erythromycin and Ampicillin.

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Relationship between Color Flow Doppler Sonographic Assessment of Hemorrhagic Anovulatory Follicles (HAFs) and of the Follicle on the Day before Ovulation in Mares

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The wall of hemorrhagic anovulatory follicles (HAFs) developed well-vascularized luteal tissue as indicated by echotexture and color Doppler signals. The aim of this study was to assess the relationships between HAFs and normal follicle vascularity evaluated by Color Doppler ultrasonography (USG) in mares. The ultrasound examinations were carried out on 15 Warmblood mares at -2 and -1 days before and 1 and 2 days after ovulation. Follicles and HAFs size was determined by measurement of the maximal cross-sectional area of follicles (MCSF) and blood supply by the maximum colored area of the luteinized tissue (MCAL) from Doppler ultrasound images. There were no significant differences in MCSF ($P > 0.05$) between days -2 and -1 before ovulation in both groups of mares with normal ovulation and with formation HAFs. Whereas at day -1, MCAL significant decreased ($P = 0.0004$) in cyclic mares with

normal ovulation and increased (P = 0.003) in mare with HAFs formation. The increase of the blood supply of the HAFs (MCAL) corresponded with vascularization at the apical area on day -1. The lack of vascularization at -1 day (normal follicles) was related to constant MCAL decreases. HAFs formed from viable preovulatory follicles that did not differ from ovulatory follicles in diameter or gray-scale echotexture. Color Doppler USG may be used to estimate the follicle growth, maturation and ovulation better than standard USG due to more an accurately determination of follicles aging. Therefore, Color Doppler USG provides more quantitative information than the standard USG in phases of the estrous cycle and ovulation.

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The Pacemakers and Conduction Pathways of Myoelectric Activity Patterns in the Porcine Uterus

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The myoelectrical activity is a result of voltage- and time-dependent changes in membrane ionic permeability and may be detected directly in myometrium by electromyography (EMG). The ICLC (Interstitial Cajal-Like Cells) contained in myometrium are suspected to display characteristic patterns of rhythmical activity and to have dual functions as pacemakers and conduction pathways for the active propagation of electrical slow waves. The aim of the study was to describe relations between uterine EMG activity and ICLC occurrence in pig *in vivo* model. The spontaneous uterine myoelectrical activity in the 10 non-pregnant, mature sows during diestrus was recorded by the combination of three electrodes connected to transmitter used in large animals. The typical pattern of rhythmic electrical activity was recorded during 3 weeks of registration. Mean amplitude (A), mean root mean square (RMS), duration of electrical activity (D), duration of pauses between activities (P), number of excitations (N) were analyzed regarding to different topographic regions. In parallel immunofluorescent studies, the distribution of specific c-kit/CD 117 receptors (display on ICLC surface) in the same topographic regions of porcine reproductive tract was determined. The significant strong correlation (Pearson's r) between ICLC density and EMG parameters such as duration of electrical activity (D) (r = –0.85, P = 0.03) and duration of pauses (P) (r = –0.81, P = 0.05) in uterine horn tip were demonstrated. No significant correlations in any of the parameters studied were observed for the uterine body and horns. The electrophysiological studies indicate that ICLC in the horn tip myometrium may participate in the regulation of slow waves durations and frequency similar to ICC in gastrointestinal tract.

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TRV2 Ion Channels in Human Testicular Peritubular Cells

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In man, several layers of peritubular cells form the wall of seminiferous tubules. They contract and thus transport immotile sperm. They also secrete numerous factors, which are involved in the regulation of testicular function, including immunological activities. A culture method enables us to study human testicular peritubular cells (HTPCs) in detail. A previous proteomic study identified a member of the transient receptor potential family of ion channels, TRPV2. TRPs are involved in thermo-, osmo-, and mechanosensation, in pain perception and in inflammation. TRPV2 was previously described in immune cells and can be activated by hypoosmolarity and mechanical stress. The identification of TRPV2 in smooth muscle-like cells of the testis implies a role of this channel in peritubular cell functions. Expression in HTPCs was confirmed by RT-PCR and sequencing. Expression levels (qPCR) decreased in advanced passages. Western blotting showed a single band corresponding to the size of TRPV2. Using the same antibody, TRPV2 was identified in testicular peritubular cells in vivo by immunohistochemistry. Functionality of this non-selective cation-channel was examined in HTPCs by monitoring intracellular Ca^{2+} levels using Fluoforte. Activators (CBD/ O-1821) increased levels of intracellular Ca^{2+}. They also evoked a cation-current in patch clamp studies. First results of prolonged exposure of HTPCs to a specific activator (O-1821) indicate that it elevates expression of inflammatory cytokines on mRNA level. This may suggest involvement of TRPV2 in the immune regulation of the human testis, but additional studies are required to elucidate the role of this channel in the human testis in health and disease.

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Relationship between Oocyte Morphology, Zona pellucida and meiotic Spindle Birefringence in unselected and selected Oocytes for ICSI and IMSI

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Birefringence of oocyte structures assessed by polarizing microscopy correlates with its quality and the developmental potential of the embryo.The aim of the study was investigate whether a similar correlation exists in the case of unselected oocytes belonging to the same cohort of oocytes used in the ICSI and IMSI procedures. In the study 329 oocytes were used and 178 of them were selected for sperm microinjection. Morphological assessment of oocytes by differential interference contrast (DIC) was done with an inverted microscopy (Leica DMi8, Germany). Birefringence of zona pellucida (ZP) and the meiotic spindle visualization (MSV) was assessed by a Nikon Ti microscope with OCTAX PolarAIDE™ system. The median value of ZP birefringence was 7.9 and 83.5% of oocytes showed high birefringence (HB) over the median. The MS was observed in 66.7% of the oocytes, in this 99.5% in HB oocytes ($p < 0.01$). The index of birefringence for cohort (BIC) was also calculated. Within the same cohort, the ZP birefringence results for unselected oocytes are closely correlated with the fertilization rate ($r = 0.27$, $p < 0.05$) , embryo quality at the days 2 ($r = 0.30$, $p < 0.05$) and 3 ($r = 0.36$, $p < 0.02$) and the clinical pregnancy rate (CPR) obtained for selected oocytes after ICSI ($r = 0.28$, $p < 0.05$) and IMSI ($r = 0.32$, $p < 0.05$) groups. A similar correlation was found for IDK. The birefringence values for ZP, MSV and BIC in conjunction with oocyte morphology in unselected oocytes may provide additional prognostic parameter in assisted reproduction programs in which the number of oocytes for IVF is restricted by the law as in the case of Poland.

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Sperm-induced Neutrophil Extracellular Trap (NET) Formation in the Bovine System

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In cattle the natural site of semen deposition is the vagina. Besides phagocytosis and secretion of immune modulators, polymorphonuclear neutrophils (PMNs) are able to form „Neutrophil Extracellular Traps" (NETs). These are web-like structures, mainly composed of chromatin. Bovine PMN were isolated via Ficoll gradient centrifugation. Frozen/thawed sperm cell suspension (SCS) of bulls with proven fertility were used. The visualization and identification of NETs was achieved by SEM or via fluorescence microscopy analysis, respectively. For NET induction, PMN and SCS were co-cultured for different time points (0, 15, 30, 45, 60, 120, 180 min). NET induction of sperm and supernatant alone was also measured. Zymosan was used to induce the formation of NETs in bovine PMN as positive control. Quantification of NETs formation was performed by spectrofluorometric analyses using an automated plate monochrome reader (Varioskan Flash; Thermo Scientific). Data were analyzed for statistical significance employing a one way Anova followed by a Tukey test ($p \leq 0.05$). SEM as well as fluorescence microscopy

analyses revealed that the exposure of bovine PMN to frozen/thawed bovine SCS trigger the formation of NETs. After quantification, the following fluorescence intensities (FI in arbitrary units, AU) were obtained for PMN plus SCS: 10.0 ± 0.6, PMN plus sperm cells: 3.2 ± 0.5 and PMN plus supernatant (extender plus minimum amount of seminal plasma): 9.3 ± 0.6. After incubation for different time points, FI were as follows; 7.2 ± 0.5, 7.7 ± 0.4, 8.1 ± 0.4, 8.4 ± 0.8, 10.8 ± 0.7, 9.7 ± 0.6, 9.6 ± 0.7 for 0, 15, 30, 45, 60, 120, 180 min. Sperm alone led to significantly reduced fluorescence intensities suggesting that the extender and the remaining seminal plasma are affecting NET formation to a higher extent. A significant increase in FI was seen until 60 min of incubation indicating that NET formation might be finished at that time point.

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Examination and Biological Significance of the Sulfatase Pathway in the Human Testis

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Large amounts of sulfated steroid hormones are produced within the human testis. As shown previously in breast tissue and placenta, these might not only be produced for excretion, but may also be re-activated in target cells by steroid sulfatase (STS). This process is called sulfatase pathway and may play a pivotal role in para- and/or intracrine regulation of testicular cells by creating a local steroid hormone supply. This requires a facilitated transport via uptake carriers and efflux transporters as these hydrophilic sulfates cannot pass the cell membrane by diffusion. Moreover, blood-testis barrier formation in the testis requires a transport through Sertoli cells (SCs) to reach germ cells (GCs). SCs are therefore expected to play a key role as gatekeepers for sulfatase pathway in human seminiferous epithelium. We analyzed the mRNA and protein expression of uptake carriers and efflux transporters like Organic Anion Transporting Polypeptides (OATP2B1, OATP3A1) and Multidrug Resistance-related Proteins (MRP1, MRP4) in testicular tissue as well as cultured SCs (FS1, HSEC). Additionally, expression pattern of STS as well as sulfonating enzymes (SULTs) were analyzed. OATP2B1, OATP3A1, and STS were detected in SCs as well as GCs, whereas MRP1 was only expressed in SCs, and SULT1E1 only in Leydig cells, respectively. By transcellular transport of [H3]DHEAS in HSEC, we showed a functional transport of sulfated steroids in vitro. Our data indicate a possible steroid synthesis via sulfatase pathway in Sertoli cells in vivo and in vitro. This may contribute to a paracrine and intracrine regulation by a local supply of sulfated and free steroid hormones inside the human seminiferous epithelium.

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Scent Detection as a Diagnostic Tool for Mastitis Pathogens: Proof of Principle

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Introduction Mastitis therapy generally includes antibiotic treatment. For the prudent use of antibiotic drugs it is essential to identify the causing pathogen. The gold standard for pathogen diagnostic in milk samples is still microbiological culturing on blood agar. As logistics and methods are time consuming a diagnose-based start of therapy is delayed by at least 24 h or based on best guess. For bacterial growth the plate has to be incubated for at least 24 h, which causes either a delay in start of therapy or leads to a "best guess" choice of antibiotic. Thus a just in time method for bacterial diagnostic would be advantageous. Odor was used as a diagnostic indicator already in ancient centuries. Nowadays electronic noses have proofed to be accurate in identifying specific odors (e.g. tuberculosis). Attempts to use this method for diagnosis of mastitis pathogens were not yet successful. Dogs have a sensitive olfactory system and are trainable. They are not only used for detection of drugs and explosives, but to identify diseases as cancer or diabetes. The hypothesis of this study was that dogs can discriminate Staph aureus in milk samples of cows suffering from clinical mastitis.

Methods Ten dogs of various breeds and sexes were trained to 1.) discriminate Staph aureus on agar plates and 2.) discriminate Staph aureus in milk samples spiked with the pathogen. At the end of the training the dogs were tested if they can discriminate Staph aureus in mastitis samples. Dogs could identify Staph aureus in the head space of agar plates against five other pathogens with an accuracy of 97.2%. The accuracy for identifying this pathogen in mastitis milk was 89%.

Conclusion In this study we demonstrated that Staph aureus can be identified by means of a specific odor.

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Quantitative Poteomics Reveals Cellular Senescence of Human Testicular Peritubular Cells

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A decrease in reproductive function has become apparent in men with advancing age. The decline in spermatogenesis may be attributable to an impaired capacity of the cells building the spermatogonial stem cell (SSC) niche to provide an appropriate environment in older males. Human testicular peritubular cells (HTPCs) form the wall of the seminiferous tubules and are emerging as an important component of the SSC niche. It is so far unknown, whether they are affected by age and whether they contribute to aging of the SSC niche in man. To characterize HTPCs and the associated secretome – also known as the senescence-associated secretory phenotype (SASP) – we analyzed in vitro-aged HTPCs (i.e. cells from advanced passages) with presenescent (early passages) from 8 individual men with respect to their quantitative protein profiles by nano-LC-MS/MS. Among more than 3,000 proteins identified, we detected extensive age-associated changes in the cellular proteome and the secretome of HTPCs. Our analysis revealed proteins considerably altered in abundance in senescent HTPCs, which are yet undescribed in this context. Furthermore, common hallmarks of aging could be confirmed, thus emphasizing the validity of our approach. The quantitative secretome alterations indicate a rearrangement of the paracrine network of the testis, in general, and specifically consequences related to the communication of peritubular cells with adjacent SSCs. Hence, changes in secreted factors may cause an age-related decline in SSC-niche function, underlining the importance of the SASP for neighboring cells and maintenance of homeostasis. Our data shall improve understanding of aging in HTPCs and delineate the consequences for male reproductive aging.

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Assessment of Anti-Müllerian Hormone in Elderly Mares in Relation to Fertility

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The process of ageing is accompanied by changes in female hormonal regulation and in reproductive activity. A hormone that has been shown to be helpful in predicting ovarian reserve in women is Anti-Müllerian hormone (AMH). The relationship between AMH and fertility has so far not been evaluated in mares. The experiments were performed in two public stud farms during the physiological breeding season from April to August. A gynecological examination was performed according to a standard protocol. Insemination protocols included the use of fresh, cooled-transported or frozen semen. Confirmation of pregnancy was made by ultrasonographic examination on day 14 to 20

and it was recorded whether or not the mare was pregnant and if the mare foaled the following year. The pregnancy rate per cycle and per season was calculated. A total of 44 horses were included in the study. Mares were aged between 12 and 21 years with a mean age of 16.3 ± 2.6 (SD) years. Results for AMH ranged between 0.01 and 2.40 ng/ml (0.74 ± 0.50 ng/ml). AMH concentrations were significantly associated with fertility. Lower AMH concentrations were found in mares that were positively scanned for early pregnancy ($p = 0.036$) and looking at AMH in 2 categories (50th percentile), mares with lower AMH levels had a higher pregnancy rate per cycle than mares with higher plasma AMH concentrations ($p = 0.033$). Foaling rate was not associated with previous AMH concentrations ($p = 0.869$). These results suggest a novel role of AMH as a prognostic marker in equine fertility.

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Evidence of Mesenchymal Stem Cells Proliferation after Transplantation into the Muscle Layer of Uterine Cervix in Swine

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Mesenchymal stem cells (MSC), during *in vitro* expansion, undergo a progressive loss of proliferative potential that leads to the senescent state, associated with a reduction efficacy in the treatment of injured tissues. The aim was to evidence the proliferation of MSC after transplantation into the muscle layer of uterine cervix in swine. Ten Polish Landrace female pigs, weighing 90–120 kg, have undergone general inhalational anesthesia (GIA) during which 40 ml of red bone marrow were gathered from the head of the humerus. The MSC from collected bone marrow were isolated and cultivated in vitro during 3 weeks as well as labeled with PKH 26 and DID. The MSC suspension was transplanted (GIA, laparotomy) into the muscle layer of the cervix and after 2 weeks the cervix was collected and fixed for immunofluorescence (IF). Immunolabeling of Ki67, a marker of proliferating cells expressed in all active phases of the cell cycle (G1, S, G2) were performed. The sample sections were labeled with primary (anti-Ki67) and fluorescent secondary antibodies linked with Hoechst, then imaged using confocal microscopy and scanning and analysis system for fluorescence respectively. Living MSC were found in all translantation places where accounted (mean % ± SEM) for DID 6.64 ± 2.30 and PKH 26 3.90 ± 1.31 of the cells. Cells positive for DID or PKH 26 showed Ki67 staining in nuclei and confirming that DID/PKH 26-labeled MSC underwent in vivo multiplication in transplantation places. The Ki67 expression was significant higher ($P < 0.0001$) in DID (35.40 ± 4.89) and PKH26 (34.81 ± 5.49) positive MSC with respect to DID/PKH 26 negative cells (13.16 ± 1.94) of surrounding tissue. There was no significant differences between Ki67 in both marked-types of MSC ($P > 0.158$).

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Idiopathic Male Infertility: The Role of Sperm Epigenetics and Nucleosome Preservation

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The components of a sperm epigenome are unique and tailored for successful fertilization and proper embryo development. Aberrations in these components are associated with idiopathic male infertility. In human sperm cells a 97% nucleosome-to-protamine exchange occurs to compact and protect the paternal DNA. In our previous work we found that the majority of sperm nucleosomes occurred in repetitive DNA elements like LINEs and SINEs. LINE-1 is a retrotransposon that makes up to 17% of the human genome and therefore it can be used as a surrogate for global DNA methylation levels. DNA methyltransferases (DNMTs) regulate methylation maintenance (DNMT1) and de novo methylation (DNMT3A/3B). The aim of this study was to analyse the LINE-1 methylation and DNMT1/3A mRNA levels (median ± SEM) in spermatozoa of subfertile patients, who underwent ART, and in healthy donors. LINE-1 methylation was determined by pyrosequencing. Regarding both groups there were no significant differences in the three investigated LINE-1 CpG sites (donors: 63% ± 1.08/45% ± 0.97/49% ± 1.14, n = 19; patients: 63% ± 0.98/44% ± 0.61/49% ± 0.75; n = 35, $p > 0.05$). However, there were in both groups individuals with strongly varying LINE-1 methylation levels (± 25%). With RT-qPCR the DNMT1/3A mRNA levels in sperm cells were determined. These levels were significantly higher in donors (DNMT1/3A: 1.49 ± 0.2/1.96 ± 0.30; n = 100) compared to patients (DNMT1/3A: 1.05 ± 0.08/0.47 ± 0.10; n = 48; $p = 0.008/p < 0.0001$, Mann-Whitney U-test). Our study demonstrates that DNMT1 as well as DNMT3A mRNA levels are significantly reduced in subfertile patients. In contrast, the preliminary methylation data in LINE-1s show no difference regarding global methylation between fertile and subfertile men.

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Characterization of Prolactin Receptor (PRL R) Expression in the Cat Mammary Gland Tissue

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Prolactin (PRL) has been first recognized as a hormone that plays an important role in breast cancer initiation and development in rodents, and, at least partly, in humans. In unaffected tissues PRL affects several physiological processes, such as mammary development, and is suspected to influence on mammary cancer, promote cancer progression and acts by binding to a specific membrane receptor – prolactin receptor (PRL R). The objective of the current study was to characterize the expression of the PRL R in the feline mammary tumors based upon immunofluorescence (IF). Samples were collected from 48 mature queens during radical mastectomy with normal (N), dysplastic (D) and neoplastic (A–C, adenocarcinoma; A, adenoma) mammary tissue and fixed for IF. The sample sections were stained with HE, labeled with primary (anti-PRLR) and fluorescent secondary antibodies linked with 7-AAD, then imaged using light and confocal microscopy and scanning cytometry respectively. The epithelial cell cytoplasm was immuno-positive while the stroma cells were mostly negative. No differences in PRLR expression (mean% ± SEM) were observed between A–C (22.13 ± 4.10), A (24.01 ± 7.30), D (20.59 ± 7.67) and N (19.00 ± 3.69) groups, although a low PRLR expression (5.52 ± 0.86) was observed in 10% normal (N), 10% dysplastic (D) and 25% neoplastic (19% A–C; 6% A) samples. A moderate PRLR expression (25.37 ± 1.46) was observed in 13% normal (N), 2% dysplastic (D) and 21% neoplastic (15% A–C; 6% A) samples whereas an overexpression of PRLR (50.42 ± 2.61) was demonstrated in 2% normal (N), 2% dysplastic (D) and 14% neoplastic (10% A–C; 4% A). Results point that PRL may prove a more important factor in mammary gland carcinogenesis that it was supposed so far.

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Purinergic Receptors in Human Testicular Peritubular Cells – Involved in Male Infertility?

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Human testicular peritubular cells (HTPCs) are the smooth muscle-like cells forming the wall of seminiferous tubules. They may, e.g.

via cytokine secretion, be involved in inflammatory processes in the testis often observed in male infertility. Accumulating mast cells in and near the tubular wall further illustrate ongoing testicular inflammation. Beyond that, mast cells are origin of the ubiquitous purinergic agonist ATP. To explore potential actions of extracellular ATP on purinergic receptors of HTPCs, a series of studies was performed.

Cultured HTPCs were found to express a variety of purinoceptor subtypes on mRNA level, among them P2X7, which is known for contribution to inflammatory signaling cascades. P2X7 expression in HTPCs was verified at the cellular level in cultured cells and in human testicular sections. Its distribution correlated with the degree of fibrotic remodeling in the wall of seminiferous tubules and strong expression was detected in the immediate vicinity of mast cells. Monitoring of cultured HTPCs loaded with a calcium-sensitive fluorescent dye revealed that the potent P2X7 agonist BzATP induced transient elevations of intracellular Ca^{2+} concentrations. Pre-incubation of cells with a specific P2X7 antagonist (AZ11645373) reduced the magnitude of the BzATP-evoked Ca^{2+} release. Stimulation of HTPCs with BzATP or ATP increased mRNA levels of inflammation-associated genes.

Taken together, these observations support the notion that P2X(7)-mediated actions in HTPCs have a say in promoting testicular inflammation. However, it is important to further characterize the other identified P2 subtypes and to determine possible interactions that may also participate in inflammatory processes.

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Spontaneous and Induced Myometrial Contractility In vitro in Genetically Selected Heifers Postpartum after Experimentally Induced Mastitis with Escherichia coli (E. c.) and Staphylococcus aureus (S. a.)

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Myometrial contractility is of crucial importance for an unimpaired uterine involution postpartum. Puerperal metritis and endometritis are common pathological states after calving in some cases demanding veterinary assistance and seriously affecting the reproductive performance. Future breeding systems might rely on genetic selection to develop animals more resistant against specific diseases. The aim of this study was to investigate potential differences in myometrial contractility in vitro examining 35 heifers, which had been genetically selected for favorable (Q; n = 18) and unfavorable (q; n = 17) parental chromosome 18 haplotypes associated with milk somatic cell score. They received intramammary challenge with 500 CFU E. c. 24 h (n = 12; Q: n = 6; q = 6) or with 10,000 CFU S. a. 96 h (n = 23; Q: n = 12; q: n = 11) before slaughter. Tissue was collected on day 39 ± 4 *post partum*. Four longitudinal myometrial samples per animal were suspended in an organ bath containing Krebs solution (KS) and air treated with 95% O_2 and 5% CO_2. The spontaneous (SC) and induced (IC; through natural PGF2α analogue [PGF], PGE2, cefapirin, or negative controls [KS]) contractile performance during 30 min intervals (T) were examined over a 3 h period. The area under the curve (AUC), mean (MA), minimal (minA) and maximal (maxA) amplitudes were calculated for every T. Q-animals displayed higher values of minA ($P < 0.05$) than q-animals during T1 and T2 (SC). No differences in SC between S. aureus/E. coli-groups were found. There were no differences among the stimulating substances (PGF, PGE2 or cefapirin) compared to KS. Strips originating from Q-animals showed higher minA when stimulated with 10-7 mmol/L cefapirin and 10-7 mmol/L PGE2 than q-animals. Further investigations are required to elucidate these differences between Q/q animals.

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Hypospadias in a Crossbred Boar

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Congenital abnormalities, cover a broad range of different conditions, occur relatively frequently in swine, especially compared to other domestic animals. Hypospadias, a congenital anomaly of the urogenital tract characterized by abnormal position of the external urethral meatus on the male phallus, is rarely described in boars. The present report describes a distal hypospadias in an 8 months old crossbred intact Pietrain x Duroc boar. Until the desired andrological examination, the boar was perceived as healthy in the herd and showed a normal performance. General physical examination revealed no abnormalities. The andrological examination supplemented with ultrasound revealed no abnormalities of the genital tract apart from the distal hypospadias. Subsequently, the boar was exposed to a sow showing signs of oestrus, in order to assess the ability to mount and to ejaculate. The boar showed a normal mating behaviour and the semen quality parameters were within the normal ranges. Taking these results together with the abnormality of the urethra in the anterior part of the penis into account, the fertility of the boar was considered being 'not restricted'. Though to the high prevalence of hypospadias in humans (1 in 200) and the known association with other congenital abnormalities such as atresia ani, it is assumed that this condition appears more frequently in pigs than currently described in literature. Therefore, further investigations should aim on examining a possible genetic background. In the present case, the pedigree of the boar will be analysed and male siblings will be clinical investigated. In addition, a monitoring for this urethral disorder of the offsprings will be conducted at the slaughterhouse. Together with genetic analyses a better understanding of this abnormality will be achieved.

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Assessment of Chilling Injury in Boar Spermatozoa Using a Competitive Oviduct Binding Assay

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Sperm binding to the oviduct epithelium is an important step on the route to fertilization. Testing the sperm's ability of binding to fresh (non-cultured) oviduct explants collected from slaughter house tissue may therefore indicate the functional competence of spermatozoa. The aim was to test the sensitivity of a standardized competitive oviduct binding assay for the assessment of chilling injury in liquid preserved boar spermatozoa. Individual ejaculates (n = 9 boars) were split and sperm labeled with either MitoTracker® Green or MitoTracker® Red. Subsequently, samples were diluted in AndroStar Plus extender and stored at 17°C or 5°C, respectively. After 24 h and 48 h storage, differentially stained semen samples stored at 17°C or 5°C were co-incubated for 15 min with the same oviduct explant under capacitating conditions. The binding index (BI), i.e. the number of sperm bound per mm^2, and the ratio of red to green sperm were calculated. In general, BI differed highly between explants (16.1–332.9; 36 explants from 14 sows). In Experiment 1 (n = 3), the BI after 48 h was higher for sperm stored at 17°C (160 ± 106) compared to sperm stored at 5°C (72 ± 50; $p < 0.05$) corresponding to a binding ratio of 69% to 31%. In Experiment 2, a slower cooling regime and AndroStar Plus 5 extender were used (n = 6). The ratio of bound sperm was less different for samples stored at 17°C (54.6%) and 5°C (45.4%) after 24 h storage; the BI did not differ. The binding index moderately correlated with the amount of sperm with intact plasma and acrosome membrane ($r = 0.53$, $p < 0.05$), but neither with motility, nor the responsiveness of sperm to bicarbonate as assessed by a calcium influx assay. In conclusion, a competitive oviduct explant assay sensitively detects chilling injury in liquid preserved boar spermatozoa.

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Impact of a Maternal Diabetes on Embryonic DNA Methylation in Rabbit Blastocyst

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In early pregnancy maternal diabetes leads to delay in embryo development and changes in nutritional and hormonal signals of the uterine environment. The current study focuses on consequences of maternal diabetes on embryonic tissue formation and its DNA methylation.

Therefore we investigated the expression and promoter methylation of epiblast lineage specifier Oct4 in 6 day old rabbit blastocysts at early gastrulation stage. The expression of Oct4 was higher in epiblast of diabetic blastocysts, accompanied by upregulation of Nanog and Sox2. Specific methylation of the POU5F1 (Oct4) promoter region was investigated by bisulfite sequencing. The Oct4 promoter was hypomethylated in hypoblasts and trophoblasts of diabetic rabbits, implying also a mark of delay in differentiation. The global DNA methylation of male and female blastocysts from diabetic and healthy rabbits was examined, employing embryoblast and trophoblast tissue separately, using LUminometric Methylation Assay (LUMA). No significant changes in global DNA methylation were observed. Furthermore we verified that concentrations of methyl group donor S-adenosyl methionine (SAM) and the product of the methylation reactions S-adenosylhomocysteine (SAH) were changed in diabetic pregnancy. SAM and SAH were measured by use of a modified liquid chromatography-tandem mass spectrometry in rabbit blood plasma collected at the day 6 *post coitum*. Our data showed that maternal diabetes mellitus affects the Oct4 promoter methylation in a specific way with consequences for Oct4 gene transcription and embryo development. Alteration of Oct4 methylation was not caused by global methylation changes.

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Urospermia in an Entlebucher Mountain Dog with ectopic ureters – A case report

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Urospermia (US) may result from retarded closure of the urinary bladder neck during semen emission due to local inflammation or insufficient α-adrenergic stimulation of the smooth muscle layers. By this urine passes the bladder neck when pre-secretion and the sperm rich fraction are reaching the pelvic urethra. In the present case US is suspected to be the result of a bilateral ectopic ureter (EU), a congenital malformation, with the ureteral orifice terminating intravesically near the bladder neck or extravesically at the prostate, deferent duct, or urethra. Case report: A 4-year-old Entlebucher Mountain Dog was presented for breeding soundness evaluation due to an unsuccessful mating 5 months ago. At the age of 2 years an abnormal course of both ureters in the area of the bladder neck had been diagnosed sonographically. As the finding was not classified as serious, the dog had been approved for breeding. The current andrological examination revealed both testes and epididymides of adequate size and shape. The collected ejaculate showed normal volume and total sperm count, but severe deficiencies in sperm motility, plasma and acrosome membrane integrity and sperm morphology. The most striking macroscopic finding was a yellowish appearance showing continuously increasing intensity in the watery prostatic secretion. Odor and pH-value revealed admixture of urine. A repeat ultrasound examination confirmed a bilateral intramural EU entering the urinary bladder about 1 cm cranial of the vesico-urethral transition with the opening pointing caudally. This finding may not indicate a causal connection between EU and US *per se*. Nevertheless the latter may be a functional indicator of a urine leakage possibly existing as additional congenital abnormality in the caudal vesical/cranial urethral area.

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Factors Affecting Semen Evaluation of Native and Frozen-thawed Goat Semen

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Parameters collected during semen evaluation are the basis to establish the suitability of an ejaculate. Therefore, the conditions must be kept as constant as possible. The aim of this study was to evaluate possible factors affecting semen analysis of native and frozen-thawed goat semen. A total of 20 ejaculates from 5 healthy peacock goat bucks were obtained using an artificial vagina. After a macroscopic evaluation 2 aliquots were assessed at different handling temperatures (20°C and 37°C) and time points (TP) (3, 10, 15 and 30 min. after collection). The pH of each sample was evaluated with indicator-paper every 10 min. for 1 h. Density and progressive motility (PM) were assessed using computer assisted sperm analysis (AndroVision®, Minitüb, Germany). Evaluation of semen viability was performed using bromophenol-nigrosine staining. For cryopreservation, a commercial semen extender (Andromed®, Minitüb, Germany) was used. After 24 h, samples were thawed and examined microscopically after 3, 5 and 10 min. In native samples, only the TP had a significant influence on viability of sperm cells ($p < 0.0001$). The number of live sperm cells already decreased 10 min. after collection. The TP had a significant effect on semen pH ($p < 0.0001$). After 10 min. a reduction of the pH could be shown at both temperatures. In frozen-thawed samples, temperature during semen evaluation had a significant effect on PM ($p = 0.053$). Progressive motility was better in samples examined at 20°C. In conclusion, handling temperature was identified as factor influencing native goat semen assessment, while temperature during semen handling had a significant effect on PM after thawing.

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Enzymes and Transporters involved in the Process of Estrogen Glucuronidation are Precisely Regulated across the Estrous Cycle and during Early Pregnancy in Pigs

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Estrogen metabolism includes the endogenous formation of inactive estrogen conjugates that are proposed to function as a reservoir for active estrogens during estrous cycle and pregnancy. Based on our study in gilts where estradiol-17β (E2) was orally applied (1000 µg E2/kg body weight/day) and estrogen metabolites were analyzed in plasma, bile, heart muscle and endometrium by GC-MS, we have good evidence that the glucuronides are the most abundant conjugates in pigs. To elucidate the potential availability of estrogen glucuronides in the endometrium during estrous cycle and early pregnancy, we analyzed the mRNA expression of enzymes (UGTs, GUSB) and transporters (MRPs, OATPs) possibly involved in glucuronide metabolism in the endometrium on days 0, 6, 10, 12, 14, and 18 of estrous cycle as well as on days 10, 12 and 14 of pregnancy. UDP-glucuronsyltransferases UGT1A1, UGT1A6 and UGT1A10 displayed minimal expression on day 6 of estrous cycle and maximal expression during late luteal phase. UGT2B31 however was only minimally expressed already during estrous. Beta-glucuronidase in turn showed highest expression at estrous and proestrous and low expression during diestrous indicating a negative regulation by progesterone. The mRNA expression

of the influx-transporter OATP2A1 significantly increased from day 0–6 and decreased again by day 10, while efflux-transporters (MRP1, MRP2, MDR1) displayed minimal expression during early diestrous. Pregnant pigs displayed differential expression of the respective enzymes and transporters mainly at day 12. Their precise regulation both during estrous cycle and pregnancy indicates a participation of estrogen glucuronides in the local availability of estrogens to fine-tune endometrial function.

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Peri-Conceptional Overweight in Male and Female Mice causes Sex-Specific Transcriptome Changes in Blastocysts which could result from differential expressed sncRNA in Spermatozoa of Obese Males

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Human epidemiological studies show that the offspring's risk to develop obesity in later life is strongly associated with maternal obesity. In a previous study, we showed that a periconceptional obesogenic exposure of female mice is sufficient to induce sex-specific abnormal health outcomes in the offspring. In adult male offspring, we found overweight, insulin resistance, hyperleptinemia, hyperuricemia and hepatic steatosis, but not in female offspring. In our current study, we investigated the transcriptome of the early embryo *in utero* of mice after maternal or paternal pre-conceptional obesogenic exposure. Microarray analysis of blastocysts from obese fathers, obese mothers and lean parents revealed that male embryos from one overweight parent, regardless of father or mother, had several upregulated genes compared to control embryos of lean parents. In female blastocysts from obese fathers, 48 genes were downregulated, whereas female blastocysts from overweight mothers only revealed a moderate differential gene expression. To address the underlying molecular mechanisms of the paternal derived transcriptome changes of the early embryo we investigated the sncRNA pattern in spermatozoa by next generation sequencing. We found several differentially expressed microRNAs and piRNAs in the spermatozoa of obese males compared to lean males. Our data indicate that overweight of mothers and fathers during the peri-conceptional period may lead to sex-dependent transcriptome changes already in the blastocyst stage. Additionally, we found that the spermatozoa of obese male mice have a different sncRNA pattern, these RNAs may act as early regulators of the embryonic transcriptome.

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Study of the Impact of Somatic Cell Nuclear Transfer (SCNT) on Transcriptome of Early Bovine Embryos

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RNA sequencing (RNA-seq) of bovine oocytes and different stages of *in vitro* produced (IVP) embryos (4-cell, 8-cell, 16-cell, blastocyst) revealed major embryonic genome activation (EGA) at the 8-cell stage [Graf et al., PNAS 2014; 4139–44]. The present study investigated the impact of cloning by somatic nuclear transfer on the transcriptome profiles of corresponding stages of bovine SCNT embryos. Three pools of 10 embryos per stage were analyzed. Lysed embryos were used to generate and amplify cDNA with the Ovation RNAseq v2 kit (NuGEN). Barcoded sequencing libraries (NuGEN rapid library kit) were sequenced on an Illumina Genome Analyzer IIx (80-base single-end reads; 20 million per library). Reads were mapped to version bostau7 of the bovine reference genome using TopHat2. RNA-seq data of IVF embryos were re-analysed accordingly, and differentially abundant transcripts (DAT) were identified using DESeq2. The number of DAT between SCNT and IVP embryos was lowest at the 4-cell stage (266), highest at the 8-cell stage (2841), decreased at the 16-cell stage (1724) and even further at the blastocyst stage (816). At the 8-cell stage, most DAT in SCNT embryos were decreased in abundance, suggesting that nuclear reprogramming lacks behind major EGA in IVP embryos. Analysis of de novo transcription (identified by intron sequence containing reads above the threshold level observed in oocytes) at the 8-cell stage revealed activation of 3548 genes in IVP, but only 1732 genes in SCNT embryos. At the 16-cell stage, SCNT embryos activated a higher number of genes (2772) than IVP embryos (1740). In summary reprogramming in SCNT embryos caused a delayed onset of transcription of multiple genes, including important regulators of early development such as NANOG, FOSL1, MTA2, and PDCD2.

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Quantification of Blood Contamination and its Influence on IL-8 Concentration in Uterine Secretion Samples

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The character and pathogenesis of subclinical endometritis (sE) in dairy cows is not fully understood which makes it difficult to set a gold standard for its diagnosis. Uterine cytology and histopathology are mainly applied as diagnostic devices. A new approach to understand the pathogenesis of sE and to facilitate its diagnosis is the examination of uterine secretions (uS). A newly developed device is used for the collection of uterine secretion samples containing a highly absorbent Merocel®-swab. During sampling slight haemorrhages may occur in the endometrium. The aims of this study are (1) to quantify the blood contamination and (2) to display its influence on the detected IL-8 concentration in uS samples. Whole blood samples of ten cows were collected. 100 µl of each blood sample were affiliated to the swab material and processed identically to the uS samples. A serial dilution of the blood samples was established with phosphate buffered saline. Of each cow 12 dilution stages of blood samples were examined photometrically at 570 nm. Mean values of optical density at each dilution stage were used for calculation of a type curve of blood dilution. To evaluate the influence of blood contamination on the presence of chemokines, IL-8 concentrations of uS and plasma samples of 30 cows were determined using AlphaLISA. The impact of IL-8 originating from blood contamination on IL-8 concentration in uS samples was calculated. The percentage of blood contamination in uS samples is estimated by its optical density using a polynomial function. Mean values of IL-8 concentration in blood contaminated uS and plasma samples were 420 pg/ml and 47 pg/ml, respectively. Considering the percentage of blood contamination in uS samples and the IL-8 concentration in plasma, the IL-8 concentration in pure uS can be calculated.

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Relationship between Uterine Contractility and Gene Expression of Hormonal Receptors in Puerperal Uteri with and without Metritis

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The aim of this study was to investigate the relationship between uterine contractility and gene expression of hormonal receptors (estrogen receptor α [ERα], progesterone re-

ceptor [PR], PGF2α receptor [FP] and oxytocin receptor [OTR]) in puerperal uteri with and without metritis. Therefore, 13 puerperal uteri of cows showing clinical signs of a metritis (n = 7) or no metritis (n = 6) were extracorporeally perfused after euthanization. Measurement of uterine contractility was performed by using four piezoelectric crystals (Sonometrics Corp., London, Ontario, Canada), which were implanted into the myometrium of the great curvature of the formerly pregnant horn in a longitudinal direction. After 1 hour of equilibration, a bolus of oxytocin (5 IU) and PGF2α (2.5 mg Dinoprost) were administered with a time interval of 30 minutes via the perfusion system into the uterine arteries. The infusion of oxytocin induced shortenings of 2 distances between crystals *in uteri* with metritis and of one distance in healthy uteri ($P < 0.05$). In all distances (n = 3) uteri with metritis showed a higher shortening after stimulation with oxytocin than healthy uteri ($P < 0.05$). After application of PGF2α no changes of crystal distances were noticed in healthy uteri ($P > 0.05$). *In uteri* with metritis one distance exhibited a shortening and a widening ($P < 0.05$). Myometrial gene expression of FP and OTR was higher ($P < 0.05$) in metritis uteri than in healthy uteri. In summary, PGF2α had no effect on uterine contractility in puerperal uteri without and with metritis. In contrast an infusion of oxytocin induced a contraction of the uteri. Similar to the gene expression of FP and OTR myometrial contractility was higher in cows with a metritis.

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Genetic Testing of Bovine Embryos Using Blastocoel Fluid as a Source of DNA

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Preimplantation Genetic Diagnosis (PGD) of embryos is a well-established technique that allows the detection of genes associated to certain diseases or traits. PGD involves obtaining a few cells from an embryo, which will then be used for PCR. Recently, Palini et al. [Reprod Biomed Online 2013; 26: 603–10] were able to diagnose the sex human embryos using only the blastocoel fluid as a source of DNA. We have also tested this technology using equine embryos, successfully diagnosing the sex of *in vitro* produced (IVP) and *in vivo* derived embryos. Therefore, the aim of our work was to study the blastocoel fluid of IVP bovine embryos, as a possible source of DNA for genetic testing and the post-freezing viability of the embryos after blastocoel fluid collection. Bovine embryos were produced *in vitro* using slaughterhouse oocytes and some of the blastocysts were collapsed, by aspirating the blastocoel fluid as described previously for equine embryos. The DNA in the blastocoel fluid was amplified by PCR using primers to detect the bovine Y-encoded testis-specific protein (TSPY) and 1715 bovine satellite (b1715Sat). Collapsed or intact blastocysts were vitrified and warmed using the open pulled straw (OPS) method. After warming, they were cultured *in vitro* for 24 h and observed under the stereomicroscope to detect re-expansion. The DNA in the blastocoel fluid from 11 out of 11 blastocysts was successfully amplified by PCR (5 males and 6 females). After vitrification, warming and *in vitro* culture for 24 h, 19/20 intact blastocysts and 11/11 collapsed embryos survived. Although these results are preliminary, we demonstrate that the DNA in the blastocoel fluid can be amplified by PCR to diagnose the sex of bovine IVP embryos. Moreover, all collapsed embryos survived vitrification, showing that blastocoel fluid collection does not impair their viability. Further experiments with a larger number of embryos will describe the efficiency of this methodology.

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Impaired Weight Gain and Disproportionate Organ Growth, but Preserved Fertility in Growth Hormone Receptor Deficient Pigs (Laron syndrome)

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Laron syndrome is a rare, autosomal recessive disorder in humans caused by loss-of-function mutations of the growth hormone receptor (GHR) gene. To generate a large animal model of GHR deficiency, we used CRISPR/Cas9 in porcine zygotes to mutate exon 3 of the GHR gene.Two heterozygous founder sows (GHR+/-) with a 1-bp or 7-bp insertion in GHR exon 3 (causing a reading frame shift) were mated with wild-type (German Landrace) boars to obtain heterozygous F1 pigs, which were intercrossed to produce GHR-/- pigs. The birthweight of GHR-/- piglets did not differ from GHR+/- or GHR+/+ littermates. GHR-/- animalsshowed a marked reduction in serum IGF1 levels (12.2 ± 9.1 vs 70.9 ± 38.2 ng/ml in GHR+/- and GHR+/+ pigs determined by RIA). Postnatal growth failure became obvious at the age of 3 weeks. At the age of 6 months, body weight of GHR-/- pigs (37.7 ± 6.4 kg) was 60% reduced compared to GHR+/+ (89.2 ± 15 kg) and GHR+/- pigs (92.2 ± 13.1 kg). Dual energy X-ray absorptiometry (DXA) revealed a markedly increased percentage of total body fat (20 ± 5%) in GHR-/- compared to GHR+/- and GHR+/+ pigs (11 ± 2%). When corrected for body weight, liver and kidney weights of GHR-/- pigs were reduced (74 ± 6% and 76 ± 12% of GHR+/+ pigs), while relative brain weight was almost doubled (190 ± 29% of GHR+/+ pigs). Mating of a GHR-/- boar with a GHR-/- sow (age 8 months) resulted in a litter of 6 healthy piglets. Their birthweight (808 ± 103 g) was markedly smaller compared to GHR-/- piglets from GHR+/- × GHR+/- matings (1331 ± 298 g). In summary, GHR deficiency in pigs results in reduced body weight gain associated with disproportionate changes in organ growth, but does not generally impair fertility.

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Pregnancy-associated Glycoproteins in Cows with retained Fetal Membranes

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Retained fetal membranes (RFM) are a common problem in bovine reproduction and severely impair reproductive health in cows. Associated with a timely release of fetal membranes are cellular changes in the maternal and fetal parts of the placenta. In a mature placenta the number of binucleate trophoblast giant cells (BNC) declines prior to parturition, while in RFM the number remains high. Since the BNC produce Pregnancy Associated Glycoproteins (PAGs) and release these into the maternal organism, we hypothesized that differences of PAG concentrations in maternal blood could be used as a diagnostic tool for RFM in cattle.

A radioimmunoassay was used to measure PAG concentrations in serum samples from cows with RFM (n = 20) and from controls (n = 68). The numerical density of immunostained PAG-positive BNC was counted in paraffin sections of placentomes, that were taken at parturition from cows with RFM (n = 20) and from controls (n = 20). At parturition mean PAG blood concentrations in cows with RFM were slightly higher than in controls (3038.09 vs. 2476.50 ng/ml), but differences were not significant. The density of PAG-positive BNC in fetal tissue was higher in RFM, compared to controls (39.5 vs. 26.5 BNC/mm^2, $p = 0.02$). Our study reveals that PAG concentrations in maternal blood do not differ significantly between cows with RFM and controls. The number of PAG-positive BNC in RFM was higher than in controls. Thus the high PAG concentrations in maternal blood of cows with RFM do not result from a "mass exodus" of BNC from the fetal into the maternal placenta prior to parturition.

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Camel whey Protein as Antioxidant and Anti-inflammatory Ameliorate the Testicular Damage in Streptozotocin-induced Diabetic mice

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Induction of diabetes using STZ in experimental animals caused deformation of normal histological seminiferous tubular structure mediated by inflammation and oxidative stress. Camel whey protein (CWP) can modulate immune functions and improves the normal inflammatory process in diabetic models. The present study aimed to explore the beneficial role of CWP against STZ induced testicular abnormalities in diabetic mice. Forty-five adult male mice weighing 25–30 g were divided into 3 groups (15 mice each). The 1st group served as control while the other 2 groups were injected with STZ (60 mg/kg b.w, i.p., 3 successive doses) for induction of diabetes. Then after 2 weeks one of the diabetic groups was treated with CWP (100 mg/kg b.w. for 4 weeks). The diabetic mice showed a reduction in insulin levels, an increase in blood glucose levels and cytokines (IL-2, IL-1B, IL-4 IL-6, and TNF-α), alterations in oxidative stress markers (LPO, CP, NO, GSH, CAT, and SOD), and structural damage in the seminiferous tubules. Supplementation of diabetic mice with CWP almost normalized the blood glucose and insulin levels and inhibited the elevation of pro-inflammatory cytokines and oxidative stress markers in the testes accompanied by an improvement in the histological structure of the seminiferous tubules. In conclusion, the supplementation of diabetic mice with CWP ameliorate the testicular damage inflicted by diabetes possibly by a mechanism suppressing oxidative stress and inflammation.

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Tubal Sperm Storage in Humans versus Animals – The Reservoir Redefined

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Upon entering the oviduct, the spermatozoa of most animals bind to the cilia of the oviductal epithelium forming what is commonly referred to as the sperm reservoir. This species specific, sugar mediated binding maintains the viability and fertilizing capacity of sperm in the female genital tract for 3–4 days in most mammals, up to 6 months in bats or even 9 years in some Australian snakes. To date sperm reservoir formation in animals has been well documented but the existence of a human sperm reservoir has not yet been described. Using newly established live cell imaging technology the aim of our study was to investigate the human sperm oviduct interaction under near in vivo conditions. All human tubal samples were obtained with ethical approval and informed consent. Oviducts from 25 patients undergoing ovariohysterectomy for benign pathologies were co-incubated with fresh or frozen/thawed sperm. Our findings showed that human sperm stay motile for up to 4 days in the oviduct. They bind exclusively to the oviductal secretory cells at specific sites of the tubal epithelium. This binding is competitive and transient in nature lasting between 1 and 90 seconds with sperm being observed to change the angle at which they are bound. This is in strong contrast to sperm binding in mammals where there is a permanent binding to the cilia until ovulation with no competition and no preference for specific binding sites in the isthmus.

Our results imply that the human sperm reservoir is unique in regard to binding sites and binding mechanisms. Sugar-mediated binding might be replaced by more transient molecular mechanisms in humans. This knowledge is pivotal in improving the results of assisted reproductive technologies (ART) in male infertility.

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Assessment of Predisposing Factors for Wound Healing Disorders after Caesarian Section in Cows

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Wound healing disorders are a common complication after Caesarian section and may relate to different techniques of surgery and local anesthesia. The aim of the present study was to evaluate additional predisposing factors. We included 39 cows (aged 1.5–9.9 years), which underwent a Caesarian section between August 2014 and March 2016 at the Clinic for Ruminants, Ludwig-Maximilians-University, Munich. In total, 65 variables (e.g. concurrent diseases, suture techniques, duration of surgery, presence of swelling, edema or fluctuation) were recorded. Statistical analyses were carried out by chi-square test (exact Fisher) and odds ratios (OR) and relative risks (RR) were calculated. We could confirm, that a "firm" palpatory clinical finding of the incision site related to significantly more severe wound healing disorders than moderate or light ones (Fisher: $p = 0.009$). However, we could not confirm that infiltration of local anesthesia in the incision site caused significantly more wound healing than paravertebral techniques (Fisher: $p = 0.669$, OR = 6.57). On the other hand we could show, that the duration of surgery had an influence on wound healing: Significantly more severe rather than moderate/light wound healing disorders could be found if the Caesarean section took more than 2 hours (Fisher: $p = 0.022$, OR = 6.65). In future a larger sample size may point out additional significant associations of influencing factors with wound healing disorders after Caesarian section.

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Trueperella Pyogenes isolated from a Cow Developing Clinical Endometritis Expressed mRNA of Fimbrial Subunits and induced a Pro-Inflammatory Response in Bovine Endometrial Epithelial Cells and Peripheral Blood Mononuclear Cells

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After parturition, different bacterial species colonize the bovine uterus. Some bacteria can induce a uterine disease, especially *T. pyogenes* is associated with clinical endometritis (CE). Virulence of invading bacteria and host innate immunity will determine the ability of a cow to defend against *T. pyogenes*. This study investigated a *T. pyogenes* strain (TP2) isolated from the uterus of a healthy dairy cow on day 15 pp, which showed signs of CE on day 21 pp. Aims of this study were to evaluate the transcription of bacterial virulence factors crucial for colonizing host cells and to investigate the effect of this strain on a pro-inflammatory response in bovine endometrial epithelial cells and peripheral blood mononuclear cells (PBMC) in vitro. TP2 was grown in BHI broth, at 6, 12 and 24h, bacteria were harvested, total RNA was extracted and subjected to RT-qPCR. Epithelial cells and PBMC did not survive a co-culture with TP2 longer than 24 h. Endometrial epithelial cells (n = 5) and PBMC (n = 3) were co-cultured up to 8 h with alive TP2 at a multiplicity of infection of 1 and 0.1, respectively. Total RNA was extracted from endometrial cells and from PBMC and subjected to RT-qPCR. The mRNA expression of fimA, fimC, fimE, and fimG was observed in TP2. Epithelial cells responded to TP2 by increased mRNA expression of CXCL3, CXCL5, IL1A, IL6, and IL8 after 8 h compared with controls. PBMC co-cultured with TP2 showed higher transcription of PTGS2 and IL8 after 2 h, CXCL3 after 4 h, and IL1A after 6 h compared with controls. In conclusion, this *T. pyogenes* strain showed pathogenic characteristics, which induced the innate immunity by increasing the pro-inflammatory responses in endometrial epithelial cells and PBMC.

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PRID Treatment of Anoestrous Milk Cows with Follicles Growth up to the Deviation Stage

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This paper presents the results of progesterone based treatment of anovulatory anoestrous cows with follicles growth up to the deviation stage. The case definition of anoestrous was no detected oestrus during 60–80 days postpartum and the presence of small follicles (< 9 mm) on ovaries without corpus luteum. The study was performed on 47 cows from one commercial herd selected by transrectal palpation and ultrasound. Cows were randomly assigned to one of two groups: 1 (n = 31) treated with progesterone insert (PRID Delta, Ceva) or 2 (n = 16) left untreated. Clinical response and reproductive dates were recorded for 120 days after treatment by rectal palpation and ultrasound. The oestrus rate (51.7% vs 19.5%) and total pregnancy rate (87.1% vs 18.8%) were higher in group 1 than in control cows ($P < 0.05$). Also treatment-conception interval 115.8 ± 34.6 vs. 234.7 ± 21.9 was shorter in treated cows ($P < 0.05$). In contrast the number of inseminations per conception (2.6 vs 1.7) was better in control group. Although, in general there is no recommendation for hormonal treatment of anoestrous cows follicular development arrested in its early stage, our results suggest that PRID protocol can be used with moderate success rate in these cases.

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Retinoic Acid Modulates Testicular Oxidative Damage induced by Cisplatin-loaded Silver Nanoparticles in Mice

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Oxidative stress has been proven to be involved in cisplatin- (CP-) induced toxicity. Silver nanoparticles modulate ATP-Binding Cassette transporter (ABC transporter) activity and enhance chemotherapy in multidrug resistant cancer. The present study was designed to evaluate the antioxidant activity of Retinoic Acid (RA) against CP-loaded Silver Nanoparticles induced testicular oxidative damage in mice. Our data indicated significant increases in lipid peroxides (LPO) and Nitric Oxide (NO) levels in testes of mice treated with CP- loaded AgNPs (2 mg/kg/week, for 2 weeks) that was associated with a significant reduction in the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) content. Histopathologically, testes of mice treated with CP loaded AgNPs showed congestion, interstitial edemas well as degeneration of spermatogenic cells with increase in the number of apoptotic cells in the seminiferous tubules. The prior administration of RA (0.5 mg/kg/twice weekly, for 2 weeks) before CP-loaded AgNPs by 24 hours significantly reduced the elevation in LPO and NO levels. However, it reduced the effect of CP-loaded AgNPs on antioxidant levels. The administration of RA before CP-loaded AgNPs injection improved the histological pictures and reduced the number of apoptotic cells. In conclusion, RT is a potent antioxidant, against CP-loaded AgNPs induced testicular oxidative damage. Further study must be done to evaluate this regime in cancer chemotherapy.

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Can post-thaw Motility of Canine Semen be Predicted by the Hypoosmotic Swelling Test?

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Integrity of sperm cell membranes is considered to be crucial for survival during cryopreservation because cells are exposed to considerable osmotic challenges. Objective of this study was to examine relationships between Hypoosmotic swelling (HOS) test results and standard semen parameters before freezing and after thawing. Semen of 35 private owned male dogs of different breeds was collected and analyzed. Standard semen parameters as well as the HOS test were evaluated in fresh and frozen-thawed semen samples. For cryopreservation CaniPRO™ Freeze A&B and for thawing CaniPRO™ Culture Medium (MOFA) were used. For grading as good quality, frozen-thawed semen had to show at least 50% progressive motile sperm cells and less or equal than 20% morphological abnormal sperm cells. Cryopreservation caused a decrease in all standard semen parameters as well as in the results of the HOS test ($P < 0.01$). In fresh semen, HOS test results were correlated to progressive motility ($\rho = 0.51$), viability ($\rho = 0.50$) and normal morphology ($\rho = 0.46$). In frozen-thawed semen correlations between HOS test and progressive motility ($\rho = 0.67$) and viability ($\rho = 0.86$) were found. Fresh semen HOS test results were correlated with post-thaw viability ($\rho = 0.50$), normal morphology ($\rho = 0.55$) and HOS test results ($\rho = 0.43$). Out of 35 ejaculates, 14 were classified showing good semen quality. For testing predictability of individual freezability, results of fresh semen parameters and HOS test results of good and poor semen quality were compared. Statistical analysis demonstrated that it is not possible to predict post-thaw motility using standard semen parameters such as progressive motility ($\rho = 0.22$), viability ($\rho = 0.33$) or the HOS test results ($\rho = 0.35$).

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Fetal Ultrasonographic Measurements near Parturition as Predictors of the Time of Birth in the Domestic Cat

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In the cat, accuracy of parturition day prediction by ultrasonographic measurement of fetal structures is decreasing towards the end of pregnancy. Our goal was to determine fetal biparietal, abdominal and eye diameter (BPD, AD and ED, respectively) by ultrasonography within 5 days of delivery to predict parturition date. Twenty-five pregnancies in 24 queens of different breeds were examined. BPD, AD and ED measurements were taken once, on the day of parturition in 13 queens presented at our clinic due to dystocia. Eleven queens were examined on 62–63 days after the first mating and 48–72 hours later if birth did not occur. Two pregnancies were excluded because of elective Cesarean section. Linear regression analysis was used for the association of BPD, AD and ED with days before birth (DBB). Fetal ultrasonographic measurements varied among and within litters. There was a significant, moderate relationship between BPD, DBB and litter size. While BPD increased with time closer to birth, litter size was inversely related to BPD. Linear regression showed a low agreement between AD and DBB, which was not influenced by litter size. Fetal ED length and width were not associated with time to parturition. Using the linear regression equation of Beccaglia et al. (2008) for BPD measurements within the last five days before parturition resulted in less accurate birth prediction (40% within 1 day and 53% within 2 days) than it was previously reported for earlier pregnancy stages. In conclusion, fetal ultrasonographic BPD and AD have low accuracy for the estimation of the day of birth when measured in the last 5 days of pregnancy. Fetal ED measured shortly before birth is not suitable for estimation of delivery date in the cat.

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Metoclopramide Administration Changes Milk Composition in Lactating Bitches

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Hypo- or agalactia in the bitch negatively affects puppy growth rate and results in increased pup morbidity and mortality. Metoclopramide (MC) is recommended to stimulate milk production through increases in serum prolactin (PRL), but this has not been confirmed in the dog. While direct assess-

ment of milk yield is not possible, monitoring milk energy and nutrient content may give an indication on the effects of MC on canine milk composition and yield. Lactose is the major determinant of milk volume by its osmotic actions, and its production is stimulated by PRL through increased glucose uptake and availability of α-lactalbumin [Ben-Jonathan et al., 2006]. We hypothesize that MC stimulates milk production by increasing milk lactose. Ten control and ten treated (MC at 0.2 mg/kg PO q6h for 6 days) healthy bitches were included in the study. Body weight, age, parity and litter size was similar between the groups. Milk was collected on Day 0 (10–24 h after parturition and before start of treatment), and on Day 4 and 6. Day 0 samples, and the combined Day 4+6 samples were analyzed for gross energy (GE) by bomb calorimetry, dry matter (DM) by drying till constant weight, and lactose by high performance anion exchange chromatography. Data was analyzed by independent and paired t-test, Mann-Whitney-U test and Wilcoxon signed rank test. In the whole population and in each group, milk lactose (g/kg) on whole milk, GE (J/g) on DM, and lactose/DM significantly increased from Day 0 to Day 4+6. Milk DM did not significantly change over time. Milk composition was similar between the groups on Day 0. On Day 4+6, lactose/DM was significantly higher in the treated than in the control group, which may indicate higher milk yield in the treated dogs based on the osmotic properties of lactose.

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Seasonal Effects on Testicular Development and Spermatogenesis in Harbour Porpoises from the North and Baltic Sea

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The harbour porpoise *(Phocoena phocoena)* is a seasonal breeding marine mammal that can be found native in the German North and Baltic Sea. The subpopulation from the central Baltic Sea was listed as critically endangered by the IUCN Red List. Like all mammals, harbour porpoises undergo distinct phases towards sexual maturity. However, knowledge about male reproductive biology such as postnatal testicular development, seminiferous tubule differentiation, age of initiation of spermatogenesis and morphological changes associated with regression and re-initiation of spermatogenesis as well as seasonal changes in testicular activity is scarce. To gain more insights in initial signs of testicular maturity, testes of male harbour porpoises of various ages that became stranded or bycaught in the years 1998–2016 were histologically investigated and divided into neonates, juveniles and adults. Within seminiferous cords/tubules of newborn and juvenile harbour porpoises only spermatogonia and Sertoli cells could be observed. Testes of adult males showed special characteristics during the course of the year. Analysis further revealed that in sexually active males different stages of spermatogenesis could be detected. Results of the present study give first insights into the regular postnatal reproductive biology of male harbour porpoises in German waters. This analysis is the baseline data for the identification of alterations in testicular development and spermatogenesis in this wild life species due to human activites and changing environmental factors. In ongoing studies, the cellular processes of seasonal changes in adult males will be investigated immunohistochemically with established Sertoli cell and germ cell markers.

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Prolonging Survival Time of Spermatozoa in the Female Genital Tract – Novel Insights

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Successful fertilization only occurs if sperm successfully survive in the female genital tract till ovulation takes place. Thus, the aim of our study was to prolong survival time of spermatozoa in the bovine female genital tract. For this purpose, we investigated sperm survival time before and after addition of specific supplements under in vitro conditions (37 oC, sperm-TALP). The effectivity of successful candidates was then evaluated under near in vivo conditions using live cell imaging in the oviduct. The following supplements were evaluated: a) protein extracts from bovine oviductal cells of cyclic cows and heifers, b) protein extracts from bovine oviductal cells by from cyclic cows after co-incubation with sperm and c) L-Arginine (0.1 mM, 1 mM, 10 mM, 15 mM, 30 mM). Our studies showed that protein extracts from oviductal cells (0.15 µg/ml) from cyclic cows were able to increase the number of motile sperm over 6 hours up to 25% as compared to control sperm incubated in sperm-TALP without supplement. Contrary there was no significant effect when using the protein extracts of heifers or after co-incubation with spermatozoa. L-Arginine (1 mM) was able to increase the number of motile sperm up to 20% and over a time interval of 8 hours under *in vitro* conditions. Live cell imaging showed that this also occurred under near in vivo conditions without affecting physiological behavior of sperm in the female genital tract. Our results show that addition of arginine and supplementation of proteins secreted from oviductal cells prolong sperm survival time in the female genital tract. This knowledge is pivotal for reducing sperm concentrations in straws and increasing commercial success in artificial insemination.

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Expression of PTGS2 and PGFS/PTGFR during Recrudescence of Canine Spermatogenesis following Downregulation with an Azagly-nafarelin Implant

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Cyclooxygenases (PTGS) and Prostaglandin-F2α synthase (PGFS) are considered to play an important role for male fertility. PTGS2 and PGFS are expressed in Leydig cells (LC) and arachidonic acid has a stimulatory effect on LC-derived testosterone production. Consequently, they are considered to be involved in regulation of Steroid acute regulatory (StAR) protein. Application of a slow release GnRH agonist implant results in a fully reversible downregulation of testicular endocrine (and germinative) function including the whole steroidogenic apparatus with StAR being the bottle neck. To further investigate the role of PTGS2 and PGFS on steroidogenesis, testicular samples were obtained from previously downregulated mature male Beagle dogs during different times of testicular recrudescence (week 0, 3, 6, 9, 12) after implant removal. Untreated adult healthy dogs (CG, n = 5) and juvenile dogs (JG, n = 3) served as controls. Expression of PTGS2, PGFS and PTGFR on mRNA level was identified in all samples, with, however, no significant differences between groups in whole testicular homogenates. Specific immunopositive staining against PTGS was found in the cytoplasm of LC and Sertoli cells (SC). For LC, staining intensity was weak in week 0 and strong in week 3; no difference was found between week 6 to 12 and CG. Whereas SC cytoplasm was most intensely stained at the basal membrane, it was equally stained from week 3 onwards. In JG, strong staining of LC and SC (adluminal) was found. Identified differences in LC-PTGS protein expression at downregulation and during recrudescence correlate with StAR protein expression and indicate a need for investigation of LC/interstitial tissue only to verify possible differences on mRNA level.

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The possible Role of the SIRT1 Enzyme in Oxidative Stress and Spindle Assembly in Bovine Oocytes – A large Animal Model for Reproductive Ageing

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Chromosomal defects and oxidative stress become much more prevalent with advanc-

ing maternal age and are considered the major factors responsible for the increased incidence of miscarriage and birth defects in women over 35 year of age. However, mechanisms that modulate the meiotic apparatus remain to be discovered. The SIRT1 enzyme deacetylates a spectrum of transcriptional regulators and can be activated by resveratrol. Therefore, the goal of our large animal model was to examine, how the SIRT1 enzyme influences oxidative stress and spindle assembly in bovine oocytes from donors of different age. COCs of prepubertal (5–6 months old), adult cows (2nd lactation) and aged cows (10th lactation) were collected by Ovum Pick-up twice a week. Medium for IVM and IVF was supplemented with 2 μM Resveratrol® (Sigma-Aldrich, Buchs, Switzerland). Standard lab protocol was used as control. SIRT1 activity was measured by using a fluorometric SIRT1 activator kit (BioVision, Milpitas, USA). The spindle assembly of oocytes was analyzed by fluorescence immunostaining under a confocal microscope (LSM510, Zeiss, Germany). Reactive oxygen species (ROS) levels were assessed in oocytes by using Dihydroethidium (Thermo Fisher, Bonn, Germany). In total 238 COCs of all 3 donor groups were analyzed. A 4-fold increase of SIRT1 enzyme activity (4 independent replicates) could be detected in oocytes of resveratrol treated groups compared to the ones out of the control groups. A high frequency of spindle defects and chromosome disorganization were observed in the oocytes of the untreated aged donor group (35.5 ± 8.7%) and a lower frequency in the resveratrol treated aged donor group (9.6 ± 3.8%, respectively 4 independent replicates). The ROS levels (12 independent replicates) were significantly lower (121 ± 34 FIU) in the resveratrol treated samples than in those of the control group (865 ± 45 FIU, $p \leq 0.05$). In conclusion, these results indicate that resveratrol is able to influence SIRT1 enzyme activity, the redox status and spindle formation in bovine oocytes of different maternal age. In this regard, SIRT1 could be a marker for reproductive ageing and useful for further understanding of human infertility caused by ageing.

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Improvement of DNA Integrity in Cryopreserved Bovine Sperm by Sodium Pyruvate

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This study aimed to assess effects of addition of the carbon source and antioxidant sodium pyruvate on sperm quality of cryopreserved bovine sperm with special attention to DNA integrity. For this purpose, in each of 23 Simmental AI bulls three ejaculates were collected. In a split sample design ejaculates were diluted by using a TRIS egg yolk extender without and with the addition of 5 mM sodium pyruvate. Both aliquots were equilibrated for 24 hours before freezing. Frozen sperm samples were thawed, and examined immediately after thawing (0 h) as well as after 3, 6, 12, and 24 h incubation at 37 °C. By using computer assisted sperm analysis (CASA) system and flow cytometric assays respectively, the percentages of rapidly motile sperm (RMS), plasma membrane and acrosome intact sperm (PMAI), sperm with high mitochondrial membrane potential (HMMP), and sperm with a high degree of DNA fragmentation (%DFI) were determined. Sperm diluted with the extender containing sodium pyruvate showed higher levels of RMS, PMAI and HMMP and lower %DFI values at all time points after thawing ($P < 0.001$) compared to sperm frozen in an extender without sodium pyruvate. The results of this study show that the addition of sodium pyruvate to the semen extender improves not only the viability, but also the DNA integrity of cryopreserved bovine sperm. Therefore, in an ongoing study we investigate the effect of sodium pyruvate on fertility of bovine sperm.

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Effect of Steroids and Luteotropic and Luteolytic Factors on Membrane Progesterone Receptors mRNA Expression in the Bovine Myometrial Cells

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We have found the variable expression of mRNA and protein for progesterone receptor membrane component (PGRMC) 1 and 2, PGRMC1 binding partner – SERBP1 (serpine 1 mRNA binding protein) and membrane progestin receptors (mPR) α, β and γ in bovine myometrium during the estrous cycle, but the regulatory mechanism of mRNA expression of these receptors in myometrium is not clear. The aim of the present study was to examine the effect of P4 (10-7;10-6;10-5M), estradiol (E2; 10-10;10-9;10-8 M), P4 together with E2 (10-6M P4/10-9M E2; 10-5M P4/10-8M E2) and oxytocin (OT;10-7M), prostaglandin (PG) E2 (10-6M), PGF2α (10-6M), tumor necrosis factor α (TNFα; 10-4M) and arachidonic acid (AA;10-5M, positive control) on gene expression for: PGRMC1, PGRMC2, mPRα, β and γ in the bovine myometrial cells (2.5×10^5/ml; n = 5) from days 6–10 and 11–16 of the estrous cycle. After 6h, the concentration of PGF2α and PGE2 in medium and mRNA expression of studied genes were determined by EIA and Real Time PCR method, respectively. Obtained data indicate that E2 (10-8M) stimulated ($P < 0.05$) expression of PGRMC1 mRNA, and P4 (10-5M) and P4 together with E2 increased ($P < 0.05$) mPRβ mRNA in the cells from 6–10 days of the estrous cycle. In the cells from 11–16 days of the estrous cycle, P4 (10-6 M) and E2 (10-10M) decreased ($P < 0.05$) only SERBP1 mRNA, but P4 in the dose of 10-5M increased ($P < 0.05$) the expression of this gene. Other factors did not affect ($P > 0.05$) the gene expression. These results suggest that steroids may regulate PGRMC1, SERBP1 and mPRβ mRNA expression in the bovine myometrial cells the day and dose-dependent effect and this way may influence on the myometrium function during bovine estrous cycle.

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A Comparison of Diagnostic Methods used for the Identification of coagulase-negative Staphylococci isolated from Cows with Mastitis

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In many countries, members of the genus Staphylococcus have now become the leading cause of intramammary infections (IMI) in cows. Apart from the major pathogen S. aureus, coagulase-negative staphylococci (CNS) are also more and more frequently isolated. In spite of the well-recognized pathogenic potential of many CNS species and their marked heterogeneity, they are frequently not identified at the species level by routine laboratory procedures. The aim of the present work was to compare various diagnostic methods used for the identification of CNS isolated from cows with IMI. The study was performed on 120 isolates of CNS recovered from milk samples collected in the years 2013–2016 in three dairy farms in Lower Silesia, Poland. The bacteria isolated were tentatively identified as CNS by colony morphology, Gram staining, catalase activity and the coagulase test. Then, 3 independent diagnostic procedures were carried out: phenotypic identification (ID 32 STAPH system, bioMérieux), genotypic identification (sequence analysis of the 16S rRNA gene and, if necessary, dnaJ gene) and MALDI-TOF-MS analysis (Bruker Daltonics, Germany). Of the methods used, the most accurate turned out to be the DNA-based ones. However, sequencing of the 16S rRNA gene alone allowed for an unequivocal identification of only 70% isolates. The remaining strains were successfully assigned to the species level by sequence analysis of the dnaJ gene. The phenotypic identification and MALDI-TOF procedure showed a lower diagnostic performance. Results of our study suggest the necessity of upgrading the existing diagnostic procedures and databases for CNS isolated from mastitis in cows.

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Production of Porcine Embryonic Chimeras by Morula Aggregation

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Chimeras have been proven as an invaluable experimental tool for studying mechanisms of embryonic development, as well as for evaluating the status of pluripotent stem cells. To optimize a chimera system based on morula aggregation using in vitro produced (IVP) pig embryos, the effect of decompaction on production efficiency and developmental potential was investigated. Furthermore, the generation of presumptive tetraploid chimeras was attempted. Compacted morulae derived from IVP parthenotes were used for the experiments. Decompaction was performed by incubation in Ca^{2+}/Mg^{2+}-free DPBS containing 0.1 mM EDTA-2Na. After that, the zonae pellucidae were removed and the intercellular junctions were further loosened by pipetting. The embryos were treated with phytohemagglutinin (150 µg/ml) and pairs of embryos were cultured individually. A pair forming a single blastocyst after 3 days of culture was assessed as complete chimera, monitored with the PrimoVision system. Presumptive tetraploid embryos were generated by electrofusion of 2-cell embryos. Most aggregations from compacted morulae resulted in two separated blastocysts, while decompaction of morulae significantly improved the formation of chimeric blastocysts (3.1%, 1/32 vs. 70.5%, 31/44; $P < 0.05$). Decompaction treatment did not affect development to blastocyst (95.5%, 84/88). Presumptive tetraploid embryos treated by decompaction efficiently formed complete chimeric blastocysts as well (85.7%, 6/7). These results indicate that decompaction treatment markedly improves the formation of porcine embryonic chimeras by morula aggregation.

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Single-cell RNA Sequencing of Bovine Day 2 and Day 3 Embryos reveals Developmental Heterogeneity and Early Differentiation Events

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RNA sequencing (RNA-seq) of pools of bovine embryos revealed major embryonic genome activation at the 8-cell stage [Graf et al., PNAS 2014; 4139–44]. The present study addressed the questions i) how heterogeneous the transcriptome profiles of early embryonic cells are; and ii) if expression profiling provides evidence for early differentiation events. Using single-cell RNA-seq, we determined the transcriptome profiles of 161 blastomeres from in vitro produced bovine embryos at Day 2 (n = 6) and Day 3 (n = 8) *post fertilization.* After removal of the zona pellucida, blastomeres were separated mechanically in Ca^{2+}- and Mg^{2+}-free PBS and lysed individually in buffer A of Prelude Dilute Lysis Module (NuGen). RNA-seq libraries were prepared using the Single Cells RNA Barcoding and Sequencing (SCRB-Seq) protocol (http://dx.doi.org/10.1101/003236). The libraries were sequenced on an Illumina HiSeq 1500 (50 nt, paired-end reads; 45000 unique molecular identifier [UMI] reads on average per library). After normalization with DESeq2, expression profiles were clustered using R package SC3 (http://dx.doi.org/10.1101/036558). Three clusters of cells were revealed: CI: 43 Day 2, 28 Day 3 cells; CII: 31 Day 3 cells; and CIII: 59 Day 3 cells. The order of these clusters was determined by computing their distances. In CI, enriched gene ontology (GO) terms were "DNA metabolic process" and "Organelle localization". In CII, but not in CIII, the most enriched GO term was "RNA processing". Our analysis suggests developmental heterogeneity in Day 3 embryos, with some delayed cells having a transcript profile of Day 2 cells. Two additional distinct clusters of cells in Day 3 embryos may indicate first differentiation events.

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Preliminary Studies on Salivary Protein Profile in Human Physiological Pregnancy and Complicated by Premature Delivery

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Easy obtainable and reasonable biomarkers of different physiological and pathological states are crucial for appropriate diagnosis. The hypothesis was stated that the analysis of salivary proteomic profile may allow for the selection of possible biomarkers of premature delivery in humans. Saliva samples were collected around 30 weeks of pregnancy from 8 patients with diagnosis of premature delivery and 8 controls with physiological course of pregnancy. Samples were subjected to 2D electrophoresis. Statistical analysis of electrophoretic gels allowed for the selection of spots of interest, which were cut from gels, decoloured and identified by mass spectrometry using MALDI TOF technique. The results of identification were compared with Swiss-Prot database. The analysis of gel pictures showed 1,393 detected spots. Out of them 59 spots were significantly different between examined samples. 32 spots expressed higher intensity of staining in premature delivery than in control samples while 27 spots showed opposite relationship. Nine spots which differed significantly were identified. Three of them expressed higher intensity of staining in premature delivery than in controls (Dedicator of cytokinesis protein 1, Metallothionein-2, Guanylyl cyclase-activating protein 1) and 6 showed opposite relationship (Epithelial-stromal interaction protein 1, Serum albumin, Tyrosine--tRNA ligase, cytoplasmic [EC:6.1.1.1], Protein chibby homolog 3, Leukemia inhibitory factor receptor, Adenosylhomocysteinase 3 [EC:3.3.1.1]). In conclusion, further research with higher number of patients is necessary in order to confirm the usefulness of saliva as the source of biomarkers of different alterations including premature delivery.

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MRI Imaging of Placenta in Feline

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Magnetic resonance imaging (MRI) has a superior soft-tissue contrast compared to other radiological imaging modalities and its structural and functional applications have led to a significant increase in performed MRI scans worldwide. Magnetic resonance imaging is considered to be safe and is not associated with risk. Nevertheless, it should be used prudently only when use is expected to answer a relevant clinical question. Magnetic resonance imaging serves as a problem-solving examination in instances where the US findings are equivocal or where additional information is needed. Animal model provides a convenient way to optimize MR protocol that is particularly important in fetus and placenta diagnostics. The aim of the study was to evaluate structure and functional parameters in feline placenta on a 3.0T MR scanner. MRI examination was performed in pregnant female cats prior to elective ovariohysterectomy. Anesthetized cat was positioned feet first, prone on the MR table. Protocols were validated with MR imaging on Discovery MR750w 3.0T. MRI examination can provide additional information about placenta function complimentary to US imaging. However feline model is challenging due to a small FOV what increases scan time, distortion, and noise level. On the other hand, the model is particularly useful for the specific study of the diagnostic methods used in humans as well as MRI is superior method for pregnancy pathologies evaluation in domestic cats in veterinary medicine.

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Long-term Exposure of Boar Spermatozoa to Seminal Plasma during In Vitro-Storage and Subsequent Thermic Stress may affect Sperm Quality

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High dilution of boar semen is associated with a reduction of seminal plasma (SP). This might restrict the reduction of sperm numbers in semen doses. The aim of this study was to examine the effect of reduced amounts of SP in semen doses with regular and low sperm numbers on sperm quality. Centrifuged semen from individual boars (n = 8) was used to produce semen doses (100 ml) in Beltsville Thawing Solution with standardized amounts of autologous SP (0.5%, 10%) and sperm numbers (1.0×10^9, 1.8×10^9). Semen doses were stored at 17°C for 144 h. Motility assessment with CASA revealed no difference between samples until 72 h storage. At 144 h, sperm motility was dramatically reduced ($P < 0.001$) in samples of half of the boars (n = 4) with 1.0 × 109 sperm in presence of 10 % SP (19 ± 9%) when compared to 0.5% SP (70 ± 7%). In samples from the other boars, motility was higher with 10% SP (84 ± 6% vs 77 ± 4%; $P < 0.01$). Samples with 10% SP contained fewer membrane intact sperm ($P < 0.05$) irrespective of the absolute sperm number. After incubation with additional thermic stress (38°C for 300 min) at 72 h storage, motility and curvilinear velocity were lower ($P < 0.05$) in samples with 10% SP compared to samples with 0.5% SP without major boar differences. In doses with 1.8×10^9 sperm, motility was 60 ± 19% (10% SP) vs 74 ± 23% (0.5% SP), while in doses with 1.0 × 109 sperm, motility was 44 ± 7% (10% SP) vs 67 ± 5% (0.5% SP). In addition, the percentage of membrane intact sperm with high mitochondrial transmembrane potential was lower ($P < 0.05$) in samples with 10% SP. In conclusion, long-term exposition of sperm to 10% SP during storage influences sperm quality in a boar-specific manner. Under thermic stress conditions in vitro the presence of 10% SP is detrimental to boar sperm quality.

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Lipopolysaccharide-induced Mastitis and Endometritis have different Effects on the Bovine Corpus Luteum

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Two studies were performed to investigate the effects of bovine mastitis and endometritis on the corpus luteum (CL). In the first study, on Day 9 of the estrous cycle (Day 1 = ovulation), 14 lactating cows received once 200 µg E. coli LPS (dissolved in 10 mL NaCL; n = 8) or 10 mL saline (n = 6) into one quarter of the mammary gland. In the second study, estrous cycles with and without intrauterine infusions of 9 mL phosphate buffered saline with LPS (3 µg/kg) every 6 h from 12 h before until 9 d after ovulation were examined in 8 heifers. In both studies, blood sampling and ultrasonography of the ovaries were performed on Days 1, 4, 8, 9, 10, 12, 15, 18, and afterwards every 2 d until ovulation. Biopsies of the CL for RT-qPCR were taken 24 h before and 6 h after intramammary LPS, and on Day 6 of cycles with and without intrauterine LPS. Repeated measures ANOVA was performed. Intramammary LPS caused severe local inflammation, an increase ($P < 0.05$) in plasma levels of cortisol and haptoglobin, but no change in those of PGF2α metabolites (PGFM) and progesterone (P4), luteal size (LTA), and luteal blood flow (LBF). Furthermore, mRNA abundance of TNFα was increased ($P < 0.05$), whereas those of TLR4 and steroidogenic STAR and 3βHSD remained similar. In contrast, intrauterine LPS induced subclinical endometritis and premature luteolysis, which was characterized by increased PGFM and decreased P4, LTA and LBF (each $P < 0.05$). Furthermore, intrauterine LPS increased mRNA expression of TLR4, decreased STAR and 3βHSD (each $P < 0.05$), and had no effect on TNFα. In summary, intramammary LPS induced systemic inflammatory reactions and alterations in luteal mRNA but no lysis of the CL, whereas intrauterine LPS induced premature luteolysis without clinical signs of inflammation.

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Epigenetic Modifiers alter the Immune Response in udder Tissues

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Mastitis accounts for large parts of antimicrobial drug use in dairy production worldwide. Due to the imminent normative to reduce the use of antimicrobial drugs in livestock, new ways for therapy and prophylaxis of mastitis are needed. Recently epigenetic regulation of inflammation by chromatin modifications has increasingly drawn attention. Currently some epigenetic modifiers have already been approved for the use in humans, however little is known about their actions in the bovine system. The aim of our study was to investigate whether three epigenetic modifiers (Vitamin D3, SAHA and S2101) influence the initial immune response towards mastitis pathogens in bovine udder tissue. Tissue explants of the teat cistern and udder parenchyma were collected from 21 cows and were preincubated in the absence and presence of epigenetic modifiers. Subsequently the tissue was stimulated with heat inactivated particles of E. coli and S. aureus. Vitamin D3 had no effect on the immune response of udder tissue *in vitro*. The epigenetic modifiers SAHA and S2101 significantly blocked the upregulation of CXCL8, TNFα, S100A9 and LAP ($P < 0.05$). However, the pathogen induced upregulation of the IL10 was not affected after treatment with SAHA and S2101. Transcript abundances for CXCL8 were validated by ELISA for IL8 and a functional chemotaxis assay. In conclusion these data show the potential of epigenetic modifiers to block overshooting inflammation in the udder. Thus epigenetic modifiers may serve in future as immune modulators in treatment and prophylaxis of clinical mastitis.

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The Population of Small RNAs in Cryopreserved Semen of Fertile and Sub-fertile Bulls

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The present study was a preliminary attempt to characterize the population of small RNA molecules in cryopreserved bovine semen, collected from bulls of diverse fertility status. For this purpose, single ejaculates from 10 mature bulls serving as sperm donors in a commercial semen production center were used. The corrected fertility index (FI) based on the non-return rates of more than 300 AI was used for the classification of the bulls in two groups: a) fertile bulls, with FI > mean FI+SD of the center's bull population, and b) sub-fertile bulls, with FI ≤ mean FI-SD of the center's bull population. From each one of the 10 frozen-thawed ejaculates, total RNA was extracted applying the standard TRIzol® (Thermo Fisher Scientific, Switzerland) protocol followed by DNase treatment. Small RNA libraries were prepared with the NEXTflex Small RNA-Seq Kit v2 (Bioo Scientific, USA) and sequenced using the Illumina high-throughput sequencing technology. After excluding sequences of rRNA, tRNA and 7SL RNA, the analysis of the data revealed 1750 unique small RNA sequences (with minimum length of 16 nucleotides) in total, with miRNA and piRNA being the most abundant classes of RNA molecules. Unique sequences with > 100 reads were mapped to species-specific precursors in the miRBase 20.0 using BLAST. Hierarchical cluster analysis showed significant differences between fertile and sub-fertile bulls regarding the frequency of the 43 most abundant RNA molecules ($P < 0.05$). Concluding, next generation sequencing revealed a wide variety of small RNA molecules in cryopreserved bovine semen. The results of this study further suggested a differentiation in the population of small RNAs in sperm collected from bulls with different fertility.

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Impaired Glucose Tolerance in Newborn Piglets Exposed to Mild Hyperglycemia *in utero*

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Elevated blood glucose during pregnancy is detrimental for the fetus as it increases the risk to develop metabolic diseases as obesity or diabetes in adulthood. However, effects of maternal diabetes and underlying mechanisms are difficult to study in humans and have not been fully clarified by animal models. Here, the effects of maternal hyperglycemia on neonatal offspring are investigated using INSC93S transgenic pigs exhibiting reduced glucose tolerance and insulin secretion as well as mild hyperglycemia. INSC93S transgenic (tg, n = 3) and wild-type (wt, n = 9) sows were artificially inseminated. Non-pregnant (np) wt sows served as controls. Fasting blood glucose was monitored weekly throughout pregnancy. Within the 3rd trimester hyperinsulinemic-euglycemic clamps and mixed-meal glucose tolerance tests were performed. At birth, wt piglets born to wt sows (wt/wt, n = 18) and wt piglets born to tg sows (wt/tg, n = 13) underwent an oral glucose tolerance test before first colostrum intake. As in humans, pregnant (p) wt sows showed a significantly reduced insulin sensitivity (–35%) compared to np-wt sows (p = 0.028), while insulin sensitivity of pregnant INSC93S transgenic sows was reduced to the same extent. Glucose tolerance was nearly, however not fully (p = 0.018) sustained in wt-p vs wt-np sows due to increased insulin secretion while pregnant INSC93S transgenic sows did not meet the increased insulin demand (p = 0.04). Fasting hyperglycemia in tg-p sows did not deteriorate throughout pregnancy. Newborn wt/wt piglets revealed unaltered body and organ weights compared to wt/tg piglets but reduced oral glucose tolerance and alterations in lipid metabolism. Metabolomics analyses will give further insight into the offspring's metabolism after exposure to mild hyperglycemia *in utero*.

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Relationships between Coefficient and Inbreeding level for Polish Warmblood Mares Participating in Stationary Performance Tests

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Inbreeding has a negative effect on the reproductive characteristics of animals. For inbred individuals, impaired fertility, reduced vitality, weaker growth and worse performance could appear. The aim of this study was to estimate the relationship and the inbreeding coefficient based on pedigree analysis of mares participating in stationary performance tests. Pedigree data from stationary riding performance tests of 1007 mares obtained in Polish training stations in 2007–2012 were combined and analyzed using INBREED procedure of the SAS package and a self-written software. The group included mares of the following breeds: Polish Warmblood (sp) (n = 469), Malopolska breed (mlp) (n = 224) and Wielkopolska breed (wlkp) (n = 314). The mean inbreed coefficients and the mean relatedness between breeds, within breeds and between training stations were determined in the population of studied mares. Mean relationships and inbreeding coefficient within the entire population of analyzed mares were 0.59% and 0.38%, respectively. An inbreeding coefficient over 10% was achieved for 4 mares undergoing stationary tests (3 mares of Wielkopolska and 1 of Malopolska breeds). In total, 76% of the mares were not inbred. In conclusion, the inbreeding coefficient in the population of half-bred mares is maintained at a low level. The obtained mean values of relationship and inbreeding coefficients can show the correctness of the method of selection of stallions.

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Impairment of the Blood-testis Barrier in a Beagle Dog Showing Intratubular Lymphocytic Infiltration

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Impairment of blood-testis barrier (BTB) integrity has been observed in inflammation, infection, trauma and experimental autoimmune orchitis, which is inducible in rodents. In the present study, an initially fertile 2-year-old Beagle dog presented a decline in total sperm number to azoospermia within 5 months, verified by twice monthly semen analyses. The dog was clinically healthy and showed normal thyroid function. Bacteriological semen examination, hematological and serum biochemical analyses resulted in physiological findings. To assay the causes of azoospermia, the dog was finally castrated. Histological examination of haematoxylin and eosin stained testicular sections and immunostaining of smooth muscle actin, claudin3, claudin11, connexin43 and vimentin were conducted to elucidate a possible impairment of the BTB. Histological examination revealed focal interstitial and intratubular lymphocytic infiltrations, an arrest of spermatogenesis at different levels, seminiferous tubules with Sertoli cell only syndrome and single giant cells. Germ cell sloughing was observed in a few tubules. There was complete absence of sperm in the epididymides. Tubules with intratubular lymphocytic infiltrations showed loss of smooth muscle actin, indicating a mechanical and functional alteration of the tubular wall. The staining patterns of the epithelial tight junction proteins claudin3/-11 and the gap junction protein connexin43 were discontinuous suggesting an alteration of the BTB at these locations, and vimentin identified somatic Sertoli cells. These findings indicate the possibility of an autoimmune orchitis leading to azoospermia. For the first time an impairment of the canine BTB and the tubular wall associated with a putative autoimmune orchitis could be demonstrated by using immunohistochemistry.

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Establishment of a Reliable Method to Generate Bovine Embryos via Intracytoplasmic Sperm Injection (ICSI)

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Intracytoplasmic sperm injection (ICSI) is a successful method of assisted reproduction technology to treat male factor infertility. In addition to its clinical usefulness, ICSI is also a valuable research tool for studying fundamental aspects of gamete interaction during fertilization. Oocyte activation is a prerequisite for decondensation of the sperm head and formation of the male pronucleus regardless of whether the spermatozoon enters the oocyte by injection or by fertilization. In cattle, additional oocyte activation is necessary for embryonic development after ICSI. However, oocyte activation might also increase the number of parthenotes. The objective of the present work was to establish a reliable method to generate bovine embryos via ICSI. Oocytes were matured for 22 h in TCM199 supplemented with BSA and eCG/hCG. ICSI was performed after denudation of cumulus-oocyte-complexes. After ICSI, the oocytes were activated by incubation in 5µM ionomycin for 5 min and subsequently cultered in TALP. The oocytes were then incubated in 2mM 6-DMAP for 3 h. Sperm-injected and control oocytes which were only activated or sham injected and activated (parthenogenetic controls) were cultured in SOF medium. After 18 h of culture embryos were stained with Hoechst 33342 to visualize pronuclear formation. First results show a correct pronucleus formation (2PN/2PB) rate of 20.8% (27/129) in the ICSI group. In the control groups, rates of chemical activation (CA) or CA and sham-injection (SHAM+CA) indicated by the generation of haploid embryos (1PN/2PB) were 51.7% (56/115, CA) and 46.7% (43/91, SHAM+CA). These data show that bovine embryos can be generated via ICSI in a reasonable number.

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Bisphenol A Induced Oxidative Damage in the Rat Testis: A possible Cause for Decline in Steroidogenesis

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Bisphenol A (BPA), an estrogenic chemical, has been shown to adversely affect human health and fertility; however, the underlying mechanisms remain unknown. The present study was done to investigate the effect of BPA on pre-pubertal male reproductive organs by histological examination and the biochemical assays of hormones and oxidative stress markers. Forty male albino rats were divided equally into 4 groups. GI was served as control and was injected SC with the vehicle (DMSO); GII, GIII and GIV were injected SC with BPA in a dose of 11.4, 57.1 and 114.2 mg/kg b.w/ day, respectively. The severity of the BPA effects observed was dose dependent, SC injection of BPA (57.1 and 114.2 mg/kg b.w/day) significantly decreased the levels of testosterone and LH in comparison with controls and significantly increased the oxidative stress markers in the testis. The histopathological changes observed in the testis treated with BPA demonstrated its potentials to induce cytotoxic and endocrine disrupting effects on the spermatogenic, Sertoli and Leydig cells. The amount of sperm in the epididymis was decreased in GIII and there were no sperm in the epididymis in GIV. These results indicate that exposure to BPA induces oxidative damage in the rat testis and impairs steroidogenesis which may lead to male infertility.

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Impact of Metabolic Status and Genetic Merit for Fertility on Proteomic Composition of Bovine Oviduct Fluid

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Over the last decades, a decrease of dairy cow fertility, comprising early embryonic loss, has been observed. To study the effects of metabolic status as well as genetic merit for fertility of dairy cattle on oocytes, embryos, and their maternal environment, two animal models have been created by the EU consortium FECUND. The "metabolic disturbance model" included lactating cows (Lact) that were compared with cows dried off after calving (Dry) and maiden heifers (MH). The "genetic merit model" involved Holstein heifers with low (LFH) and high fertility (HFH) index and heifers from the Montbéliarde (MBD) breed. Providing the microenvironment for oocyte, sperm and early embryo, oviduct fluid (OF) plays a pivotal role for fertilization and early embryo development. We performed a holistic proteome analysis of OF samples from ampulla and isthmic regions of both models using nano-LC-MS/MS analysis and a label-free quantification approach. Substantial quantitative differences among the OF proteomes were detected. Among the 2,240 proteins identified, a total of 216 differed significantly in pairwise comparisons. The majority of proteome differences was found in isthmus OF samples of MBD vs. LFH (52 proteins), MBD vs. HFH (47), and MH vs. Lact (19). Evaluation of this dataset using bioinformatic tools revealed that affected proteins are predominantly assigned to the GO terms translation, immune response and cytoskeletal protein binding. Therefore, the dataset comprises a pool of proteome changes associated with fertility, supporting the assumption that immune processes are involved in early embryonic loss.

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Genetically Selected Cows differ in Periparturient Clinical Performance

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High incidences of periparturient diseases in dairy cows have a negative impact on economic efficiency, frequency of antibiotic treatment and animal welfare. Genetic selection for disease resistance is a sustainable tool for improving dairy farming. The aim of our study was to evaluate uterine involution and diseases (incidence and severity) in Holstein heifers having genetically been selected via single nucleotide polymorphism typing for alternative parental chromosome 18 haplotypes associated with favourable (Q) or unfavourable (q) udder health. Heifers (n = 36, 18Q/18q) originating from different German farms were kept in individual pens at the Clinic for Cattle in Hanover and supervised from day (d) 259 post insemination (p.i.), around calving until slaughter on d39 ± 4 postpartum (p.p.). Health status was monitored daily, rectal palpation and transrectal ultrasonography was performed once a week p.p. In case of disease, animals were treated according to good veterinary practice. Occurrence of retained fetal membranes, metritis grade I and II were recorded. Comparisons between groups were performed using Chi-squared test and unpaired t-test. No differences between groups were detected concerning day of calving p.i. (Q: d278.6 ± 3.1 vs. q: d275.7 ± 7.8), day p.p. when uterus was detectable in the pelvic cavity via transrectal palpation (Q: d19 ± 6.7 vs. q: d20 ± 3.8), incidence of retained fetal membranes ($P > 0.1$) or uterine weight at day of slaughter (Q: 0.669 ± 0.095 kg vs. q: 0.622 ± 0.123 kg). However, Q-heifers showed reduced incidence of metritis grade I and II compared to q-heifers ($P = 0.006$). The causal relationship between genotype and immune competence needs further investigation. However, genetic selection might be profitable for breeding cows with better periparturient clinical performance.

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The Impact of Nutritional Status on Histotroph Composition during the Periconceptional Period in Mares

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Embryo development in the horse is unique with respect to its prolonged preimplantation period. Prior to attachment, the uterine luminal fluid or so-called histotroph is the embryo's sole source of nutrients. It can be directly altered by nutrition and may serve for the identification of metabolic biomarkers during the periconceptional period. We aimed at determining the influence of maternal nutritional status on histotroph composition. Ten mares were selected from a herd of broodmares at the research station in Chamberet, France, and assigned to 2 groups (n = 5/group) based on body condition score (BCS, INRA-HN-IE 1997 scale) and subcutaneous fat thickness (FT) (FT_BCS = [FT*BCS]/[FT+BCS]), as introduced by [Superchi et al., 2014]). The normal and overweight group had a FT_BCS of 0.87–1.07 and 1.09–1.26, respectively ($P = 0.01$). Plasma and uterine fluid were collected on day 7–8 after ovulation. In both, obesity related hormones (leptin, adiponectin, insulin) were determined by RIA. LC-MS was used to characterize the metabolic profile and GC-MS for the fatty acid content. There was no effect of FT_BCS on hormones. There was a trend ($P = 0.07$) towards higher fatty acid concentration in plasma of animals with a higher FT_BCS, with conjugated linoleic acid being significantly different (2.9 ± 0.5 vs 3.5 ± 0.3 µg/g, $P \leq 0.05$). Plasma analysis showed differential abundance ($P \leq 0.05$) of 34 metabolites involved in e.g. energy metabolism, cell signaling and energy storage, compared to 17 differentially metabolites in histotroph, with an overlap of 2. Our results suggest that nutritional status impacts plasma and histotroph composition via different pathways. FT_BCS effects on the histotroph in which the embryo develops demand further investigation.

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Motility of Stallion Epididymal Sperm after 48 hours of Cooled Storage using two different Extenders

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Collection of epididymal sperm can be a last chance to preserve genetic material of valuable breeding stallions. Twenty epididymides were collected after routine castration of macroscopically normal gonads from ten healthy, 2.5 years old stallions of different breeds. Sperm from the cauda epididymidis were collected and diluted to 25×10^6 sperm/ml with Equi Plus (group 1) or Gent extender (group 2) for chilled semen (Minitüb, Tiefenbach, Germany). Total motility (TMOT, %), progressive motility (PMOT, %), curvilinear velocity (VCL, µm/s), average lateral head displacement (ALH, µm) and beat cross frequency (BCF, Hz) were evaluated. Differences were evaluated by Wilcoxon signed rank test and p-value < 0.05 was considered significant. In group 1, TMOT (61 [19–87] and 54 [24–79]), PMOT (57 [15–86] and 50 [19–78]), and VCL (145.2 [119.8–174.1] and 143.4 [122.1–160.1], p > 0.05) did not differ between H0 and H48, respectively, while ALH increased [3.2 (2.6-3.8) and 3.5 (3.0-4.0), p < 0.05] and BCF decreased (37.9 [33.1–43.0] and 32.2 [27.3–37.5], p < 0.05). In group 2 TMOT (24 [7–76] and 21 [6–48]), PMOT (16 [2–61] and 13 [2–40]), and BCF (32.5 [27.3–36.7] and 30.9 [27.7–34.4] for H0 and H48, respectively) did not differ among time points while VCL (165.4 [118.5–182.0] and 137.4 [94.6–168.1]) and ALH (3.9 [3.1–4.5] and 3.4 [2.8–4.0]) were higher at H0 than at H48, respectively. At H0, all motion characteristics differed between the 2 groups, whereas at H48, TMOT and PMOT differed between the two groups, but none of the kinematic parameters. While motility did not change over 48 hours of cooled storage using either extender, kinematic values decreased except from ALH in group 1, which increased. Concerning motility, the egg yolk containing extender appeared not ideal.

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Development of a Flow Cytometry Assay to Assess Bacterial Count in Boar Semen

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The aim of the study was to develop a new flow cytometry assay for the determination of the bacterial count in commercially processed boar semen. In total 224 fresh boar semen samples collected and processed at an AI Station were analyzed. The number of live bacteria was determined by using flow cytometry 15 minutes after staining with SYBR Green I and Propidium Iodide. In the first part of the study 111 fresh boar semen samples were spiked with pure cultures with defined numbers of bacteria commonly detected in boar ejaculates *(Staphylococcus, Streptococcus, Aeromonas, Pseudomonas, Proteus, Klebsiella, E. coli)* and analyzed on the day of sperm collection. A strong correlation between the measured amounts and the expected numbers could be observed (p < 0.01, Pearson correlation coefficient = 0.97). In the second part of the study 113 fresh boar semen samples were analyzed. On the day of collection the samples were analyzed via flow cytometry and the serial dilution cultures for the Most Probable Number (MPN) technique were prepared and subsequently assessed after 48 hours of incubation. The bacterial counts determined with both methods were only moderately correlated (p < 0.01, Spearman's rho = 0.28) with higher values detected by the MPN method (MPN: 145,923 ± 300,921 bacteria/ml; flow cytometry: 37,549 ± 59,538 bacteria/ml). In summary flow cytometry is a suitable and fast method to determine bacterial count in boar semen. Differences in the results of both techniques may be related to the time of measurement. While with the flow cytometric method the bacterial count is measured directly after collection of semen, the classical microbiological culture techniques are evaluated 48 hours later.

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Preservation of Sperm Chromatin during Dried Storage

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Sperm chromatin structure and level of condensation determine accessibility for damage, and hence success of fertilization and development. Subjecting sperm to preservation protocols and storage may cause oxidative stress and DNA damage. The aim of the current study was to evaluate stallion sperm chromatin structural stability, for diluted semen maintained at 37°C as well as after freeze-drying and dried storage. Sperm chromatin structure was evaluated using various assays on the same samples. Chromatin structure of sperm diluted in skim milk extender rapidly degraded during storage at 37°C. DNA fragmentation index (DFI) values increased from 11 ± 3 to 77 ± 11% after 24 h storage. An increase in DFI-values correlated with a decrease in halo sizes observed with the sperm chromatin dispersion assay, and an increase in comet tail length observed with single cell gel electrophoresis. Despite that no membrane intact sperm were recovered after freeze-drying, sperm chromatin of freeze-dried sperm was found to be largely intact (DFI-values: 5–8%). Sperm freeze-dried in TRIS+ without supplements exhibited rapid changes in chromatin structure during dried storage at 37°C, reaching DFI values of 68 ± 33 and 95 ± 5% after 2 and 4 weeks storage, respectively. Also, sperm freeze-dried in skim milk extender or TRIS+ supplemented with glucose/albumin were found to have DFI-values of 83–65% after 1 month storage. However, if freeze-drying was done in TRIS+ supplemented with disaccharides with or without albumin DFI-values remained 9–11%. Taken together, sperm chromatin structure can be preserved during long-term dried storage at elevated temperatures, after freeze-drying with non-reducing sugars such as sucrose or trehalose.

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Mammary Squamous-Cell Carcinoma (SCC) in the Mare

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Mammary tumors are common in dogs and cats but are very rare in other domestic animals, especially in mares with the exception of a few reported cases of carcinomas. The aim of this case report is to describe the clinical, cytological and microscopic findings of a mammary squamous-cell carcinoma (SCC) in an 18-year-old, grey, thoroughbred mare. A clinical examination was conducted 10 days after parturition, demonstrating cachexia, depression and dehydration associated with the SCC. The right halves of the mammary gland demonstrated a firm enlargement and purulent discharge. Oedema of the ventral abdomen and right hind limbs was present. The metastasis to the regional lymph nodes, vulva and vestibule vaginae were observed and locally aggressive biological behavior of the tumor was confirmed. Cytological evaluation of the mammary secretion revealed the prevalence of degenerated neutrophils, macrophages, giant cells and no bacteria. Part of the cells demonstrated pyknosis and karyolysis, the others indicated polymorphism, anisokariosis and atypia. After euthanasia, samples were collected from mammary tissue and vulva and were fixed for histology and immunofluorescence (IF). The sample sections were stained with HE, labeled with antibodies (anti-prolactine receptor [PRL R]), anti-neprilysin (CD10, anti-vascular endothelial growth factor [VEGF]) and linked with Hoechst, then imaged using light and confocal microscopy. SCC was confirmed histologically. CD10 and VEGF reactivity were localized on either stromal and neoplastic cells bodies. Epithelial cell cytoplasm was PRL R immuno-posi-

tive, while stroma cells were mostly negative. Semi-quantitative research is needed to evaluate differences between SCC and unaffected equine mammary gland tissue.

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Testicular Biopsy as a Diagnostic Tool in Sub-fertile Bulls

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Genomic selection in cattle resulted in the need for Artificial Insemination centers to supply their breeders with semen of young bulls, which are often just yearlings. If such a young bull fails to provide satisfactory semen a great interest arises to assess possible testicular defects. Clinical and ultrasound examination can provide some information regarding the testicular status. Histological evaluation of the testicular parenchyma adds further information. However, it should be without detrimental effects on sperm production to allow future sperm harvesting. Preliminary results of the evaluation of possible detrimental effects of testicular biopsy in bulls were presented. Biopsies were collected from 8 young sub-fertile bulls of different breeds (13–27 months old), housed and biopsied at their respective home station. At the puncture site a local anesthesia was administered subcutaneously and an introducer needle was inserted percutaneously into the testis. Through this needle a 14 gauge biopsy instrument was introduced and a biopsy was collected. Samples were fixed in Bouin's solution, embedded in paraffin and stained with Hematoxylin-Eosin. Later, testes from the bulls were retrieved from abattoir and prepared for histological evaluation. Bulls mounted within 1 week after biopsy again and produced further semen. In one bull the appearance of free fluid within the scrotum was reported. Within the testis of this bull a hematoma was evident after slaughter. In other bulls the biopsy sites were only visible as a scar on the tunica vaginalis or small adhesions between tunica vaginalis and testis. To conclude, a 14-gauge testicular biopsy for evaluation of spermatogenesis can be performed in bulls with little adverse effects on testicular integrity. This project is funded by the FBF.

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Bovine Salpingitis – Effects on Oviductal Microarchitecture and Fertility

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The oviduct is pivotal for successful reproduction. The effects of inflammatory alterations in the oviduct on reproductive success are largely unknown. Therefore, the aim of our study was to elucidate the effects of inflammation on sperm and oocyte transport and gameto-maternal interaction. For this purpose, oviducts revealing mild (n = 35), moderate (n = 30) and severe (n = 22) inflammation were removed from cows immediately after slaughter. Specimens were investigated using live cell imaging, stereomicroscopy, histomorphology and histochemistry. Our studies showed that 70% of cows affected by uterine inflammation exhibited infection in the oviducts. Moderate and severe inflammation caused a significant increase in the thickness of tubal folds (Anova *post hoc* Dunnett`s test, $p < 0.05$). Severe inflammation was characterized by luminal accumulations of glycoproteins, increased apoptosis, loss of tight junctions, and shedding of epithelial cells. Particle transport speed (PTS) was 31.3% (± 19.2 µm/s) reduced in oviducts with salpingitis as compared to controls. The coefficient of variation of the PTS was 11.5 times higher in inflamed oviducts as compared to the controls (Mann-Whitney-U-test, $p < 0.05$). Spermatozoa were stuck in mucus preventing them to form a sperm reservoir resulting in reduced survival time and loss of fertilizing capacity. Our results imply that inflammation of the oviduct has a major negative impact on bovine reproductive success by compromising oocyte and embryo transport and by reducing survival time and fertilizing capacity of sperm. Thus, novel concepts for improving success rates of assisted reproduction have to implement the use of biomarkers for diagnosis and specific medications for treatment of salpingitis.

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Uterine and Oviduct Activity During Early Pregnancy in Sows Registered by Telemetry Method

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The aim of the present study was to record the myoelectric activity of isthmus and ampulla parts of oviduct and uterine horn in sows during the estrous phase and early pregnancy period via telemetry recording system. A total of 8 non-pregnant pigs surgically fitted by TL10M3-D70-EEE implants positioned between the abdominal muscles, and 3 silicone electrodes sutured on the left oviduct (isthmus and ampulla) and the ipsilateral uterine horn and the bioelectrical activity of organs was recorded. The blood samples were collected in order to monitor P4 and LH levels. The estrous cycle was synchronized with eCG and hCG and animals were subject to artificial insemination (AI) 24 and 48 hrs after gonadotropin injections. After that period the sows were slaughtered, electrodes were checked for their position and the reproductive system gently flushed for embryo collection. Examination of reproduction tract carried out post mortem indicated that telemetry recording system and material (bipolar electrodes) did not have a negative influence on related tissues. The duration of activity periods was significant higher in the isthmus and ampulla during early pregnancy than those detected in estrus phase ($P < 0.0001$). In the uterus, no significant difference ($P > 0.05$) was observed in the mean duration activity during estrus and early pregnancy periods. Analysis of oviduct and uterus electromyography (EMG) activity in sows in estrous phase as well as early stages of pregnancy explicitly suggests that telemetry makes it possible to obtain results in vivo enabling assessment of myoelectrical activity of parts of reproductive system which were the focus of this study.

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The Evaluation of Placental Function in first 105 days of Gestation using 17beta-estradiol and PAG Concentrations in Dairy Cattle

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The pregnancy estrogen level of fetal origin is much higher than that of ovarian origin, which makes it possible to use this parameter for assessing the status of the embryo and the placenta. It was also demonstrated that the concentration of PAG's, secreted by the trophoblast cells is proportional to the mass of the placenta and therefore can be an indirect method of the function of trophoblast assessment in the first trimester of pregnancy. The data analyzed were derived from 50 multiparous dairy cows. Animals were divided into 3 groups: pregnant (n = 19), non-pregnant (n = 11) and late embryonic mortality (n = 10). Transrectal ultrasonographic examination, blood and urine samples were obtained 105 days of pregnancy. RIA method was used to determine PAG concentration in blood, milk and urine and to determine E2 concentration in plasma. In the performed analysis, a statistical significant positive correlation (Sr + = 0.73) between E2 in blood and PAG concentration in plasma was found. There was no significant correlation between E2 concentrations and PAG in other materials, milk and urine of P and NP group. Confirmed correlation of those 2 factors with

placenta function is also evident in the LEM group. The existence of statistically significant differences between the groups was confirmed. The same results were obtained between PAG concentration in the groups of LE and HE in blood, milk and urine. The greatest variations in the concentrations of PAG between groups NE and HE were measured from blood ($p < 0.0001$), then urine ($p < 0.001$) and in milk ($p < 0.01$). Based on obtained results in 40% of cows diagnosed with LEM late embryonic mortality were observed disorder of the secretory function of the placenta resulting in impaired secretion of PAG molecules and E2.

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Expression of CBE1 (ciliated bronchial epithelium 1) in Human Spermatogenesis

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CBE1 (ciliated bronchial epithelium 1), which was described related to motile (brain, lung, testis) and sensory ciliated cells (kidney, heart), is a largely uncharacterized protein. Expression databases show that CBE1 is highly testis-enriched in amniotes. To analyse the precise cellular localization in the human testis, we performed immunohistochemistry, in situ hybridization and RT-(q)PCR to detect CBE1 protein and mRNA in testicular biopsies showing normal and impaired spermatogenesis such as an arrest at the level of spermatocytes and round spermatids. In normal spermatogenesis, CBE1 mRNA is expressed in pachytene primary spermatocytes and the protein is localized within the flagellum of elongating spermatids from step 5 onwards. Immune-electron microscopy showed CBE1 clearly associated with microtubules in the flagellum and at a lower level at the manchette. The mRNA expression is significantly reduced in samples with an arrest of spermatogenesis at level of round spermatids compared to normal spermatogenesis. Oligozoospermic patients with immotile spermatozoa and severe hypospermatogenesis show reduction of mRNA expression and almost immuno-negative spermatids indicating an important role of CBE1 in the development of sperm motility. Further studies in testicular biopsies showing impaired spermatogenesis and ejaculates of patients with diverse clinical manifestations will clarify the possible causal relationship of CBE1 to male infertility.

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The Testicular Expression of KATNB1 during Human Spermatogenesis

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Katanin, a microtubule-severing protein, consists of an enzymatic p60 (KATNA1) and a regulatory p80 subunit (KATNB1). A missense mutation in the highly conserved WD40 domain of the Katnb1 gene causes defects in spermatogenesis (Taily mouse). Homozygous Taily males are infertile due to a decreased sperm production, sperms with abnormal head shape and an absence of progressive motility. This phenotype is similar to the human oligoasthenoteratozoospermia syndrome (OAT). Therefore, we analyzed the expression pattern of the regulatory p80 subunit during human spermatogenesis on mRNA (RT-PCR, RT-qPCR and *in situ* hybridization) and protein level (immunohistochemistry). We observed that KATNB1 mRNA is exclusively expressed in germ cells. In normal spermatogenesis, mRNA is localized in pachytene primary spermatocytes. KATNB1 is quantitatively reduced in maturation arrests at level of primary spermatocytes and spermatogonia. The KATNB1 protein was detected in type B spermatogonia and co-localized with Golgin A2 in the Golgi complex of pachytene spermatocytes. Additionally, KATNB1 is co-localized with pericentrin in the cleaving centrosome immediately before the first meiotic division (stage 5). The p80 subunit was also detected in early round spermatids in the dictyosome above the acrosomal cap. The expression and localization of KATNB1 support its role in spindle formation as well as in formation of microtubule-based structures during spermiogenesis. These data are consistent with the demonstrated role of KATNB1 in mouse spermatogenesis and suggest the strong conservation of function even between distantly related species.

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Comparative Transcriptome Analysis of Bovine Uninucleated Trophoblast Cells and Trophoblast Giant Cells

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In the bovine placenta, intimate feto-maternal contact is restricted to multiple discrete placentomes, which are composed of the fetal cotyledon and the maternal caruncle. Weakly invasive binucleated trophoblast giant cells (TGCs) permanently fuse with individual caruncular epithelial cells to form short-lived trinucleated feto-maternal hybrid cells. TGCs are continuously regenerated from uninucleated trophoblast cells (UTCs). Aim of this work was to identify transcripts which are associated with TGC differentiation. To this end, UTCs and TGCs were obtained from 3 bovine placentas (days 118–130 of gestation) using an optimized fluorescence activated cell sorting procedure. For transcriptome analysis, total RNA from UTC and TGC pools was labeled and hybridized to Affymetrix Bovine Gene 1.0 ST Arrays. Lists of differentially expressed genes (DEGs, n = 2452) and genes that were expressed only in UTCs (n = 682) or TGCs (n = 780) were derived from the microarray data sets and subjected to gene ontology (GO) term enrichment analysis using DAVID software. Our analyses revealed that DEGs were most significantly associated with biological processes including "intracellular signaling cascade", "extracellular matrix organization" and "regulation of cell migration". Genes that were expressed only in UTCs did not reveal remarkable GO terms or pathways. Interestingly, however, of 780 genes expressed only in TGCs, 110 genes were associated with the GO term "G-protein coupled receptor protein signaling pathway" and 72 genes with the GO term "olfactory receptor activity". This suggests that chemotaxis mediated by olfactory receptors may be important for TGCs.

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Testicular Volumetry using a Caliper, two-dimensional (2D)-, and three-dimensional (3D)-Sonography to determine Daily Sperm Output (DSO) in the Stallion

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Measurement of testes size is an important part of the stallion breeding soundness evaluation since testes size is associated with sperm production. The number of sperm produced by the stallion may impact fertility and, thus, the number of mares that a stallion can breed. Thus, accurate prediction of daily sperm output (DSO) is thought to pro-

vide meaningful information for reproductive management of breeding stallions. Measuring the size of the testes can be performed either by traditional orchidometry using a caliper to measure scrotal dimensions (length, height, width) or by measuring testicular dimensions using 2D-ultrasound imaging. Estimation of DSO is calculated based on total testicular volume (TTV) derived from testicular dimensions (4/3π [L/2] [W/2] [H/2]) and in conjunct with a formula for maximal (0.024[VolL+VolR]–0.76) and minimal expected DSO (0.024[VolL+VolR]–1.26). We hypothesize, that 3D testicular sonography allows a more precise measurement of testicular volume than caliper and 2D. Testicular dimensions from 52 breeding stallions were obtained by caliper and 2D. 3D-scans were analyzed by VOCAL-software to determine testicular volume. Values for DSO were collected from semen records. TTV showed significant correlations between methods (3D vs 2D, $r = 0{,}75$; $p < 0.001$, 3D vs caliper $r = 0{,}63$; $p < 0.001$, 2D vs caliper $r = 0{,}56$; $p < 0.001$). Correlation of TTV and DSO was high for 2D ($r = 0{,}639$; $p < 0.001$), and for 3D ($r = 0{,}604$; $p < 0.001$), and moderate for the caliper technique ($r = 0{,}46$; $p < 0.01$). Sonographic testicular volumetry measures are more accurate and can be done quicker than the caliper technique; furthermore, the parenchyma can be directly visualized, and specific parenchymal landmarks can be better paced by 3D vs 2D.

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Neoplasia in Human Testis: Evidence of Functional Polarization of Macrophages and Dendritic Cells

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Introduction In human testicular germ cell neoplasia, i.e. GCNIS and seminoma, infiltrating immune cells are frequently found. These immune cells have previously been identified as lymphocytes (T- and B-cells), macrophages and dendritic cells. We analysed the subpopulation of macrophages (M) and dendritic cells (DC) including respective cytokine and chemokine expression in order to further insights in the regulation of tumour growth.

Material/Method We evaluated Bouin-fixed, paraffin-embedded tissue samples from seminoma (n = 10), preinvasive germ cell neoplasia in situ (GCNIS, n = 10) and normal spermatogenesis (Nsp, n = 10) by immunohistochemistry and immunofluorescence using antibodies against CD11, CD68 (M1), CD163, CD206 (M2), CD1c, CD11c (mDc1), CD11c, CD141 (mDC2) and CD123, CD303, CD304 (pDC). Transcripts encoding relevant chemokines (CCL15, CCL2, CCL5, CCL18, CCL22) and cytokines (TGF-β, IL-6) were evaluated by qRT-PCR from cryopreserved tissue samples.

Results Compared to Nsp we found high numbers of M1 and M2-polarized macrophages as well as pDCs and mDCs in GCNIS and seminoma. We also detected an upregulation of chemokines (CCL2, CCL5, CCL18, CCL22) and cytokines (TGF-β, IL-6), but a downregulation of CCL15 chemokine.

Conclusion Our data suggest that in contrast to Nsp, tumor tissue shows a high expression of immune cell attracting chemokine pattern which differs from other tumours and cytokines being important for the recruitment and differentiation of immune cells. Most interestingly, the downregulation of CCL15 immune cell attracting chemokine in testis neoplasia might indicate a functional importance of CCL15 in normal testis tissue, which still has to be evaluated.

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Efficacy of Transvaginal Ultrasound-guided Twin Reduction in the Mare is Dependent on Age and Day of Gestation

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In mares, advanced twin pregnancies result in abortion, stillbirth or delivery of non-viable or severely compromised foals in most of the cases. Moreover mares frequently suffer from dystocia and fetal membrane retention causing low fertility in the following breeding season. Therefore reducing twin pregnancies to a singleton pregnancy is recommended. Twin reduction is more successful the earlier it is performed in gestation. Transvaginal ultrasound-guided aspiration (TUA) of amniotic fluid is the method of choice to obtain a singleton pregnancy before the fetal phase if twin pregnancies persist after day 25 of gestation. During 2 following breeding seasons, 58 TUAs were performed between day 30 and 49 of gestation in 3–21 year old mares. Success was defined as a singleton pregnancy diagnosed 4 weeks after TUA. The aim of the present study was to evaluate the effects of the mare's age and the day of gestation on outcomes. A singleton pregnancy was found for 67% of the cases after TUA. Success rates were 91% in case mares were younger than 7 years, whereas this decreased down to 72% and 44% for mares aged 8–15 and older than 15 years, respectively. Twin reductions performed days 30–35 of gestation showed success rates of 76%, while this number decreased to 62% if done days 36–40 and 38% for days 40–49. For mares younger than 7 years, the TUA efficacy was not affected by the day of gestation. In contrast, for older mares TUAs were less successful with increasing gestation age. Mares aged 8–15, showed a significant decrease in the success rate if done after day 35 of gestation. In the group of mares older than 15 years, performing TUA after day 40 resulted in total pregnancy loss.

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Effect of Exogenous Estradiol Benzoate Administration on Uterine Blood Flow in Postpartum Dairy Cows by Transrectal Doppler Ultrasonography

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The objective of this study was to quantify the uterine blood flow after estradiol benzoate administration in dairy cows by transrectal Doppler sonography. Six Holstein-Friesian cows were subjected for daily examination for 10 successive days starting from 4th week *post partum* (PP). All cows were examined clinically for vaginal mucous scoring, endometrial cytology and Doppler transrectal ultrasonography to quantify the blood flow to the uterine arteries ipsilateral and contralateral to the previously pregnant uterine horn. At the 3rd day of experiment, healthy cows administered with intramuscular injection of 10 mg Estradiol benzoate. Blood samples were collected for the assessment of estradiol-17β (E2) concentrations. At each examination, pulsatility index (PI), resistance index (RI), time average maximum velocity (TAMAX), blood flow volume (BFV) and uterine arteries diameter were recorded. The PI and RI indices decreased while the TAMAX velocity, BFV and diameter of ipsilateral and contralateral uterine arteries increased significantly in response to E2 treatment. There was a high correlation between both the ipsilateral and contralateral uterine arteries in all studied parameters ($R = 0.860$, $p < 0.0001$, $R = 0.922$, $p < 0.0001$, $R = 0.651$, $p < 0.0001$, $R = 0.879$, $p < 0.0001$, $R = 0.861$, $p < 0.0001$ for the PI, RI, TAMAX, BFV and uterine arteries diameter, respectively). It can be concluded that the higher levels of estrogen may be responsible for the higher time average maximum and blood flow velocities, increased diameters and decreased pulsatility and resistance indices of the uterine arteries during the reproductive cycle of the non-pregnant dairy cows.

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Effect of Stage and Quality of Biopsied Bovine Embryos obtained by Repeated Superovulation on Developmental Rates after Conventional Freezing

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Procedures for freezing biopsied bovine embryos are of critical importance for breeding programs involving preimplantation embryo genetic screening. The aim of this study was to evaluate the effect of embryo stage and quality on post-thaw *in vitro* development of biopsied embryos conventionally frozen in 1.5 M EG. All embryos were collected from one Simmental cow repeatedly superovulated (n = 7) at intervals of 5–8 weeks. Briefly, the animal received a PRID-alpha® at a random stage of the estrous cycle (d0) and was superstimulated with FSH (Pluset®) administered twice daily in decreasing doses from d 5–8. Cloprostenol (Estrumate®) was administered on d7/d8 and PRID® removed on d 8. Fixed-time AI was performed 36, 48 and 60 h after PRID® removal. Embryos were collected by flushing 7 d after 1st AI. Morulae (M), early blastocysts (EB) and blastocysts (B) evaluated by morphological criteria (IETS) as grade 1 (G1) and 2 (G2), were biopsied with a microblade in order to separate 5–10 blastomeres to provide sufficient DNA for analysis. Biopsied embryos were cultivated for 24 h in SOF+ Estrus cow serum (ECS) to blastocysts before freezing. After thawing, the embryos were cultivated for 48 h in SOF+ECS to evaluate re-expansion and development. Data were analyzed with a general linear model (Proc GLM, SAS). A total of 142 ($\bar{x}$ = 20.3) ova/embryos were recovered of which 120 ($\bar{x}$ = 17.1) were fertilized and 107 ($\bar{x}$ = 15.3) evaluated as viable. From the 80 biopsied embryos, 33 were M (20 G1 and 13 G2), 27 EB G1 and 20 B G1. Frozen-thawed biopsied G1 M, EB and B had similar development (80.0%, 85.2% and 65.0%; $p > 0.10$). However G2 M resulted in lower development (7.7%; $p < 0.001$) compared to G1 M. Results indicate that conventional freezing is suitable for biopsied G1 M, EB and B.

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Variation of Genomic estimated Breeding Values within Genotyped Full-sibs of Fleckvieh bovine Embryos

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In cattle, the interest of genotyping embryos for selection on the basis of their genomic estimated breeding values increased in many countries in the last years. The genomic total merit index (gTMI) represents the overall breeding value of the embryo. ET and OPU/IVP enable to provide more embryos for selection, and now available embryo genotyping tools allow to select the best candidate before the transfer to recipients. In the present study the variation of gTMI within full-sibs morulae and blastocysts recovered on d 7 from the uterus of 2 FSH-superstimulated German Fleckvieh donor cows inseminated with elite bulls was examined. Embryos of two donors (D1, 9 embryos; D2, 11 embryos) were biopsied in isotonic NaCl solution supplemented with 1.5% PVP without BSA (biopsy medium) by separating 5–10 blastomeres with a microblade to provide sufficient DNA for amplification and subsequent embryo genotyping without compromising their developmental potential. Each biopsy was transferred directly into the assay tube containing 2.5 µl TE-buffer. Amplified DNA (REPLI-g® Mini Kit, Qiagen) was sent to GeneControl GmbH (Grub, Germany) for SNP analysis using Illumina 50K chip technology. The gTMI from dam/sire were 111/125 and 85/107 for D1 and D2, respectively. Call rates within the embryos ranged for D1 from 0.874–0.939 ($\bar{x}$ = 0.898) and D2 from 0.858–0.967 ($\bar{x}$ = 0.915). The gTMIs of the embryos ranged from 110–120 and 89–104 within D1 and D2, respectively. Results demonstrate that bovine d7 morulae and blastocysts can be successfully screened and selected for breeding on the basis of their genomic estimated breeding values.

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Methylation of Progesterone Receptor Isoform A and B Promoters in the Reproductive System of Cows

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Progesterone receptor (PGR) isoform B (PGRB) acts as a potent activator of genes that are dependent on progesterone (P4), whereas isoform A (PGRA) is a weak activator of these genes. When both isoforms are expressed in a cell, PGRA acts as an inhibitor of PGRB, thereby reducing the effects of P4. Therefore the aim of this study was to check if the promoters of both isoforms can be methylated and to determine the percentage of the methylation of each receptor isoform. The studied tissues were corpora lutea (CL) and endometrial slices from cows on days 1–5, 6–10, 11–16, and 17–20 of the estrous cycle. Isolated genomic DNA was bisulfite converted and amplified using methyl specific PCR with primers for methylated and unmethylated sequences. Determination of the percentage of the methylation was carried out using HpaII and Mspl, a pair of restriction enzymes with different sensitivity to methylation at a specific locus. Agarose gel electrophoresis of methyl specific PCR showed partial methylation of PGRA and PGRB promoters in CL and endometrium during the estrous cycle. The percentage of methylation was higher for PGRA than for PGRB promoter in CL about 2.5–3 times ($P < 0.001$–0.0001) and 0.7–2.5 times in endometrium ($P < 0.05$–0.01) during the estrous cycle. Obtained data may indicate that higher promoter methylation of PGRA isoform can be a mechanism for regulation of PGRA inhibitory activity against PGRB and this way influence the regulation the P4 action in CL and endometrium.

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Prostaglandin E2 (PGE2) is a Testicular Peritubular Cell-derived Factor involved in Human Testicular Homeostasis

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In the testis, smooth muscle-like peritubular cells build the wall of seminiferous tubules. Transport of immotile sperm to the rete testis has been regarded as their major role. We have established a culture procedure for human testicular peritubular cells (HTPCs) and a subsequent proteome-analysis of HTPCs has revealed among others cyclooxygenase 1 (COX1) and 2 PGE-synthases (PTGES2 and 3). This implies that HTPCs are a source of PGE2, which consequently may be a physiological factor in the human testis. Western blots confirmed that HTPCs express COX1 and ELISA-measurements showed that they constitutively secrete PGE2. Indomethacin, a prototype non-steroidal anti-inflammatory drug (NSAID), significantly reduced PGE2 synthesis. Immunohistochemistry (IHC) showed COX1 and specific receptors for PGE2, EP1–4 in human testicular samples. EP1, EP2 and EP4 were revealed in HTPCs in vivo and in vitro by RT-PCR and/or IHC. Results of collagen gel contraction assay indicate that PGE2 does not acutely contract HTPCs, yet qPCR stud-

ies showed that it increased the mRNA levels for the contractility proteins, smooth muscle actin (SMA) and calponin in a concentration-depended manner. HTPCs, like Sertoli cells, produce glial derived neurotrophic factor (GDNF), which regulates the renewal of spermatogonial stem cells (SSCs). PGE2 increased GDNF mRNA and GNDF levels in the culture medium. Thus, HTPCs are sources of PGE2, which may contribute to testicular homeostasis. This includes maintenance of the contractile phenotype of peritubular cells and, via GDNF, a fine tuning of the SSC niche. Blockage of PG-production e.g. by NSAIDs, steroids or other substances may thus have unwanted testicular side effects.

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Divergent Genotype in Holstein Heifers influences initial Staphylococcus aureus Shedding after Experimentally induced Mastitis

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Staphylococcus aureus (*S. aureus*) is the most important pathogen causing chronic intramammary infections and subclinical mastitis. Besides its large economic impact it also raises concerns over the use of and resistance towards antimicrobial drugs as well as animal welfare. Specific breeding programs seem to be a promising tool for improving animal resistance against production diseases. The aim of the study was to evaluate differences in the bacterial load after experimental *S. aureus* challenge in divergent genotypes. Holstein Friesian heifers were selected for favourable (Q) and unfavourable (q) parental chromosome 18 haplotypes regarding somatic cell count, used as surrogate trait for mastitis susceptibility. The animals (n = 24; Q = 12, q = 12) were kept in individual pens at the Clinic for Cattle in Hanover. On day 36 ± 3 *post partum* the heifers received an intramammary S. aureus challenge in both hindquarters. The following 96h bacterial recovery was assessed in quarter milk samples every 12h by plate count. No effects on bacterial shedding have been detected between the hindquarters after using one way ANOVA and Tukey post test (Q: P = 0.094; q: P = 0.14). However, using an unpaired t-test, q-heifers showed a significant > 10 fold higher shedding of *S. aureus* than Q-heifers 12h p.i. (Q: 1.02 logCFU/ml ± 0.32 vs q: 2.2 logCFU/ml ± 0.27; P < 0.01). This could no longer been observed at time points 24 h until 96 h *post infection* (p.i.). Whether the initial lower bacterial load after 12 h in Q-animals relates to an improved resistance against pathogens will be investigated in follow-up studies. In conclusion, genetic selection might be an opportunity for breeding cows with improved resistance towards mastitis pathogens.

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Interferon-τ induced Gene Expression in Hepatocytes during Early Pregnancy in Angus Heifers

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The conceptus-signal Interferon-τ (IFNτ) induces specific hepatic gene expression (ISG) in heifers. The aim of this study was to confirm the ISG induction *in vivo* on day 18 of pregnancy and to investigate if hepatocytes react to IFNτ. Specifically, it should be confirmed *in vitro* if primary bovine hepatocytes showed an increase of ISG mRNA expression after stimulation with IFNτ. Angus heifers (n = 13) were cycle synchronized. The day of ovulation (day 0) was defined by ovarian ultrasonography and verified by the absence of progesterone (P4). The heifers were artificially inseminated either with sperm or with seminal plasma as a mock control. Blood samples for pregnancy detection were taken on day 18, 21 and 24 after insemination, and the concentration of P4 and pregnancy associated glycoproteins was determined. A liver biopsy was taken on day 18 for qPCR analysis and immunohistochemistry. Primary bovine hepatocytes were isolated and incubated with 200 ng IFNτ *in vitro* for 2 hours. The ISG in the liver increased in pregnant versus non-pregnant heifers (p vs np; relative abundance in ΔCT = ISG 15: 22.2 ± 2.6 vs 3.8 ± 1.1; MX 1: 39.6 ± 8.9 vs 6.6 ± 1.9; MX 2: 0.9 ± 0.1 vs 0.2 ± 0.3; OAS 1: 71.3 ± 15.2 vs 29.9 ± 8.3). *In vitro* the primary bovine hepatocytes showed an increased mRNA expression of ISG after IFNτ stimulation (p < 0.0001) (IFNτ vs control; ΔCT= ISG 15: 465.1 ± 20.7 vs 27.7 ± 2.2; MX 1: 442.4 ± 13.2 vs 50.6 ± 5.9; MX 2: 75.6 ± 5.3 vs2.6 ± 0.2; OAS 1: 180.7 ± 6.4 vs 30.8 ± 3.1). Furthermore OAS 1 was localized in hepatocytes. In conclusion the findings confirm that IFNτ reaches the liver in early pregnancy of cattle and the results show *in vitro* as well as in vivo that bovine hepatocytes react to IFNτ.

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Predictive Role of AMH in ICSI Treatments

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It is already known that serum AMH level reflects the number of antral and pre-antral follicles present in the ovaries and that it is a relevant marker of ovarian reserve and thought to be a predictor of ovarian response in humans like other parameters e.g. FSH an antral follicle count. It may be a useful marker to predict treatment outcome. To find a predictive model of the treatment outcome of GnRH antagonist stimulated ICSI cycles we analyzed in this retrospective single-center study stimulated E2 and AMH levels of 249 women with a mean age of 34.06 years undergoing ICSI treatment in our clinic. Different dynamic parameters were assessed during the treatment as markers of success and compared to the hormone parameters. AMH level was strongly correlated to the number of follicles (rs = 0.39, p < 0.001) and isolated oocytes (rs = 0.70, p < 0.001) and immature oocytes (rs = 0.27, p < 0.001). It also correlates with the pregnancy rates (rs = 0.21, p < 0.05). Linear regression between AMH, E2 levels and pregnancy rate revealed no stronger correlation. Interestingly AMH correlates significantly to formation of multiple PN (rs = 0.27, p < 0.001). No correlation could be found between basal E2 and dynamic parameters. This demonstrates AMH measurement predicts response of patients and is also correlated to the treatment outcome and other dynamic parameters during stimulation and embryo culture. The correlation of AMH and stimulated E2 revealed the potential predictive capacity of AMH to reduce OHHS.

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Cell type-specific Analysis of Endometrial Gene Expression in the Mare During the Time of Initial Recognition of Pregnancy

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The results of endometrial gene expression studies during the time of conceptus migration (days 8 to 16) did not provide clear conclusions on possible mechanisms of maternal recognition of pregnancy (MRP) in the mare. This finding called for a deeper analysis of cell type-specific gene expression in the endometrium in response to the equine embryo, which could be the key for improved understanding of the mechanism of MRP. In a pilot study we used Laser Capture Microdissection (LCM) to collect samples for the luminal epithelium (LE), glandular epithelium (GE), and stroma from endometrial biopsies taken from 5 mares on day 12 of pregnancy and day 12 of the estrous cycle, respectively. RNA was isolated in order to perform RNA sequencing. The preparation and sequencing of RNA-Seq libraries was successful for about 2 thirds of the libraries. Interestingly, data analysis showed that gene expression differences are greater between cell types than between pregnant and cyclic state. Differen-

tial gene expression between pregnant and cyclic state was mainly found in the LE but also in GE and stroma. The comparison with a previous RNA-Seq data set for whole biopsy samples derived from day 12 of pregnancy revealed the specific origin of the observed gene expression differences. Furthermore, we found that a number of genes are specifically differentially expressed (DE) in only 1 cell type. Some genes not DE when looking at the whole biopsy were DE in 1 cell type and showed similar levels in the other cell types in comparison of pregnancy and cyclic stage. These results support our hypothesis of the existence of a specific response in different endometrial cell types in the mare and call for further studies.

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Improving Cryopreservation Success of Shipped Boar Semen

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The demand for cryopreserved boar semen for export or gene banking is constantly increasing. To offer the opportunity of semen freezing also to small boar studs, a procedure allowing cryopreservation of pre-diluted semen shipped over night was established. Using a modular study design, different holding and equilibration times, extenders and additives that may help to sustain the integrity of spermatozoa during and after cryopreservation were compared. Normospermic ejaculates of 42 Pietrain boars were pre-diluted 1+1 (v/v) with BTS extender and shipped over night to the laboratory at IFN Schönow. Quality control post-thaw (PT) included assessment of motility (CASA; 10, 30 and 120 min at 38°C), mitochondrial activity (Rhodamin123/PI), membrane status (FITC-PNA, -PSA/PI) and DNA integrity (SCSA) using flow cytometry. Total sperm motility at 10 min PT was significantly ($P < 0.05$) higher for 2h than for 24h of a post-arrival holding time at 17°C (36.5 ± 11.2 % vs 30.7 ± 13.2 %). There was no difference between 2, 4, 24 and 48 h of equilibration at 5°C. Motility was significantly ($P < 0.0001$) higher in extenders based on lactose (L) or trehalose (T) compared to Androstar® CryoPlus (43.9 ± 10.1 % and 41.7 ± 6.9 % vs 27.1 ± 6.4 %). Contrary to literature, addition of 100, 500 and 1000 mM zinc sulfate heptahydrate to either cooling, freezing or thawing extender did not alter motility. Flow cytometry parameters were not influenced by any freezing modification. In conclusion, for highest PT sperm quality and for best practicability we recommend 2h of post-arrival holding time at 17°C, 4h of equilibration at 5°C and the use of lactose extender for cryopreservation of pre-diluted shipped boar semen.

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Histochemical Investigation of the Epididymal Duct Basal Cells of the Domestic Rabbit *(Oryctolagus cuniculus domesticus)*

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The epididymis contains a diversity of functions including the concentration, maturation and storage of sperm. To collect more information about the different cell types we examined the morphology of the epididymis of the rabbit. Special attention is given to the basal cells. To my knowledge little information regarding presentation and variation of rabbit basal cells is available. Epididymes from three rabbits of different ages were used. They were removed during orchidectomy in a veterinarian clinic in Munich, Germany, dissected into small pieces and fixed in Bouin's solution. After embedding according to routine protocols we prepared serial sections (5 µm) of the epididymes. They were investigated using overview stainings like Hematoxylin-Eosin and Goldner and immunohistochemical stainings for keratins (K5, 14, 18) and claudins (Cl 1,3). The investigated keratins produced a specific expression pattern in the different cell populations, particularly in the basal cells. These cells showed a marked positive staining with K5 and K14. Cl 1 was localized along the entire length of the lateral plasma membranes between adjacent principal cells as well as at the interface between principal and basal cells. Cl 3 was localized apically at the epithelial border in all regions of the rabbit epididymis. The investigation provides accurate description of the different cell types of rabbit epididymal duct and environment. Cl 1 seems not only to be localized in the realm of tight junctions. It appears along the entire length between adjacent epithelial cells and basal cells and might play a role as an adhesion molecule to adhere principal cells to basal cells?

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Intracytoplasmic Injection of Equine Sperm Induces Paternal Pronuclear Formation in Bovine Oocytes

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When oocyte availability is limited, interspecies intracytoplasmic sperm injection (ICSI) is a useful method to study early events of fertilization. It was shown that equine sperm are capable to induce pronuclear formation in matured oocytes of different species such as cat, swine and cow [Rodriguez et al., Reprod Fertil Dev 2014; 27: 95]. The objective of the present work was to study male pronuclear formation after interspecies ICSI of equine sperm into matured bovine oocytes. Ovaries from cows were obtained from a local abattoir and cumulus-oocyte complexes were matured for 22 hours in TCM-199 supplemented with BSA and eCG/hCG. ICSI was performed in 10 µl droplets TCM-199 using frozen-thawed semen, which was stained with MitoTracker® Green FM to visualize the midpiece. A single sperm was immobilized by the application of several piezo impulses and then injected into a matured oocyte, also using electric pulses to draw a hole in the zona pellucida, open the oolemm and to activate the oocyte. After the ICSI procedure, the injected oocytes were cultured for 18 hours in SOF medium. To identify the success of activation and pronuclear formation they were stained with Hoechst 33342. Parthenogenetic (sham-injected) controls were included. First results show a paternal pronuclear formation rate of 23.5% (16/68) in the ICSI group. No pronuclear formation could be observed (0/65) after sham-injection. In conclusion, equine sperm cells can decondense and form paternal pronuclei in bovine oocytes after ICSI.

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A Nonhuman Primate Model for the Study of Testicular Peritubular Cells

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Human testicular peritubular cells (HTPCs) are smooth-muscle-1 like cells, which form the wall of the seminiferous tubules and are being associated with the transport of immotile sperm. New data indicate that these cells play important roles and are involved in male (in)fertility in a complex way. For example, by producing GDNF (glial cell line derived neurotrophic factor) they may contribute to the spermatogonial stem cell niche. Whether they age and whether their functions may decay is not known. To be able to examine such a possibility and to control confounding influences (lifestyle, age, nutrition and the medical history of patients), we turned to a relevant primate model, the marmoset monkey (*Callithrix jacchus*). Marmoset monkeys are nonhuman primates used as model organisms in reproductive research and share many biologically relevant aspects with humans. We successfully isolated monkey testicular peritubular cells (MKTPCs) from young adult animals. MKTPCs, like their counterpart in vivo, express characteristic markers, e.g. smooth muscle-actin and the androgen receptor, but lack markers for Leydig cells (LH-receptor) and Sertoli cells (FSH-receptor). A LC-MS/MS pilot study revealed that the secreted and cellular proteins of MKTPCs considerably overlap with the ones of HTPCs. This supports the relevance of the marmoset

monkey as a translational model. We therefore obtained MKTPCs also from old animals and are in the process of studying them. It is becoming clear from increased cell size and increased β-galactosidase activity that these cells have aged. We expect detailed insights into ageing related changes of testicular peritubular cells from the ongoing studies involving MKTPCs and HTPCs.

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The Luteal Capacity of Bovine Corpora lutea is Simultaneously Influenced by their Location, their local Relationship to the Predecessor, and the Ovulatory Follicle's Lifespan

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With the aim to better understand the influence of spontaneously varying pre-oestrus conditions on the secretory capacity of the later *Corpus luteum* (CL), ovarian function was studied between d-12 and d12 (d1 = oestrus) in Swiss Brown dairy cattle (daily progesterone-RIA in peripheral blood; ovary palpation at intervals of 1–2 days; 393 datasets). The findings revealed a basically superior luteal activity of right-sided corpora lutea (r-CL) as compared to their left-sided counterparts (l-CL). This asymmetry, being evident when CLs do not have a direct fully active predecessor (first normal oestrus cycle after parturition), proved to be by far less obvious in regularly cycling cows in which, all in all, just the regression of r-CLs was delayed. The local relationship between consecutive CLs was found to influence the P4 secretion; the luteal capacity of successors in ipsilateral position being significantly impaired. The median lifespan of ovulatory follicles (OF) was equal on both ovaries, and no effect of the developing OF on the activity of a concomitant CL could be seen. Yet, the OF's lifespan, and especially the time the vesicle is exposed to an ipsilateral CL, are factors which influence the ensuing luteinisation. In conclusion, a basic difference in activity of bovine l-CLs and r-CLs seems to be blurred by local effects of the CL on OFs that happen to develop on the same ovary. As a result, CLs indirectly interfere with the activity of ipsilateral successors.

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Simultaneous long-term Profiles of Sulfonated Androgens, Estrogens and Progestogens in postpubertal Boars measured by liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS)

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Sulfonated steroids (s-St) have been traditionally regarded as inactive metabolites but are increasingly recognized as a pool of precursors for the local production of active steroids in specific target cells. Moreover, direct effects without preceding hydrolysis have been observed. We use the boar as a model to study the still widely unknown s-St physiology as it exhibits an unusually broad s-St spectrum primarily of testicular origin. Previous attempts to characterize s-St metabolism in boars mainly applying GC/MS or immunoassays were limited by high expenses, low sensitivity, low specificity or the lack of methods. The use of LC-MS/MS in steroidomics enables the analysis of free and intact sulfated steroids with highest specificity and good sensitivity, making possible the simultaneous measurement of numerous analytes in higher numbers of samples. Profiles (6 hours, 20 min intervals) were established for sulfonated 5-androstene-3β, 17β-diol (ADS), androsterone (AS), dehydroepiandrosterone (DHEAS), epiandrosterone (EAS), epitestosterone (ETS), estrone (E1S), estradiol-17β (E2S), pregnenolone (P5S), 17αOH-pregnenolone (OHP5S) and free testosterone (T) in 4 unstimulated and 4 hCG-stimulated boars. Moreover, single measurements were performed in testicular efferent and afferent blood to differentiate between testicular vs. extratesticular origin. Highest concentrations were found for EA-S, followed by ETS, ADS and DHEAS, which generally exhibited higher levels than E1S and AS. Lowest concentrations were found for E2S, P5S and OHP5S. Sulfonated T, dihydrotestosterone and cholesterol also included in the analytical profile were undetectable. Profiles of all quantifiable s-St showed simultaneous wave-like patterns virtually synchronous to spontaneous or hCG-stimulated T pulses.

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What is the Body Temperature Measured by Ingestible Temperature Loggers in Healthy Bitches after Parturition?

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The first days after parturition are characterized by many claims raised by nursing the puppies, uterine involution, milk production and hormonal changes. Some authors consider body temperatures higher than 39.5°C in this period to be normal, others don't. The objective of this study was to identify the physiological ranges of body temperature in the first 7 days p.p.. This method enables the collection of continuous data with a reduced potential bias due to the handling of animals and less yielding of stress. The study was performed with 20 private-owned bitches of different breeds. During each experiment the bitch daily swallowed temperature loggers (ANIPILL, BodyCap, Hérouville Saint-Clair, France) for 7 days. Core body temperature was measured every 15 minutes, stored by the loggers and also sent telemetrically to a monitor. Bitches were defined to be healthy if the total count of leucocytes was not higher than 12.0 x 1000/µl in blood samples taken on day 3 and 7. In total, 8 out of 20 bitches were defined as healthy in the first 3 days after parturition. The other animals had to be excluded because of C-section (n = 5), leucocytosis (n = 5), drug administration (n = 1) and mastitis (n = 1). Mean body temperatures were (mean ± SD) 38.9°C ± 0.40 on day 0 p.p. (end of birth until end of day of parturition), 38.9°C ± 0.44 on day 1 p.p., 38.9 °C ± 0.35 on day 2 p.p., 38,7°C ± 0.31 on day 3 p.p., 38,8°C ± 0.30 on day 4 p.p., 38.6°C ± 0.35 on day 5 p.p., 38.5°C ± 0,27 on day 6 p.p. and 38.4°C ± 0.34 on day 7 p. p., respectively. Within the first 3 days 3 out of the 8 healthy bitches showed temperatures above 39.5°C. We conclude that temperature of bitches in the first days p.p. does not differ from those of healthy dogs in general, while the appearance of short of febrile episodes seems to be physiological.

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Assessing Influence of Barn Climate on Reproductive Performance of AI boars using Big Data Management

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Barn climate fluctuations, especially temperatures above 25°C and high air humidity cause depressions of sperm output and quality that vary boar specific. To predict critical production dates caused by this, for 12 month barn climate data (temperature [T], air humidity [H], air pressure [P], brightness [B]) of 3 barns in one boar stud in south of Germany were recorded using data loggers (MSR145, MSR Electronics GmbH, Switzerland). Additionally, production and spermatological data of raw semen as well as sperm kinetics (SpermVision® Automorph, Minitüb GmbH, Germany) were documented. During measurement period, 9,145 climate data records per barn, 11,590 ejaculate data records of 304 AI boars and 67,344 kinetic data records were collected. Data analysis was performed with SAS statistics, calculating linear mixed models and spearman correlations. The production parameter weekday, ejaculation interval, breed and age of boar showed significant ($P < 0.0001$) impact on output and motility. The course of seasons translated into quarters and its effect on sperm output and the kinetic parameter velocity and distance average path (VAP, DAP) could be demonstrated clearly ($P = 0.0021$ and $P = 0.0122$). In detail, barn climate (T, H and T × H) and weekday influenced sperm kinetics VAP and DAP ($P < 0.0001$). An effect of P and B on sperm output and quality could not be shown. Morphologic defects correlated negatively

(r_s = –0.4; P < 0.0001) with sperm motility. In summary, we were able to verify the influence of barn climate, boar breed, age of boar and production proceeding on quantity and quality of ejaculates. Continuous acquisition of climate, production and spermatological data as well as sperm kinetics is the foundation of developing a prediction model, which can help to cope with times of sperm depression.

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Bovine Cystic Ovary Disease: How Does it Affect Tubal Functional Integrity and Gameto-Maternal Interaction?

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Cystic ovary disease (COD) is a major factor contributing to poor reproductive efficiency of lactating dairy cows. However, the precise effects of follicular and luteal cysts on oviductal function are still unknown. As the oviduct is pivotal for gamete transport, fertilisation and early embryonic development, the aim of our study was to analyse the effects of ovarian cysts on the functional integrity of the oviduct using light microscopy, stereomicroscopy, histochemistry, scanning electron microscopy and quantitative and qualitative live cell imaging. Our results showed that 90% of the bovine female tracts collected in the abattoir revealed COD on the right ovary. The ampullar epithelium from cows with luteal cysts revealed a constant predominance of ciliated cells lacking the ability to form secretory cells required for embryo nutrition after fertilization. Synthesis of glycoproteins and acidic mucopolysaccharides was increased within the ciliated cells of the ampulla. Whereas the attachment of the cumulus-oocyte-complex to the ampullar epithelium and the formation of the sperm reservoir in the isthmus was not negatively affected in cows with ovarian cysts, ciliary beat frequency and particle transport speed were increased in the tubal ampulla as compared to controls. Our results imply that ovarian cysts impair formation of secretory cells in the ampulla. Ciliated cells do increase their secretory activity but are not able to synthesize glycogen which is essential for successful pregnancy. At the same time the transport of the early embryo is compromised. Thus, successful therapeutic strategies for treatment of ovarian cysts include not only successful induction of ovulation and fertilization, but optimizing the conditions for the early embryo in the oviduct.

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Time lapse Technology for Embryo Selection at the Pronuclear Stage in Countries where the Culture of Supernumerary Embryos is limited: Where is the Benefit?

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Tripronucleate embryos (3PN) after intracytoplasmatic sperm injection (ICSI) have a low developmental potential, giving rise to poor-quality embryos which are associated with significantly decreased implantation, pregnancy and life birth rates. Accordingly the transfer of 3PN embryos should be avoided making accurate identification of such embryos highly desirable. In the present study we analysed whether time-lapse technology can be used to properly identify 3PN embryos that would have been erroneously classified as normal if looking at them at a specific time interval. We performed a retrospective time-lapse movie evaluation from patients undergoing ICSI from September 2013 to December 2013. Eighteen of these patients had at least 1 3PN embryo and were therefore included in the subsequent study. Different times of appearance of the individual pronuclei of 29 3PN embryos were analysed using the EmbryoScopeTM as a time-lapse imaging device. Other analysed parameters were first division, number of cells on day 3, compaction and blastulation. In 48.3% of all 3PN embryos the appearance of all 3 pronuclei occurred at the same time. In contrast, in 51.7% the 3rd pronucleus appeared later than the other 2 (more than one hour later in 44.8% and more than 5 hours later in 10.3%). Of all 3PN embryos, 9 were subjected to further culture, 44.4% of them until day 3 and 55.6% of them until day 5. Only 33.3% presented a regular first cleavage and had 8 or more cells on day 3. From the embryos cultured until day 5, only 20% showed signs of compaction and began to blastulate. These findings may encourage other centres under restrictive legislation to introduce time-lapse technology into their laboratory routine, since this technique can also be used for early embryo selection. An example is the accurate identification of 3PN embryos, avoiding further culture or even transfer of these embryos.

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Comparison of different Reanimation Methods in New born Calves after Dystocia under Field Conditions

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The aim of this study was to compare the use of two different hand-powered vacuum pumps and the suspension by the hind legs within the scope of resuscitating newborn calves after dystocia. The present study represents the first summary based on findings from examinations performed under field conditions. A total of 60 calves (born with dystocia) from different farms were examined. They were randomly divided into three groups with 20 calves per group. To remove mucus and fetal fluid from the respiratory tract of calves, following operations were performed: Group I: HK-Beatmungspumpe (Rheintechnik), Group II: Calf Aspirator and Resuscitator (McCulloch Medical), Group III: Only method of suspension by the hind legs of the calf. After these procedures in the groups, the vitality (Time to Sternal Recumbency, T-SR) of the calves and the incidence of diseases like diarrhea, pneumonia and omphalitis in the first weeks of life were recorded. After delivery, the girth of the muzzle of 55 calves was also measured. Considering the study results, there was no significant difference of the T-SR between the groups. However the calves that were treated with a hand-powered vacuum pump in tendency showed a shorter T-SR (p = 0.07). In conclusion, the present study was not able to show that the use of hand-powered vacuum pumps is superior to the suspension by the hind legs.

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Effect of eCG Administration on Day 6 *post partum* in Cows on Serum Concentration of Estradiol

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In modern dairy industry, the amount of postpartum anestrous has increased in the last decades. In recent studies a positive influence of eCG administration could be detected on early resumption of ovarian activity, resulted in an improved reproductive performance by day 6 *post partum* [Rostami et al., 2011, Vojgani et al., 2013]. These studies revealed enhancing ovarian follicle growth and early ovulation in *post partum* cows but did not provide any estradiol serum-level in cows. The aim of this study was to collect data on estradiol serum levels on day 6 *post partum* and after eCG administration on day 10 *post partum*. 63 dairy and beef cows were included in this study. The animals were divided into two groups, healthy and dystocia/puerperial disorders. These groups were further divided into 2 subgroups; animals treated with eCG and control. Blood serum samples were taken on day 6 *post partum* followed by an injection of 480 I.E. eCG or nothing respectively. On day 10, a second serum sample was taken. The estradiol concentrations were determined by radio-immonoassay. There was no statistically significant increase in the estradiol-concentration ob-

served in any group. Although the literature showed that the reproductive performance of dairy cattle, including an enhanced follicle growth to a dominant stage, could be improved by administration of eCG, no rise in estradiol concentration could be detected in this study. The cause of the discrepancy of the study results and the literature is unclear. Possible is that in contrast to the results found in the literature, either no effect of eCG could be achieved or the eCG effects are not mediated by the stimulation of the follicular growth.

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Serum Level of Neuropeptides in Cows with Torsio uteri

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Introduction Torsio uteri *intra partum* is a common reason for dystocia in dairy cows. This disease resembles the displacement of the abomasum as both diseases are associated with a torsion of a hollow organ. Due to the formerly shown changes in the neuropeptic content of the abomasal wall, the topic of this study was to examine the neuropeptide serum levels of cows suffering from torsio uteri.

Materials and Methods Blood samples of 20 cows with torsio uteri, 36 healthy controls and 15 cows intra partum without torsio uteri were examined and neuropeptic contents for substance P (SP) and vasoactive intestinal polypeptide (VIP) were measured by means of commercially available ELISA kits. For all cows Interleukin 1β (Il 1β) levels were determined and compared between groups.

Results Significant differences between groups could be shown for the SP concentrations in cows with torsio uteri and cows calving without torsio uteri ($P < 0.0001$). SP serum levels in cows sub partu were markedly higher than in those suffering from torsio uteri. Comparing the healthy controls with cows during calving also resulted in significantly higher SP levels in cows sub partu ($P = 0.002$). Concerning VIP and Il 1β no significant differences between the groups could be detected.

Discussion and Conclusion Remarkable amounts of SP are released into the blood. SP being a biomarker and mediator for pain suggests the hypothesis that cows with torsio uteri do not experience as much pain as cows that are calving without dystocia. A possible reason could be the lack of opening of the cervix in cows with uterine torsion. Further research is needed to proof this assumption.

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Evaluation of Equine Cervical Function Based on the Histological Evidences of Smooth Muscle Tissue

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Proper cervical contractility and relaxation is a major factor in mare's fertility and uterine defense mechanisms. Histologically, the cervix consists mostly of connective tissue, smooth muscle and extracellular matrix. Smooth muscles and Cajal-like interstitial cells (ICLC) are responsible for contractility. Cervical disorders are predisposing factors for fluid accumulation and contamination of the uterus, might impair fertilization as well as influence pregnancy and parturition. The purpose of this study was to demonstrate ICLC in the equine cervix to complement the knowledge on mare's reproductive tract conditions with detailed information regarding cervical function in diestrus. Fresh whole thickness samples of the uterine body and cervix were collected from 30 slaughtered mature mares with no signs of reproductive tract pathologies. The samples were stained with hematoxylin eosin (HE), Masson's Trichrome and immunofluorescent labeling (IF) with primary (anti-vimentine and anti-CD117) and secondary antibodies (conjuncted with AF488 and AF647) and imaged by using light and confocal microscopy, respectively. Cells with morphologic and immunologic phenotypes similar to the cells of Cajal of the gastrointestinal tract were identified inside of the uterine body and cervix. The CD117 and vimentine positive cells were localized in the muscle layers as fusiform, triangular or starlike-cells with dendritic processes, which formed a cellular network and were classified as ICLC. The fusiform ICLC were the predominant cell type in the corpus uteri. Vimentin reactivity was mainly localized within the cell processes, and CD117 has a patchy pattern in the cell body. In conclusion, the double IF labeling protocol was optimized with respect to ICLC identification in equine reproductive tract.

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Morphokinetics of Preimplantation Mouse Embryo Development using Time Lapse Monitoring of Embryo Development (TLMED)

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The purpose of this study was to determine the preimplantation developmental potential of embryos from different mouse strains, using TLMED technique. A total of 457 embryos were collected from 80 female mice, 10 strains: 129S1/SvW, AKR/W, C57BL/6W, C57BL/10W, C3H/W, C3Hwad/W, CBAmut/W, A.CA/W, BN/aW, BALB/cW. Mice were superovulated, mated and euthanized according to the standard procedure. Only 301 morphological normal embryos (zygotes, 2–3 cell) were analyzed. Embryos were cultured in CSC medium containing 10% SSS for 96–120 hours in three gas atmosphere incubator containing 6% CO_2, 5% O_2, and 89% N_2. The morphokinetic assessment was performed using the Primo Vision Time Lapse Embryo Monitoring System. From C3H/W, C3Hwad/W, 129S1/SvW, and C57BL/6W it was obtained respectively 63% (24/38), 72% (28/39), 66% (21/32) and 87% (28/32) of blastocysts with a cleavage average time over 1189 and 1243, 986, 1399, 1418 min from 3rd and 2nd cleavage respectively. These strains showed the highest preimplantation developmental potential. C57BL/10W, CBAmut/W and BN/aW are characterized respectively by average timing of 3rd cleavage – respectively: 1418, 681, 1255 min and by lower n, % and quality of embryos. The blastocysts rate was 71% (12/17), 23% (7/30), 61% (34/56). AKR/W, BALB/cW and A.CA/W embryos showed deeply compromised embryo developmental showing respectively: 2% (1/26), 0% (0/22), 0% (0/9) and timing of third cleavage over 2061, 527 and 1861 min. Embryos from different mouse strains showed clear differences in their developmental potential and timing. The time of 3rd cleavage is the principal morphokinetics parameter for prediction of developmental and implantation potential of tested mice strains.

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Assessment of the Thyroid Hormone Status During Late Pregnancy and Early Lactation

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Thyroid hormones are important regulators of metabolic adaption during pregnancy and in early lactation. However, less data is available from modern high-yielding dairy cows. The aim of the study was to assess thyroid hormones (Triiodothyronine [T3], Thyroxine [T4]) and Thyroid stimulating hormone [TSH] during transition from late pregnancy to early lactation as well as to examine, whether basic thyroid ultrasound is feasible in cows and may reflect the thyroid hormone status. Holstein Friesian heifers (n = 12) were sampled twice weekly from day 259 ± 2 after artificial insemination to day 34 ± 2 after parturition and T3, T4 as well as TSH were analyzed. At the end of the clinical puerperium an ultrasonographic examination of the thyroid gland was conducted. A subsequent dissection of the animals allowed the determination of thyroid size, weight and volume which were correlated to the ultrasonografically detected thickness of the thyroid gland. Concentrations of T3 and T4 significantly decreased with a nadir during the first and second week postpartum ($p < 0.05$) while TSH remained constant ($p > 0.05$), excepting a peak around calving. Interestingly, the highest inter-individual variance for T3, T4 and TSH was detected at time of parturition. Neither the thickness of the thyroid gland assessed ultrasonographically nor the data measured during dissection showed significant correlations with hormone concentrations ($p > 0.05$). Moreover the thyroid gland altered its shape if the cow changed the position of the head. This derogated the usefulness of this method. The low thyroid hormone levels after calving can be related to the negative energy balance postpartum. Moreover, an important effect of thyroid hormones on the individual capacity for metabolic adaption around calving is suggested.

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Assessment of Successful Fertilization in Bovine Blastocysts via Neuronatin Transcript Analyses

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Oocyte activation is a prerequisite for decondensation of the sperm head and formation of the male pronucleus regardless of whether the spermatozoon enters the oocyte by injection or by fertilization. In several species including cattle the injection procedure itself is insufficient to induce oocyte activation. Therefore additional stimuli are needed which may also initiate parthenogenetic development. The aim of this study was to validate the paternal contribution to embryonic development employing the analyses of neuronatin (NNAT) mRNA expression, a paternally expressed gene, via RT-PCR. Cumulus-oocyte-complexes were isolated from abattoir-derived ovaries. After 24 h of maturation, fertilization was realized using a standard protocol [Parrish et al. 1995]. Presumptive zygotes were cultured in SOFaa for 8 days. To generate parthenogenetic blastocysts, matured oocytes were activated by incubation in 5 µM ionomycin for 5 minutes and in 1.9 mM 6-dimethylaminopurine for 4 h. Afterwards, treated oocytes were cultured in SOFaa for 8 days. RT-PCR was performed for six embryos stemming from the IVF-group and three embryos out of the chemical activated group. NNAT mRNA was expressed in five blastocysts out of the IVF-group, one blastocyst did not show NNAT mRNA expression suggesting that it was derived from parthenogenesis. NNAT mRNA could not be detected in all blastocysts derived from chemical activation indicating that only the maternal genome was present. These results clearly demonstrate that NNAT mRNA expression is an appropriate method to prove the paternal participation in early bovine embryo development.

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The Effect of Progesterone on the Endometrial Expression of Estrogen mRNA in the Dairy Cows

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Estrogen acts through endometrial steroid receptors (oestrogen α-ER and Estrogen β-ER), influence the timing of upregulation in proper as well as failure luteolysis. This study examined the expression patterns of steroid receptors (α-ER, β-ER) in the bovine endometrium during intravaginal progesterone treatment in dairy cows. Uterine biopsy samples were collected over the proestrus before intravaginal progesterone administration (progesterone insert, 1.38 g, 7 days) and after 7 days from 25 HF nonlactating cows. From clippings from endometrium full RNA was isolated using columnary method. Complete RNA was then undergone a reverse transcription reaction using oligo T primers to receive mRNA transcripted onto cDNA. cDNA was then get multiplied using cyclic polimerase reaction using paired starters (ESR-α-GAPDH and ESR-β-GAPDH) specified for each examined sequence using QuantStudio Real-Time PCR system. Progress of the reaction of polimerisation of complimentary strands for each sequence was registered on a diagram of growth of the fluorescence intensity that occurs during attaching a marked nucleotide to the matrix. Significant differences ($P < 0.0001$) in gene expression in the endometrial samples were found between α-ER and β-ER independently from progesterone administration. The mRNA expression (mean ± SD) was significant higher before (α-ER 0.23 ± 0.098; β-ER 0.03 ± 0.021) than after (α-ER 0.19 ± 0.292; β-ER 0.02 ± 0.031) treatment both for α-ER ($P < 0.0001$) and β-ER ($P = 0.016$). Seven day progesterone administration affected oestrogen endometrial receptors expression the major α-ER and the second one β-ER. The results confirm the inhibitory effect of progesterone on estrogen receptors expression.

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Diversity of DQA2 Gene in Relation to Somatic Cell Count (SCC) of Milk and Subclinical Mastitis Occurrence in Low-input Dairy Sheep Farms

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This study aimed to explore the diversity of the ovine major histocompatibility complex (MHC) DQA2 gene in relation to Somatic Cell Count (SCC) of milk and subclinical mastitis occurrence in low-input dairy sheep farms, with regard to different biotic (age class of ewes, lactation month) and abiotic (management [MS] and milking system [MLS]) risk factors. Samplings were carried out in 10 extensively and 10 semi-intensively managed low-input dairy sheep flocks, with three milking systems: milking parlour machine (MPM), portable milking machine (PM) and hand milking (HM). Over 2 consecutive lactations, milk samples (n = 9,624) were collected monthly in each flock, from 20 ewes (10 primi- and 10 multiparous ewes). SCC was assessed in milk samples with pH ≥ 6.00, using the FossomaticTM system (FOSS®, Denmark) and samples with SCC ≥ 400×10^3 cells/ml (n = 2,541) were processed with bacteriological examination. Blood samples were collected from the studied ewes (n = 800) onto FTA Whatmann™ Cards. Following DNA extraction from the FTA cards, PCR-SSCP analysis of DQA2 was performed to ascertain genotype. Only 2 alleles were found in > 10% of the blood samples: alleles B1 („0602“) and K („0301“), that were identified in 215 and 244 sheep respectively, with an occurrence of 0.139 and 0.158. The occurrence of any of the alleles did not differ between ewes with SCC < 400×10^3 and SCC ≥ 400×10^3 cells/ml of milk or between different MSs, MLSs, age class of ewes or lactation months. MHC DQA2 allele B1 („0602“) occurred with a lower frequency in ewes with subclinical mastitis ($p < 0.05$). To our knowledge, this is the first report exploring the effect of DQA2 gene on subclin-

ical mastitis of sheep reared under low-input management systems.

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Embryo-Maternal Interaction in Roe Deer

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Embryonic development is a complex, well-orchestrated process taking place in different compartments of the female reproductive tract. Maternal recognition of pregnancy (MRP) is considered a critical event for embryo survival and establishment of pregnancy. The embryo continuously faces changes of its microenvironment, the histotroph, which contains different constituents important for embryo survival and development. We aim at unravelling how the embryo of roe deer, a mono-oestrous seasonal species, signals its presence. Therefore, samples from the endometrium of hunted roe deer during the period of diapause and the reactivation of embryonic growth were examined (n = 5 for endometrium of embryos < 1 mm, 1–2 mm, > 2 mm, and n = 3 for elongated embryos, sampled between early November until end of December). This is comparable to the time period when MRP takes place in other ruminants. A targeted approach was used to determine the differential mRNA expression of genes known to be involved in MRP of cattle, pig, and sheep. Genes involved in the classical IFN-signalling, e.g. IRF2, ISG15, and MX1, conceptus elongation, e.g. AKR1B1, HSD11B1, PTGS2, PTGES, and PTGER2, implantation, e.g. MUC1, ESR1, and PGR, and amino acid and glucose transporters, e.g. SLC1A5, SLC2A1, SLC5A1, SLC7A2, and SLC15A3 were analysed by qPCR. Our first preliminary data show that not even minute amounts of IFNτ seem to be secreted by the embryo, since upregulation of IFN-stimulated genes in the maternal endometrium, as observed in other ruminants is lacking. In conclusion, it remains to be shown which other signals, e.g. oestrogens, may play a role in MRP in roe deer. The endometrial response to signals from the embryo may provide a conclusive fingerprint.

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Relations between Plasma Anti-Muellerian-Hormone (AMH) Concentrations of Holstein-Friesian Heifers and their later Fertility as Dairy Cow

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Plasma levels of Anti-Muellerian Hormone (AMH), produced only from granulosa cells, reflect the total number of oocytes and also the number of antral follicles of an individual (ovarian reserve). Recently it was published that high AMH levels could be positively related to fertility in cattle. In this study we aimed to prove, whether AMH levels of Holstein-Friesian heifers could be used as a predictive and independent biomarker for their subsequent fertility as dairy cows. Therefore 1043 plasma samples and data were collected from post puberal heifers of 5 farms and correlated to their fertility parameters. Plasma samples were analyzed with an ELISA-Kit (DSL-10-144400, Beckman Coulter, USA) for their AMH concentration. Overall, the studied heifers had 0.493 ± 0.3 ng/ml AMH in the plasma. We firstly checked possible but unknown impact factors on the individual AMH levels of the heifers. We found no significant effects of the farm, age, weight, height, BCS, progesterone levels and pregnancy status at sampling or their later milk yield, fat and protein on the AMH levels ($r < 0.1$, $p > 0.1$). For 672 heifers fertility parameters of their first lactation could be acquired and analyzed. We found no correlations between the AMH levels and the heifers' age of first breeding or calving, services per conception as heifer or cow, days to first service post-partum, days open to pregnancy or conception rates ($r < 0.1$, $p > 0.1$). Our results support the notion that AMH levels of post puberal heifers could be considered as mainly independent of environmental and individual factors at the time of blood sampling. However, our results showed that a single assessment of the ovarian reserve in heifers by AMH plasma concentrations, measured by a human ELISA kit is not a suitable parameter or biomarker to predict fertility in dairy cattle.

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Functional Implication of Taste Receptors and Gustatory Signaling Molecules for Male Reproduction

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Male fertility is characterized by a sequential process which can be subdivided into 3 substeps: The proliferation and differentiation of male germ cells in the seminiferous tubules of the testis, the travelling of motile sperm through the different segments of the epididymal duct, and finally, the long lasting journey of sperm in the female genital tract which allows sperm-egg fusion. The recent observation of an expression of taste receptors and coupled downstream signaling components, such as the taste G protein α-Gustducin and the cation channel TRPM5 in non-taste tissue led to the question whether gustatory signaling molecules are also expressed in the male reproductive system and whether proper sperm function is influenced by taste signaling components. Combining complementary molecular, cellular and reproductive biology approaches we found that the 2 subunits of the umami taste receptor dimer (Tas1r1/Tas1r3) as well as some members of the Tas2r family of bitter receptors and components of their downstream signaling cascade, such as Gustducin and TRPM5, are expressed during late phases of spermatogenesis as well as in mature spermatozoa. Moreover, employing a Tas1r1-deficient mCherry reporter mouse strain, we found that Tas1r1 gene deletion resulted in spermatogenic abnormalities, including an increase in the rate of apoptosis of developing germ cells. Furthermore, a significant increase in spontaneous acrosomal reaction was observed in Tas1r1 null mutant sperm which was accompanied by an elevated level of the second messenger cAMP and an increase in cytosolic Calcium. Since production and maturation but also sperm's fertilizing competence strongly depend on their ability to detect diverse chemical ligands in their microenvironments, it will be essential to uncover the physiological function of taste receptors for male germ cell's ability to adequately respond to distinct external stimuli in their surrounding milieu.

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Comparative Study of Automatic and Manual Thermogram Analysis of Bovine Udders with induced E. coli-Mastitis

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Infrared thermography is a simple and noninvasive method to measure changes in bovine udder surface temperature and thus a helpful tool for early mastitis detection. However, the manual analysis of thermograms is very time-consuming. The aim of this study was to compare whether the automatic segmentation and evaluation of thermal images gains comparable results. Five healthy Holstein-Friesian cows were challenged with E. coli into the right hind quarter. In each case, acute mastitis was induced. Thermograms were taken every two hours in a period of 24 hours before and after challenge. The images were interpreted manually, using a polygon-tool. Automatic image analysis based on Active Shape Model approach was implemented by Fraunhofer Institute. For each image, values for average and maximum udder temperature were calculated and compared by boxplot-analysis. Manually determined temperatures were higher than automatically analyzed values (median = 0.80K; lower quartile [q1] = 0.76K; upper quartile [q3] = 0.87K). Both methods detected peak values for maximum udder temperature 13 and 15 hours *post infectionem* (p.i.): 2.20K (13 h p.i.) and 2.19K (15 h p.i.) difference from median maximum temperature (37.44°C; q_1 = 37.27°C;

q_3 = 37.61°C) for manual analysis, as well as 2.33K (13 h p.i.) and 2.08K (15 h p.i.) for automatic segmentation (median 36.38°C; q_1 = 36.19°C; q_3 = 36.61°C). It is presumed that higher temperatures in manual analysis occur due to inclusion of warmer regions, e.g. udder-thigh cleft, whereas automatic segmentation leaves these regions out. Still, temperature curves for each method show similar progresses. Thus, automatic segmentation of infrared images is a quick and promising approach to enhance early mastitis detection.

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Maturational Potential of Feline Oocytes with different Morphological Characteristics

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Since many felid species living in the wild are threatened from extinction, assisted reproductive techniques (ART), such as in vitro maturation (IVM) and fertilization (IVF) gain more importance to support reproduction of captive cats. Due to its similarities to many wild living species the domestic cat (felis catus) serves as a model for all feline species. Ideally oocytes with optimal quality, defined by homogenous almost black ooplasm and intact cumulus cell layer, are used for ART. But since oocytes are not always sufficient in number, maturational potential of oocytes with poorer quality is of interest. Oocytes were matured *in vitro* in 3 different groups: Oocytes with optimal quality as positive control (control; n = 149), oocytes with grey, uneven or patchy ooplasm but intact cumulus cell layer (oopl-; n = 242) and oocytes with partially missing or damaged cumulus layers which had a high quality ooplasm (cum-; n = 122). After IVM (M199 based medium, 5.5% CO_2, 38.5 °C) the nuclear stage of the oocytes was determined with propidium iodide staining. Maturation rate was defined as relation of MII oocytes to the total number of oocytes per group. The control (75.84%) and the cum- (73.39%) group showed similarly high maturation rates. In contrast, the maturation of oopl- oocytes was significantly lower (38.91%). In the cum-group, the number of parthenogenetic cleavage (13 of 122) after maturation was strikingly high. To further evaluate the developmental potential of the oocytes IVF was performed. First fertilization results confirm the trends being achieved during IVM. A strong selection of oocytes for ART with even dark cytoplasm as a quality marker is important, whereas full intactness of cumulus is negligible.

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Characterization of Cystic ovarian Follicles in Dairy Cows and Heifers

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Reproductive dysfunction observed in lactating cows has been attributed to metabolic adaptation which is needed to ensure energy demand for lactation. This is especially critical during the post partum period of negative energy balance marked by high rates of oxidative stress; however, conception is highly desirable. Between 6 and 30% of first-lactating and especially multiparous cows develop a cystic ovarian follicle (COF) during the early post-partum period. Cystic ovarian follicles interfere with normal ovarian activity and thus prevent conception and prolong inter-calving interval. In the present study we developed a technique to induce COFs by ultrasound-guided follicle injection. We injected different cyclooxygenase inhibitors, such as indomethacin and flunixin at different time points relative to the (expected) ovulation in different concentrations. Ovulation is successfully inhibited by injection of 50 µM indomethacin 16 hours post-GnRH with 100% efficiency. The remaining non-ovulated follicular structure resembles those of a COF. We compared these COFs with classical developing corpus lutea at days 1 and 5 after the (expected) ovulation. We measured gene expression patterns of marker genes of the cyclooxygenase pathway as well as endocrine parameters (such as PGE2 and 8-iso-PGF2α). Our data indicate that PGE2 is massively down-regulated after injection of indomethacin, thus preventing an essential step for proper ovulation.

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Expression of the Nuclear Glucocorticoid Receptor in Peritubular Cells of the Human Testis and Activation of Target Genes by Dexamethasone

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The receptors for glucocorticoids (GCs) were described in the testis, among others in peritubular cells, i.e. the cells forming the wall of seminiferous tubules. Peritubular cells are emerging as important players of human testicular functions. They are contractile and transport sperm, are involved in immunoregulation of the testis and contribute to the spermatogonial stem cell niche. Whether GCs are involved in these functions is not known. After confirming in testicular biopsies of healthy men that peritubular myoid cells are immunopositive for the nuclear glucocorticoid receptor (GR), we explored its expression in cultured human testicular peritubular cells (HTPCs). We detected mRNA of GRalpha, but not beta, and protein of GR in these cells. Stimulation with the synthetic GC dexamethasone (Dex; 1 µM) resulted in a ligand-dependent GR nuclear translocation and the expression of the GC-responsive target genes, including PLZF and FKBP5. The GR antagonist RU486 blocked these actions indicating that GR is fully active in these cells. Further qPCR screening studies of HTPCs revealed that Dex influenced the expression of known and formerly reported candidate genes associated with HTPC function, amongst others, smooth muscle actin, calponin, cyclooxygenase 2 and angiotensin receptor type I. Thus, our studies demonstrate that HTPCs express functional GRs, which are linked to functions of peritubular cells. GC-signaling thus likely plays an important role in the normal adult testis. It remains to be shown, if this system is altered in states of infertility and if elevated GC levels, as associated with stress or medication, may impair male fertility.

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Transient Oligo-Astheno-Teratozoospermia in a 3-year old German Shorthair Pointer: A Case report

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Acquired infertility may develop occasionally, but in many cases the causal background remains obscure. Oligo-astheno-teratozoospermia is a condition including concurrent oligozoospermia, asthenozoospermia and teratozoospermia and may end up with azoospermia.

Case report: A 3-year-old German Shorthair Pointer of 37 kg body weight, which had a history of 11 successful breedings, was presented for semen freezing. The accompanying breeding soundness evaluation revealed a generalized pyodermia also affecting the scrotum. At morphological examination of the genital tract increased scrotal temperature and pain as well as scrotal lesions and slightly swollen scrotal lymph nodes were detected. The general condition and libido were undisturbed. Microscopic semen analysis yielded a total sperm count of 315×10^6, 40% progressively motile spermatozoa, 29% dead spermatozoa and 94% morphologically abnormal spermatozoa indicating disturbed testicular and epididymal function probably due to periorchitis. In the sperm rich fraction a low content of Staphylococcus pseudintermedius, α-hemolytic streptococci, β-hemolytic streptococci and coagulase-negative staphylococci were identified. Complete hematologic analysis showed no deviations. Regarding pyodermia a 4-week oral therapy with cephalexin was performed accompanied by a 1-week anti-inflammatory therapy with carprodyl and scrotal cooling directed against periorchitis. At control semen analysis 9 weeks after the end of treatment all ejaculate

parameters were within the reference ranges. This case report demonstrates that early diagnosis of testicular and epididymal dysfunction and specific etiological treatment even in case of severe semen alterations may lead to complete restauration of fertility.

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Functional Characterization of a Calcium Sensor in a Transgenic Pig Model

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Calcium plays a crucial role as second messenger in many physiological and pathological processes such as oocyte fertilization, neurotransmitter release, muscle contraction and endothelial cell activation, which are characterized by an increase of the intracellular calcium concentration. Intracellular Ca^{2+} changes can be monitored by loading cells with calcium sensitive fluorescent dyes such as Fura-2, but their usage is often compromised by reproducibility, toxic cleavage products and leakage. Therefore, we generated transgenic pigs carrying the genetically encoded calcium sensor Case12 under the control of the ubiquitously active CAG promoter for a stable transgene expression. Case12, a fusion protein of calmodulin, M13 and green fluorescent protein, changes conformation after Ca^{2+} binding, resulting in an increase of the green fluorescence signal. After somatic cell nuclear transfer using a mix of stably transfected kidney cells 8 living Case12 transgenic piglets were born. After expression analysis via epifluorescence microscopy and Western Blot using fibroblasts and endothelial cells isolated from five different founders, best expressing founder cells were used for further studies. Functionality of Case12 transgenic endothelial cells could be shown by treatment with ATP, resulting in a fast increase followed by a decrease of the fluorescence signal, what is the typical picture seen after ATP treatment. Furthermore we used Case12 cells to show endothelial activation during rejection processes in xenotransplantation, suggesting that cells from Case12 transgenic pigs could be used for monitoring changes of intracellular Ca^{2+} concentration during (patho-)physiological processes.

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The Effects of a Herd Health Management Program on Selected Reproductive parameters in a Dairy Herd in Poland

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The aim of the study was to evaluate the effectiveness of a herd health management program (HHMP) in dairy cattle herd. The study was conducted during 2012–2015 in the herd in northeastern Poland. In the experiment were 211 of HF breed cattle. Control group was a herd in 2011 (without HHMP). HHMP was conducted by the method with a use of Agranova computer program which had following functions: recording and analyzing data, creating statistics, evaluating financial losses due to reproduction failures, reminding about certain events (examinations, calvings, drying off, estrus, injections etc.). Veterinarian did following rectal ultrasound examinations once a month in all cows: post partum (10; 30; 60 ± 5 d.), pregnancy detection (30 and 60 ± 5 d.), not inseminated > 90 days *post partum*, cows not in calf inseminated ≥ 3 times, heifers at the age of > 400 days. Each examinations were followed by therapy, estrus/ovulation synchronization protocol or decision to cull the cow was made. Effectiveness of the therapy was evaluated during next visitation. Comparison of reproductive parameters between the control group of the study (2011) and the last year (2015) revealed improvement of intercalving period (IP) (468 vs 424 d.), age of first calving (945 vs 782 d.), insemination index (2.1 vs 1.5 AI/pregnancy), culling index (0.19 vs 0.05) together with increased mean annual milk yield (6639 vs. 8984 liters/cow). Economic evaluation showed decrease in mean costs of extended IP of 64.7%. Results indicate the effectiveness of used HHMP as a method of improving the profitability of milk production.

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Effect of Selenium and Vitamin E Supplementation on Semen Quality, Glutathione Peroxidase Activity, and Total Antioxidant Capacity in Dogs with Lowered Fertility

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Twenty clinically healthy dogs with poor semen quality (low total sperm count and percentage of progressive forward motility, increased percentage of morphologically abnormal sperm) and lowered fertility were used in this study. Lowered fertility was defined as low pregnancy rate and/or smaller than expected litter size. Ten dogs were supplemented daily by selenium (0.6 mg/kg organic selenium yeast) and Vitamin E (5 mg/kg) per os for 60 days. The control group consisted on 10 males without supplementation. Semen was collected by manual manipulation from all dogs on day 0, 30, 60 and 90. The sperm concentration and motility parameters were evaluated using computer assisted sperm analyzer (Hamilton Thorne, version IVOS 12.3). The morphology of spermatozoa and the percentage of live and dead spermatozoa were assessed microscopically by Diff-Quick stain and nigrosin/eosin stain, respectively. Activity of glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAC) in spermatozoa were determined spectrophotometrically. The concentration of spermatozoa, the most motility parameters determined (MOT, PMOT, VSL, BCF, RAPID) and the percentage of spermatozoa with normal morphology and of live spermatozoa increased significantly ($p < 0.05$) after 60 days of supplementation. There was an increase ($p < 0.05$) in GSH-Px-activity and TAC in spermatozoa. In conclusion, supplementation with selenium and Vitamin E for a period of 60 days improve the quality of semen and enhance the antioxidant status of semen in dog with lowered fertility. More studies on a larger number of animals are needed to confirm these results.

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EP3 Correlates with Poor Prognosis of Endometrial Carcinoma Patients

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Background The underlying mechanisms leading to endometrial carcinoma remain unknown to date. The expression of prostaglandin E2 (PGE2) receptor 3 (EP3) is decreased in colon and prostate cancer. Furthermore, EP3 reduces tumor cell proliferation, migration and invasion. The aim of this investigation was to analyze the expression of EP3 in endometrial cancer and its effect on clinical features and patients' prognosis.

Materials and Methods We retrospectively analyzed 140 endometrial carcinoma patients. Routinely processed tissue samples were immunohistochemically stained using anti-EP3 antibodies. Specific staining was analyzed by designating a percentage of positive glandular epithelial cells to each sample.

Results Detailed medical records of 140 endometrial cancer patients including age, stage of disease and WHO gradings were available. EP3 staining differed significantly according to WHO tumor grading: Tumors graded G3 displayed the highest expression, while tumors graded G1 displayed the lowest expression ($p = 0.011$). No differences were evident regarding the histological classification and FIGO stages. Grouped according to lymph node or organ metastasis or relapse, the staining intensity also was showed no significant differences. Using ROC curves, a cut-off of 77.5% was identified defining low (< 77.5% positive glandular epithelial cells) and high (> 77.5% positive cells) EP3 expression. Kaplan-Meier analysis indicated, that the overall survival (OS) of patients

with a high EP3 expression in their respective tumors was poorer (8.19 vs 14.83 years, p = 0.004) as was the progression-free survival (PFS) (7.88 vs 14.13 years, p = 0.011). A multivariate analysis showed EP3 expression as independent prognostic marker for PFS (p = 0.042) besides age, stage and recurrence.

Discussion Here we demonstrate for the first time, that EP3 expression in glandular epithelial cells increases with the malignancy of tumor stage and correlates with poor patients' prognosis. These results are contradictory to previous studies and indicate that EP3 might act in a cell and tissue type specific manner and is a potential therapeutical target in endometrial cancer.

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Comparison of two Methods for Automated Heat Detection in Cattle

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Heat detection is one of the most important management factors on dairy farms. To assist the farmer on modern dairies, different automated systems for heat detection are available. Two methods investigated in this study were based on a continuous automatic analysis of locomotion activity in a freestall barn on a 24/7-basis. A new heat detection system involving machine vision (MV) was compared with a commercial activity detection system (AD). The MV system combined high-resolution GigE-cameras and 2D matrix codes for marking individual cows. Progesterone determination in skimmed milk (EIA) was used as a reference for both methods. The study was carried out in a 75 cow dairy farm over 6 months. 173 heat periods of 53 cows and 149 non-estrus periods (diestrus or early pregnancy) until day 28 were detected by progesterone profiles. The median increase in moving activity in relation to a activity average of the preceding 10 days was 111.6% by MV and 121.8% by AD. From a practical point of view, the MV system showed a sensitivity of 80.3% and a specificity of 45.6%. The AD system however showed a sensitivity of 84.4% and a specificity of 66.4%. The median of the required time to mount and maintain the devices were 38.4 minutes for markers in MV system and 2 minutes for the AD system. The median marker durability in MV system was 35 days, in AD system no device had to be changed during the trial period. In conclusion, both systems are applicable of distinguishing "estrus" and "no estrus" in dairy cows. However, the novel MV system showed no advantage over the established commercially available AD system. The project was supported by Schaumann Foundation.

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In vitro polysialylated Cervical Mucins Counteract the Cytotoxicity of Extracellular Histones

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Neutrophil extracellular traps (NET) can be formed during the invasion of pathogens to arrest and kill bacteria and fungi. However, numerous studies demonstrated that various diseases are directly related to this meshwork of DNA, histones and antimicrobial factors. Furthermore, the female reproductive system responds to the insemination with a severe invasion of neutrophils and NETosis. The binding of sperm to released NET could be associated with a decreased fertilization. The semen included DNases destroying the DNA-meshwork but the extracellular histones are still present representing the major cytotoxic component of NET during these processes. Recent studies demonstrated that polySia chains attached to N-glycans of NCAM and ST8SiaII are present on the surface of sperms in ejaculates. These polysialylated proteins may counteract the cytotoxicity of released histones. We wanted to combine the capacity of sialic acid polymers to counteract histone mediated cytotoxicity with the possibility to reduce adhesion of neutrophils using mucins. To this end, we examined mucins for their capability to serve as a target for bacterial polysialyltransferases. Intriguingly, bovine cervical mucins can be efficiently elongated by bacterial polySiaT *in vitro*. The resulting polymers can comprise of more than 50 sialic acid residues and succeeding cell experiments demonstrated the biological activity of elongated mucins against histone-mediated cytotoxicity. Thus, polysialylated mucins may represent a novel element to counteract pathological processes mediated by extracellular histones.

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Sonographic Characterization of Changes on Bovine Placenta, Uterine Wall and Foetal Membranes during Pregnancy

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The objectives of this study were to describe the sonographic anatomy of placenta, uterine wall and foetal membranes and to determine measurable changes during normal pregnancy in cows. Transrectal examinations were regularly performed using an 8 MHz linear transducer between week 6 and 43 of pregnancy. Size and appearance of different structures such as the endometrium, myometrium, height and width of placentomes, the thickness of the uterine wall with adherent chorion leave (CTUP) and the echogenicity of the foetal fluids were assessed. Measurement of uterine wall and placentomes were done close to the cervix, in the uterine corpus, in the middle and close to the tip of the pregnant and non-pregnant uterine horn. The height and the width of placentomes in the pregnant uterine horn showed a good correlation with the gestation period (height r = 0.78, width r = 0.83, both p < 0.0001). The size of placentomes increased steadily until week 27 of gestation followed by a flattening of the growth curve until week 31 of gestation reaching a plateau until week 43. In the non-pregnant horn smaller placentomes were found compared to the pregnant horn (p < 0.01). Placentomes in the area of the tip of the pregnant horn were significant smaller (height and width) than those in the middle of the horn, corpus or close to the cervix. Endometrium, myometrium and CTUP showed no measurable alterations throughout pregnancy. While the echogenicity of the allantoic fluid has not altered during pregnancy the amniotic fluid became more echogenic (p < 0.0001). Ultrasonographic monitoring of placentae may be a valuable diagnostic tool for the characterization of the placenta in bovine pregnancies.

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