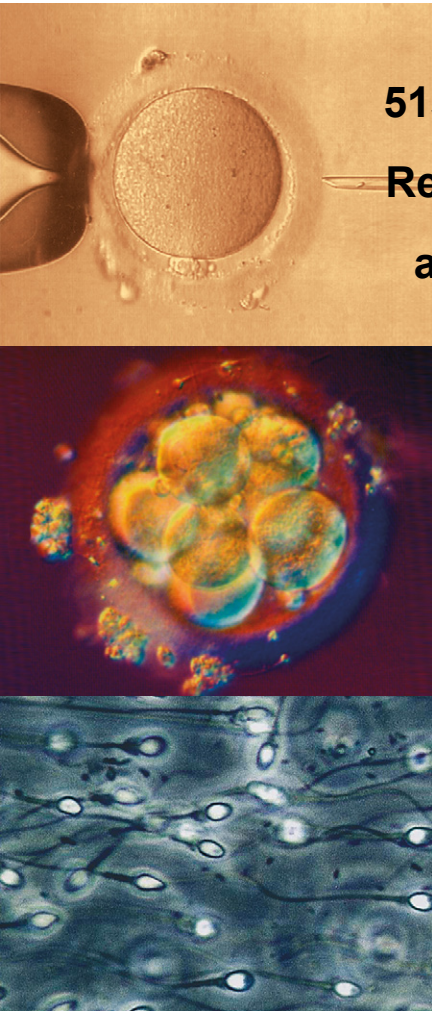


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**51st Annual Conference of Physiology and Pathology of
Reproduction and 43rd Mutual Conference of Veterinary
and Human Reproductive Medicine 21st-23rd February**

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51st Annual Conference of Physiology and Pathology of Reproduction and 43rd Mutual Conference of Veterinary and Human Reproductive Medicine 21st–23rd February, 2018, Hannover

Abstracts*

01

Pathological classification and etiological prevalence of cervicitis and vulvovaginitis in ewes suffering from infertility problems

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The purpose of this study is to investigate reproductive problems which may occur in ewes using pathological and bacteriological examination. One hundred ewes slaughtered in abattoirs at Sohag Governorate, Egypt, were included in this study. Their age ranged from 3–6 years. According to the owners complain, these animals suffered from many infertility problems such as repeat breeding and anoestrus. After slaughtering, the genital tracts were examined grossly. Bacteriological swabs were taken from the lumen of the uterus, cervix and vagina for bacteriological examination. Cervical and vaginal tissue samples were taken and processed for histopathology. The cervical lesions were classified into acute catarrhal cervicitis (7 cases), chronic catarrhal cervicitis (4 cases), acute necrotic cervicitis (1 case), and acute fibrinonecrotic cervicitis (2 cases). Vaginal and vulvar lesions were classified into acute granular vulvovaginitis (8 cases), chronic granular vulvovaginitis (2 cases) and ulcerative vulvovaginitis (2 cases). *Staph. aureus* was isolated from two cases (3.33%), *Staph. aureus* with *Streptococcus species* isolated from one (1.66%) case, *Streptococcus species* was isolated from three cases (5%), *Proteus species* was isolated from one case (1.66%), *E.-coli* associated with *Salmonella species* were isolated from one case (1.66%). The association between pathological and bacteriological findings was discussed.

02

Von Willebrand factor influences blood vessel architecture and the expression of Integrin α V β 3 and Ang-2 in the porcine uterus

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Aim Von Willebrand Factor (VWF) was shown to influence angiogenesis mainly via Integrin α V β 3 (ITG) and Angiopoietin-(Ang-) 2 *in vitro* and in mice. Since women with Von Willebrand Disease (VWD) suffer higher rates of miscarriages, we studied the blood vessel conformation and expression of VWF, ITG and Ang-2 by immuno-histochemistry (IHC) and qPCR of corresponding genes in uteri in a porcine model.

Methods Uteri were harvested from 6 sows with a natural mutation of the VWF-gene (2 heterozygous carriers [HC], 2 homozygous for VWD, 2 wildtype [WT]-animals). Phenotype and genotype were attested by blood VWF level and molecular genetic analysis. IHC and qPCR were conducted using *CD31* as housekeeping gene.

Results Blood vessels in the lamina propria of WT-sows were smaller than in the VWD-animals, which showed groups of dilated and thin-walled arteries and veins. VWF expression in VWD- and HC-pigs was reduced by 95% compared to WT-pigs, which was reflected by IHC. The mRNA expression of *ITGAV* and *ANG2* was more than doubled in the VWD-pigs, while the HCs ranged in between. In IHC stainings, ITG and Ang-2 showed varying staining patterns among the genotypes.

Conclusions The altered blood vessels in the WT-pigs can be classified as angiodyplastic, implicating an impaired angiogenesis. VWF seems to stabilize ITG at the apical membranes in WT-sows. In VWD-pigs, a stronger cytoplasmic staining of ITG is presumably caused by increased internalization of the protein. Ang-2 staining patterns are probably results of a varied, VWF-dependent storage capability.

03

Ovarian characteristics of the roe deer (*Capreolus capreolus*) during diapause

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Embryonic diapause, a period of developmental dormancy described in more than 130 species, is still not completely understood. In the roe deer, the only known ungulate that exhibits this phenomenon, the rut period occurs from mid-July to early August. Thereafter, implantation takes place not until late December/early January. Little is known from the female gonadal stage during this period. Here, we evaluated the roe deer as a model to study ovarian characteristics during the diapause period. Ovaries from a total of 47 hunted animals between October and January were collected and considered for the analyses. The ovarian surface area (mm²), total follicle number and corpora lutea (CL) diameter were evaluated according to the month of sampling. The estimated surface area of right (r) and left (l) side ovaries did not change during the studied period of diapause (Oct: [r] 60.8 ± 6.6, [l] 77.3 ± 8.2; Nov: [r] 82.8 ± 8.6, [l] 72.4 ± 8.6; Dec: [r] 74.0 ± 6.0, [l] 70.8 ± 6.6; Jan: [r] 67.1 ± 6.1, [l] 67.25 ± 6.2 mm², respectively) ($p > 0.05$). Contrary, the average total number of follicles decreased with the progression of diapause (Oct: 30.6 ± 4.4, Nov: 26.6 ± 3.0, Dec: 13.6 ± 2.6 and Jan: 17.2 ± 2.9) ($p < 0.05$). Additionally, CL were present in approximately half of the animals on both ovaries (26/47: 55.3%), and in all animals CL (2–3) were present during the diapause period. Average CL diameters for Oct, Nov, Dec and Jan were 6.2 ± 0.2, 7.3 ± 0.2, 7.1 ± 0.2 and 6.1 ± 0.4 mm, respectively ($p > 0.05$). Our results indicate that despite a lack of change of ovary size, the follicular development diminishes over time during the period of diapause in the roe deer.

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04

Human testis cancer control by immune cells – potential role of tumour infiltrating lymphocytes

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Introduction In human testicular germ cell neoplasia, i.e. seminoma and pre-invasive germ cell neoplasia *in situ* (GCNIS), infiltrating immune cells (T and B cells, macrophages, dendritic cells) are frequently present. Recent studies indicate that functional polarization and the respective subtypes of tumor infiltrating lymphocytes (TIL) including regulatory T cells (Treg) influence cancer development and immune-surveillance. Therefore, we aimed to identify and characterize subsets of T cells, i.e. Treg, in seminoma and GCNIS in comparison to non-neoplastic testes.

Material/Method Human testis samples (seminoma, GCNIS +/- lymphocytic infiltrates [ly], impaired spermatogenesis [hyp] + ly, and normal spermatogenesis [nsp]; n = 10, each) were analyzed by immunohistochemistry/fluorescence (markers: CD3, CD4, CD8, CD20, CD68, CD11c, CD25, and FOXP3). For cytokine expression profiles, quantitative RT-PCR was performed.

Results Preliminary results revealed that CD4+/FOXP3+ T cells (Treg) are located in immune cell infiltrates in neoplastic and non-neoplastic testicular tissue. Treg in nsp showed a scattered distribution of individual cells. Increased transcript levels of Treg-related cytokines IL-10 and TGF- β were associated with testicular pathologies.

Conclusion Our data suggest that TIL in testicular neoplasia comprise a subset of Treg cells. Detailed functional characterization of TIL in testicular neoplasia will help to elucidate the complex mechanisms of „immune editing“ during testis cancer development.

05

Transport of exfoliated epithelial cells in the immature epididymal duct is driven by smooth muscle cell contractions

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In the context of sperm transport, contractions of the adult epididymal duct (EpD) are well known. Some reports also described contractions of the EpD during development, but their function, regulation and potential impact on fertility during adulthood are unknown. We investigated human prenatal epididymis and identified cellular structures in the lumen of the EpD as exfoliated epithelial cells using antibodies against neutral endopeptidase as a marker for epithelial EpD cells [Thong et al. 2014]. After birth, exfoliated cells were also found in the EpD. Time-lapse imaging revealed directional movement of these luminal cells. The smooth muscle cell (SMC) contracting agent noradrenaline accelerated the transport while the relaxing drug sildenafil decelerated it. These effects on the transport were associated with contractions of the ductal SMC layer. Systematic analyses revealed comparable spontaneous contractions of the immature and adult EpD in caput, corpus and cauda. As shown above for the transport of cells, contractile frequency was also increased by noradrenaline and decreased by sildenafil. Our data suggest organized waste disposal in the EpD. This mechanism might be important during development to avoid infertility by luminal obstruction as hypothesized for cystic fibrosis.

06

Liquid preservation of biopsied *in vivo* derived bovine embryos

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Recently, we have developed an efficient procedure that is able to preserve biopsied, *in vitro* produced bovine embryos for up to seven days under hypothermic conditions. The storage medium is composed of TCM199 in air supplemented with 25% fetal bovine serum [Blad-Stahl N, et al. FBS. Reprod Dom Anim 2017; 52 (Suppl.1); 11]. The aim of the present study was to preserve biopsied, *in vivo* generated bovine embryos for up to seven days under hypothermic conditions (0–4°C) via the same protocol. Superovulated heifers were flushed at Day 6. Transferable embryos were biopsied, followed by a liquid preservation (LP) in TCMair supplemented with 25% FBS. Non-biopsied embryos served as controls. The live-dead staining was applied to calculate the total cell number and the live-dead ratio. Survival and hatching rates were also documented; survival/hatching rates: embryos without LP (biopsied 100% [5/5]/80% [4/5], control 100% [5/5]/100% [5/5]); embryos with LP (biopsied 33% [4/12]/0% [0/12], control 67% [8/12]/8% [1/12]). Without LP, total cell numbers were

similar in embryos after 3 days of culture (biopsied 121.8 $\pm$ 29.5; control 122.4 $\pm$ 29.0). These embryos showed a live-dead-cell ratio of 13.8 $\pm$ 6.5 and 17.1 $\pm$ 7.7, respectively. Immediately after LP, total cell numbers were 77.2 $\pm$ 27.2 (biopsied) and 73.3 $\pm$ 15.4 (control) and the live-dead-cell ratio was 8.5 $\pm$ 3.8 and 11.3 $\pm$ 4.5. After 3 days of culture after LP, the results were as follows: total cell numbers 81.5 $\pm$ 31.6 (biopsied) and 84.1 $\pm$ 20.0 (control); live-dead-cell ratio 0.2 $\pm$ 0.3 and 0.7 $\pm$ 0.7. These data show that *in vivo* derived embryos do not survive LP as do the *in vitro* counterparts. (The financial support of the Förderverein Bioökonomieforschung e.V. [FBF] is gratefully acknowledged.)

07

Reliability of an automatic heat detection system using tri-axial accelerometer in dairy cattle on pasture

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Heat detection in dairy cattle is determining reproductive performance and economic output on dairy farms. Many automatic heat detection aids have been developed to assist this difficult and time consuming task. Sufficient studies for the use in pasture-based systems are lacking. Results from studies conducted in research facilities with livestock maintained indoors cannot be transferred to pasture management due to differences in animal activity and weather effects. Dairy cows (n = 106) in an Irish commercial seasonal-calving herd managed at pasture were fitted with an tri-axial accelerometer (HerdInsights, Alanya Ltd, Cork, County Cork, Ireland) during the breeding season. The system generated estrus alerts automatically. Alerts were validated through transrectal ultrasound examination and milk progesterone measurements. For classification of false negative alerts, visual observations of the farmer were listed and the number of theoretically expected oestri was determined. Examination results were used to confirm the occurrence of oestrus and calculate efficiency, accuracy and sensitivity of detection of oestrus. Efficiency of the automatic heat detection system was 86.8% for the first 21 days after the start of the breeding season, 98.1% for 42 days and 100% for 63 days. Accuracy of the automatic heat detection was 72.2% and sensitivity was 93.3%. The data collected confirmed the suitability of a first-tested automatic heat detection system for dairy cows on pasture. In addition to the pasture management, the multimetric analysis of behavioural data was key for a satisfying sensitivity. Due to a high proportion of false positive alerts, the use as a stand-alone system for heat detection cannot be recommended.

Expression of Connexin 43 and androgen receptor in testes of azoospermic dogs

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Azoospermia represents one of the most common reasons for infertility in the male. Although this finding is common in dogs, too, the underlying testicular changes are poorly characterised. We recently identified immune cell infiltration in 9 of 10 testicular biopsies from dogs suffering from non-obstructive azoospermia (NOA). Here, we aimed to obtain further insights into canine NOA (n = 10) by investigating the expression of the androgen receptor (AR) and the gap junction protein Connexin 43 (Cx43). Five healthy dogs with normal semen quality served as controls. Immunohistochemistry against AR and Cx43 was performed and evaluated including quantification of the immunopositive area (PIA) and the staining intensity (mean gray scale) by means of ImageTool 3.0. For AR, Sertoli, Leydig, peritubular and perivascular cells, and some spermatogonia were immunopositive. PIA and mean gray scale (p = 0.0013) were significantly lower in the tubuli of azoospermic dogs (PIA p = 0.0097; mean gray scale p = 0.0013), whereas only the mean gray scale differed significantly regarding interstitial AR expression (p = 0.0092) from the controls. For Cx43, immunopositive staining was found in the parabasal compartment within the Sertoli cell cytoplasm in the controls, but staining was diffuse in the tubuli of the azoospermic dogs. Neither PIA nor mean gray scale differed significantly between groups. Whereas especially tubular AR expression seems to be reduced in testes of azoospermic dogs compared to healthy controls, Cx43 distribution, but not quantity was affected in our samples indicating a disruption of the blood testis barrier. (We acknowledge funding of the GfK for this project.)

Characterization of inositol trisphosphate receptors in boar spermatozoa

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An inositol 1,4,5-trisphosphate receptor-gated channel (ITPR) is implicated in the regulation of Ca²⁺ from intracellular membrane stores in sperm of several species. The aim of this study was to characterize the protein expression and localization of ITPR in boar spermatozoa and to determine whether ITPR

is involved in the regulation of intracellular Ca²⁺ concentration. In Western blot analysis of sperm from 2 boars (2 replicates per boar) ITPR was detected with a size bigger than 250 kDa. Using fluorescence microscopy ITPR was localized mainly at the post-acrosomal and neck region of spermatozoa. Changes in free intracellular Ca²⁺ concentrations were monitored with Fluo-4 in viable sperm using continuous flow cytometric measurements for six minutes (n = 6 boars). After addition of Thimerosal, an ITPR sensitizer, a 2–3-fold increase in Fluo-4 fluorescence intensity indicated a rise in the intracellular Ca²⁺ concentration within 80 sec after addition irrespective of the extracellular Ca²⁺ concentration. Pre-incubation with ITPR-inhibitor 2-aminoethoxydiphenyl borate (2-APB) for 15 min before Thimerosal addition delayed the Thimerosal-induced rise in the intracellular Ca²⁺ concentration by 20 sec only in presence of 2 mM extracellular Ca²⁺. In conclusion, boar spermatozoa express ITPR and this receptor-gated calcium channel has regulatory function on intracellular calcium levels. The need of increased extracellular (and intracellular) calcium levels for the modulator to take effect indicates a co-regulatory function of Ca²⁺ on the inositol 1,4,5-trisphosphate receptor-gated channel in boar spermatozoa.

Effects of postpartum intrauterine treatments on reproductive performance of dairy cows

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Main objective of this study was to determine effects of intrauterine infusion of antibiotics or antiseptics on fertility in first lactation postpartum dairy cows. In a controlled field trial 380 dairy cows, 17–30 days in milk (DIM), were randomly assigned to 3 treatment groups and one control group. Vaginal discharge and uterine position were scored according to a scale ranging from 1 to 4, as described in the literature. In group LUGOL (n = 118), cows received Lugol's iodine solution, diluted with physiological saline solution (150 ml, 0.7%) in form of an intrauterine treatment. In group ANTIBIOTIC (n = 89), cows received 5.5 g oxytetracycline hydrochloride, 0.2 g trimethoprim and 1.1 g sulfadoxine combination, solubilized in physiological saline solution (150 ml), in form of an intrauterine treatment. In group PERACETIC (n = 81), cows received peracetic acid solution, diluted to 5% with distilled water (150 ml), in form of an intrauterine treatment. Cows that did not receive any treatment were regarded as control group (CONTROL, n = 92). Reproductive performance measures

showed significant differences between the PERACETIC and other groups. Conception rates to all services and percentages of cows being pregnant by 260 DIM were significantly lower in group PERACETIC than in group CONTROL (p < 0.01). Pregnancy rate in groups ANTIBIOTIC, LUGOL, PERACETIC and CONTROL were 62.9%, 52.5%, 33.3% and 56.5%, respectively. Culling rate was higher in group PERACETIC than in the other groups (p < 0.01). The results of this field trial suggest that postpartum peracetic acid treatment is detrimental to fertility in dairy cattle.

Evaluation of different methods for IgG measurements in foals

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Failure of transfer of passive immunity is one of the main risks for infectious diseases in neonatal foals. The transfer of colostral immunoglobulins can be evaluated by measuring the IgG in the blood. In this study we compare different methods to check IgG concentrations in the blood of foals. Immunoglobulins were evaluated by SNAP-Test (SNAP Foal IgG Test, IDEXX) and ELISA, as well as the gammaglutamyltransferase activity and total protein was measured. The ELISA was the gold standard. Blood samples from 54 foals were taken before first suckling and after 12 hours post natum. No statistically significant correlation between the IgG concentration 12 hours post natum and the sex of the foal, the date of birth, the time until the first contact with the udder, the age of the mare, the numbers of foalings or the duration of gestation could be detected. There was a statistically significant correlation between the results of the SNAP-test and the measurement of the total protein (p = 0.00001, r = -0.5), the activity of gammaglutamyltransferase (p = 0.0064, r = -0.37) and the IgG concentration with ELISA (p < 0.0001; r = -0.54). The results of the SNAP-Test showed a very high accuracy with the ELISA (96%). In the ranges < 400 and 400–800 mg/dl the accuracy was 100%, whereas at values > 800 mg/dl the accuracy was 98%. The results confirm that the SNAP-test can routinely be used for the evaluation of the IgG in foals.

Fertility of bitches after caesarean section – preliminary results

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Studies about the fertility of dogs after caesarean (c)-sections are rare. Aim of this study was to evaluate fertility parameters of bitches which gave birth by caesarean section (group 1) and compare them with bitches

which underwent natural parturition (group 2). Owners of breeding bitches were asked to complete an online form to gain the information. The fully completed forms from 20 bitches after c-section and 20 bitches after natural birth were evaluated. For 14 bitches in group 1 it was the 1st birth, only 6 bitches had previous litters. In group 2 only 4 bitches had a previous litter, for 16 bitches it was the 1st parturition. None of the bitches had a previous c-section. Thirtytwo of the 40 bitches belonged to the breeds Boxer, Boston Terrier, Border Collie and Golden and Labrador Retriever. The four remaining dogs of group 1 belonged to the breeds Giant Schnauzer, Scottish Terrier, Mini Bullterrier, Petit Brabancon, whereas the four remaining dogs of group 2 belonged to the breeds Australian Shepherd, Great Dane, Poodle and Continental Bulldog. The mean weight of the dogs was 23.7 ± 10 kg (group 1) and 23.8 ± 12.4 kg (group 2), respectively. The mean age at the first c-section was 3.6 yrs. The indications for c-section were uterine inertia (8), transversal presentation of the fetus (6), single puppy (2), dead fetus (1), fetomaternal disproportion (1), uterine spasm (1) and incomplete abortion (1). The mean interval between heats was 7.1 ± 1.7 months in group 1 and 7.5 ± 1.9 months in group 2. The interval from birth to next heat was 7.3 ± 2.1 months in group 1 and 7.6 ± 2 months in group 2. In the majority of bitches, the 2nd heat after c-section or natural parturition, respectively, was used for next mating. All bitches in both groups conceived. In group 1 6.0 ± 2.4 puppies were born by c-section and 5.6 ± 2.0 in the following litter, whereas in group 2 7.1 ± 1.8 puppies were born in the first and 6.5 ± 2.6 puppies in the following birth. In 10 dogs of group 1 the following pregnancy ended up by c-section. Indication for c-sections were uterine inertia (3), fetomaternal disproportion (2), dead puppy (1), uterus rupture (1), uterus torsion (1) and posterior longitudinal presentation with ventral position (1). Only one elective c-section was performed due to owners wish without medical indication. The dogs belonged to the breeds Golden Retriever (4), Border Collie (2), Boxer (1), Great Schnauzer (1), Scottish Terrier (1) and Mini Bullterrier (1). Only 2 dogs of group 2 had a c-section in the following pregnancy. These results indicate that caesarean section does not have a significant impact on subsequent fertility or litter size. However, the results might suggest that after a caesarean section the need of another caesarean delivery in the next pregnancy might be increased.

13

Application of real-time ultrasound elastography in the mare's uterus

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Elastography is a new noninvasive technique for evaluation of tissue elasticity using conventional real-time ultrasound equipment with modified software. This new technique can be used to assess tissue elasticity by detecting tissue deformities occurring after sequential movements of compression and relaxation determined by the operator with the ultrasound probe. The aim of this study was to evaluate real-time qualitative ultrasound elastography as an adjunct to conventional sonography for the reproductive organs in mares. The ultrasound examinations were carried out on 6 Warmblood mares at the age from 6–20 years. All mares underwent imaging with an ESAOTE MyLab Alpha system using a linear 3–11 MHz probe. The *corpus uteri* was examined in different stages of estrus cycle and the Average Percentage of Pixels of Each Color (APPEC) was calculated. For the qualitative analysis, a categorical assessment was performed based on a grading scale of 1–4 (1 = mostly hard, 2 = intermediate hard, 3 = intermediate soft, 4 = mostly soft), whereas tissue stiffness was depicted by a color scale (blue = hard, red = soft). There were no significant differences in elastography between mares in the same stage of cycle ($p > 0.05$). There were significant differences ($p < 0.05$) in elastography between estrus (more yellow/red areas) and diestrus (more blue and green areas). APPEC scales (mean% $\pm$ SEM) in estrus vs diestrus were $44.8\% \pm 6.34$ vs $32.6\% \pm 3.57$ by scale 1, $27.2\% \pm 4.21$ vs $5.1\% \pm 1.32$ by scale 2, $7.2\% \pm 2.06$ vs $24.8\% \pm 2.27$ by scale 3 and $22.8\% \pm 4.54$ vs $34.83\% \pm 3.57$ by scale 4. The real-time qualitative ultrasound elastography is a feasible adjunct to examine elasticity of the mare's uterus.

14

Interstitial Cajal Like Cells (ICLC) as a marker of pathological changes in mare's myometrium

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Dysfunction in contractile activity of mares reproductive tract prevent the processes of fertilization, recognition and maintaining an early pregnancy, implantation, development of the embryo and mechanisms of self-purification of the uterus, which may result in early death of embryos. Simultaneously higher risk

of embryo death was observed in correlation with progression of endometriosis. The aim of the study was to verify suggested correlation between the progression of endometriosis and dysfunction in contractility of the mare uterus. The alteration in Interstitial Cajal Like Cells (ICLC) density was estimated by immunofluorescent methods (IF) as a cellular indicator of ability to generate and propagate electrical slow waves. The uterine samples obtained from 60 mares were stained with hematoxylin eosin (HE), Masson's Trichrome Stain (MTS), labeled with specific c-kit/CD117 markers and imaged using light and confocal microscopy as well as quantified under scanning cytometry. Samples stained with HE and MTS were classified according to Kenny and Doig [Kenney and Doig, Equine endometrial biopsy. In: Morrow DA (ed). Current Therapy in Theriogenology, W.B. Saunders, Philadelphia, 1986, 723–9] into group I, IIa, IIb and III. In corresponding samples density (mean% $\pm$ SEM) of c-kit positive cells has been analyzed. In corpus uteri, significantly higher ($p < 0.0001$) ICLC density was demonstrated in group I ($4.20\% \pm 0.45$) in contrary to group III ($2.11\% \pm 0.12$). No differences between groups IIa and IIb were found. We suggest, that pathogenesis of endometriosis is connected with decreased of number of peacemaker cells which are crucial for proper contractile activity of the uterus. The density of ICLC may be considered as a useful marker of pathological changes in the mare's myometrium.

15

The effects of repeated anesthesia on fertility and stress parameters in common marmosets (*Callithrix jacchus*)

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Common marmoset monkeys are valuable model organisms in biomedical research. However, it is important to minimize stress for the monkeys, also in order to obtain research data that are not biased by stress. We derived embryonic stem cell lines from natural marmoset embryos, which were obtained by noninvasive or minimal-invasive uterus flush regularly performed once per month. The reproduction cycle was recorded via progesterone profiling of blood samples. Five to eight days after ovulation the uterus was either flushed minimal-invasive with a transabdominal puncture of the uterus or non-invasive via a transvaginal, inserted catheter under short-term anesthesia. The number of retrieved embryos was used as a fertility parameter. Cortisol levels in blood samples taken at the initiation of the experiments and at least 2 years later, served as a stress parameter. Eight animals were used in up to 50 embryo retrievals. The average number of retrieved embryos did not decline with an increase in the number of anesthetics. Decade-wise analysis of embryo retrievals surpris-

ingly revealed that the embryo retrieval rates even increased during the last 10 embryo retrievals. Mean cortisol levels showed a clear inter-individual variation over all timepoints. Importantly, cortisol level from the initiation time point quickly decreased in nine females and remained at a relatively low level for approximately 2 years. These data indicate that long-term use of clinically healthy female common marmoset does not impair fertility nor enhances serum cortisol levels and animal numbers can be reduced according to the "3R" principle.

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The genotyping by ADSRRS – fingerprinting of *Staphylococcus aureus* isolated from milk of cows with mastitis in the North-East Region of Poland

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Staphylococcus aureus is an important etiological factor in cows' mammary gland inflammation. Its significance systematically increased in the studied region of north-eastern Poland. The aim of the study was the phenotypic and genotypic characteristics of *S. aureus* strains in this region, which could explain the increase in *S. aureus* in mammary gland infections in the examined region. Isolated strains were evaluated on the basis of colony and cell morphology, susceptibility to selected antibiotics, including methicillin. The ability to produce betalactamase, lipase, decomposition of mannose, ribose and mannitol has been studied and the pathogenicity; i.e. the adhesion to the epithelial cells of the mammary gland, which allows colonization of tissues and the spread of infection and the formation of slime and biofilm that promotes the survival of bacteria in the environment. Forty-five strains were genotyped with ADSRRS-fingerprinting. Genotypic assay has identified different amplification profiles, consisting of 9 to 13 DNA fragments of 200–1600 bp. Nine genotype groups were identified, of which D genotype was predominant. Strains of this genotype in 41.4% produced betalactamase, which contributes to greater resistance by antibiotics in practice. Genotype D strains exhibited greater adhesion than those of the other genotypes and up to 55.2% of these strains produced slime and 69% produced biofilm. Genotypic analysis showed that in the first year of the study 14.29% belonged to genotype D, while in the following year genotype D was already 73.64%. It can be concluded that the significant increase in infections with these *S. aureus* was related to the appearance of high pathogenicity, ability to spread and great ability to infection. In conclusion, ADSRRS-fingerprinting technique could be a useful tool for the screening of genome differentiation and helpful in epidemiological studies of *S. aureus*.

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Cranial stumps of premature oviducts redeveloped into fully differentiated oviducts in laying hens

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In birds that are kept as pets, hysterectomy is a therapy for abnormal laying activity. The aim of this study was to investigate whether hysterectomy is a possibility to stop egg production in laying hens as well. A total of 36 hens were hysterectomized, 18 of them in the 12th week of age, 18 in the 14th week of age. The hysterectomy was performed as follows: An incision with a length of about 1.5 cm was made at the left side of the hen, between the last rib and the vertebral column. The oviduct was grasped with forceps and, after preventing hemorrhage via ligatures, cut at the cranial and caudal end and taken out. The incision was sealed with single stitches. In some cases, a small cranial stump of the oviduct, less than 1 cm in length, was left in the hen to avoid ruptures. After the surgery, all hens were examined daily via ultrasonography daily to see whether there were any follicles in the abdomen. In the 22nd week of age we found calcified eggs in the ultrasound of some of the hysterectomized hens. We decided to terminate the study and euthanized all hens. In the following dissection we saw that all laying hens in which a cranial stump of the oviduct had been left possessed a fully differentiated, newly developed oviduct. Only the connection to the cloaca was missing. We assume that the cranial part of a premature oviduct is able to develop into each cell type in laying hens, including uterus cells with the ability to form egg shells.

18

Activation of apoptosis pathways in human spermatozoa – relationship between apoptosis, semen parameters and outcomes of assisted reproduction techniques (ART)

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Apoptosis plays an important role in the testis by controlling number and eliminating defective germ cell during spermatogenesis. Ejaculated spermatozoa, particularly in infertile men, have been shown to display numerous features that are typical of apoptosis in somatic cells including Fas expression, ROS production, activation of caspases,

DNA fragmentation, reduction in mitochondrial membrane potential, plasma membrane translocation of phosphatidylserine and permeability. The goal of this study was to investigate which pathways of apoptosis (after treatment) can be activated and whether caspases can be autoactivated (after 3 h incubation) in the sperm of 19 healthy men, (normozoospermic) and in 27 infertile patients with impaired semen quality. Moreover, it was examined if there is any difference in levels of activated caspases-8,-9,-3/7 between the two groups. The correlation between semen parameters and the outcomes of ART was established. Anti-Fas treatment resulted in apoptosis type I both in normal and abnormal sperm reflected by a significant increase in the activation of caspase-3/7, -8 and -9. A significant increase of the percentage of spermatozoa containing activated caspase-9 in normal and abnormal sperm was found after betulinic acid treatment. Autoactivation was observed in both groups. No significant difference were found in the increase of the percentage of spermatozoa with activated caspase-3/7, -8 and -9 after induction of type I, II, apoptosis and apoptosis autoactivation between normal and abnormal sperm. Only few significant correlations between activation of caspases and 1) the semen parameters, 2) the ART were observed.

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FTO protein linked with obesity and insulin resistance development

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Introduction Genome-wide association studies (GWAS) linked single nucleotide polymorphisms (SNPs) within introns of Fat mass-and obesity-associated gene (FTO) with obesity and type 2 diabetes. The role of FTO, product of this gene, in this association is still unclear. The aim of present study was to find target tissues and type of cells relevant for obesity and diabetes type 2 development. Porcine tissues were used since pig is regarded as an optimal model for human to study metabolic disorders.

Material and Methods Porcine tissues were sampled after 6 months of different dietary treatments: control (C, according to NRC dietary requirements), low energy (LE, 50% energy intake, n = 6) and high energy (HE 150% energy intake). Each group consisted of 6 pigs. FTO expression level was measured by Western blotting, mapping by

in-tissue cytometry and visualized in confocal microscopy.

Results Western-blot analysis revealed high FTO expression level in the cerebellum, hypothalamus and kidney, and low expression in the gastrointestinal tract (apart of salivary gland), muscle and adipose tissue regardless of energy intake. In-tissue cytometry confirmed that in some tissues, FTO was abundantly expressed in the specific areas or in the selected type of cells (high in insulin producing beta-cells, near to intralobular bile ductuli and the Kupffer cells, in the medullar part of adrenal gland). Moreover, the level of this protein is regulated in some tissues as in the adipose tissue, pancreas, adrenal gland by energy intake.

Conclusions Diet dependent changes of the FTO level confirm the hypothesis that FTO may directly influence for obesity and diabetes type 2 development. In tissues where the level of FTO is low, FTO may occur in the specific type of cells and its abundance may be of help to better understand its role.

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Different semen extenders and varying seminal plasma concentrations affect bovine NETs formation

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In cattle, the natural site of semen deposition is the vagina. However, artificial insemination introduces variable amounts of seminal plasma (SP) into the uterus. Polymorphonuclear neutrophils (PMN) are able to form „Neutrophil Extracellular Traps“ (NETs) extruding their DNA. The aim of the present study was to investigate the effect of different semen extenders supplemented with various proteins or varying SP concentrations on NETs formation. Semen extenders from 2 companies were supplemented either with no animal protein or egg yolk or an egg yolk like substance. SP was added to the incubation medium in concentrations of 1, 3, 5, 10, 15 and 20%. After incubation of PMN and extenders or the different amounts of SP, DNA quantification was performed by spectrofluorometric analyses via PicoGreen staining. Relative fluorescence intensities (FI) calculated from at least 9 experiments were statistically analyzed employing ANOVA followed by a Tukey test. The 2 animal protein-free extenders showed similar results. A significantly higher FI was observed in one of the extenders supplemented with egg yolk. The same held true for one extender completed with an egg yolk like substance. Relative FI significantly increased from 1 to 5% SP, followed by a slight decrease up to a concentration of 20%. These data indicate that NETs formation is dependent on the composition of the extender

itself and the protein source used by different companies. Furthermore, NETs formation is also dependent on the dose of SP used. From these results it can be speculated that semen extenders plus additives and SP may contribute to reduced fertility. (The financial support of the Förderverein Bioökonomieforschung e.V. [FBF] is gratefully acknowledged.)

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Administration of pegbovigrastim reduced the incidence of acute puerperal metritis in primiparous cows in a German Holstein dairy herd

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Recently, it has been shown that treatment with recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol (PEG rbG-CS, pegbovigrastim) is a well-tolerated approach to overcome periparturient immune suppression in dairy cows [Canning et al. J Dairy Sci 2017; 100: 6504–15] by increasing the number of polymorphonuclear neutrophils (PMN) in peripheral blood and exocytosis of myeloperoxidase by stimulated PMN [Kimura et al. J Dairy Sci 2014; 97: 4842–51]. Thus, we analyzed the effects of periparturient pegbovigrastim injections (ImrestorTM, Elanco Animal Health) on the incidence of acute puerperal metritis (APM) and the antibiotic doses necessary to treat APM. In a Saxon dairy herd, 169 highly pregnant heifers were randomly assigned to the treatment group (IMR: n = 82) who received 15 mg pegbovigrastim subcutaneously 10 ± 3 days before the anticipated calving date and within 24 hours after calving (label use), or to the untreated control group (Co: n = 87). Using logistic regression and Cox regression models, administration of pegbovigrastim was demonstrated to reduce the incidence of APM (IMR: 22.7%, Co: 43.9%, p = 0.003; HR [Co] = 2.32, 95%-CI = 1.71–2.92, p = 0.007). Moreover, number of antibiotic doses per calving to treat APM was lower in pegbovigrastim group (IMR: 0.32 ± 0.66, Co: 0.59 ± 0.75, p = 0.005). Milk yield and milk compounds on the first test day, and incidence of clinical mastitis during the first 30 days in milk did not differ significantly. These results encourage to further research on effects of pegbovigrastim in prevention of uterine diseases.

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Application of an ELISA pregnancy test of PAG in a herd of beef cattle in Poland

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Pregnancy-associated glycoproteins (PAG) are secreted by the binucleate giant cells of the ruminant placenta and then enter maternal circulation. Their presence in maternal serum has long been recognized since day 22nd day after postmating. PAG concentration in maternal serum has been recognized as an indicator of pregnancy which may be useful in the reproductive management of cattle. ELISA pregnancy test detects a subset of PAG as a management tool designed for dairy cows. The potential for using the PAG ELISA test in beef cows, especially in big herds in pasture-mating system when other methods for detecting early pregnancy in cattle (rectal palpation (RP) and USG examination) are difficult to perform. This study demonstrated application of an ELISA pregnancy test of PAG in a herd of beef cattle in Poland. The serum PAG ELISA, RP and USG results were compared for pregnancy diagnosis in beef cows with previous bull exposure. Serum samples were collected over breeding season in 2016 from Limousin beef cows (n = 95) maintained in pasture-mating system. Cows were exposed to a bull and underwent pregnancy examination (RP, USG) between 1 and 7 month after mating. The presence of PAG in serum was determined using antigen-capture ELISAs. Pregnancy status of open and pregnant corresponded to serum SN values of < 0.30 and ≥ 0.30, respectively. When compared to RP and USG finding the performance of serum PAG ELISA was sensitivity of 95.6% (97.8%–100.0%) and specificity of 100% (100%–100%). The positive and negative predictive values were 100% (100%–100%) and 55.6% (71.4%–100%), respectively. We conclude that serum PAG ELISA is accurate in predicting pregnancy and is useful for breeding management in pasture-mating system of beef cows.

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Association between sperm epigenetics and male subfertility: retrotransposon suppression and nucleosome preservation patterns

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Aberrations in the sperm epigenome are associated with male subfertility. Our previous work revealed that the majority of sperm nucleosomes retained in sperm chromatin occurred in repetitive DNA elements like

LINEs and SINEs. LINE-1 (L1) is a retro-transposon, which is normally suppressed by DNA methylation. Changes in L1 methylation can affect functions of sperm chromatin. In somatic cells the heterochromatin marker H4K20me3 is associated with repression of LINEs and SINEs. The aims of this study were to analyse if spermatozoa of subfertile patients (ICSI) and healthy donors differ in L1 methylation and to investigate the presence of H4K20me3 in mature sperm and healthy human testis tissue. L1 methylation was analysed by Elisa and pyrosequencing. H4K20me3 was investigated through immunohistochemistry staining and western blot. L1 methylation was significantly increased in immotile sperm of donors compared to motile sperm of patients ($n = 84$, $p < 0.01$, Mann-Whitney U-test) and donors ($n = 76$, $p < 0.01$, Mann-Whitney U-test). In patients, who achieved a pregnancy, the fertilization rate was significantly positive correlated to L1 methylation ($n = 21$, $p < 0.01$, $r = 0.56$, Spearman rank correlation). H4K20me3 was detectable from spermatogonia up to early elongating spermatozoa. Western blot confirmed the presence of H4K20me3 in mature sperm. Our study shows that immotile spermatozoa possess significantly increased L1 methylation. Fertilization rate after ART is significantly positive correlated to global L1 methylation. Moreover H4K20me3 is present at various stages of human spermatogenesis and retained in spermatozoa of healthy donors.

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Comparison of prolactin receptor (PRL R) expression and VEGF in feline mammary gland carcinoma, a preliminary study by confocal microscope

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Prolactin (PRL) is relevant in many tumor types (mammary gland tumors, prostate tumors, colon and anus tumors). PRL is considered as an oncogene activator. PRL could have a possible role in angiogenesis (affecting endothelial cells directly or indirectly via VEGF or affecting the FGF2/stat5). Some studies indicate a possible prognostic role of VEGF in feline mammary tumors and the role of growth factors and their receptors in promoting tumor proliferation. This study presents preliminary information about interactions between the expression of PRL R and VEGF in mammary gland adenocarcinomas in female cats. Samples were collected from 11 mature queens during radical mastectomy with adenocarcinoma III grade 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. For VEGF detection sections VEGF (C-1) monoclonal primary antibodies linked with Alexa Fluor 660 were used. In all 11 studied samples with adenocarcinoma high expression of VEGF was observed (mean $\pm$ SD) VEGF (21.39 ± 6.13). The inhomogeneous group of evaluated tumors were divided into two populations with high PRLR expression (45.07 ± 21.64) and with low PRL R expression (4.74 ± 4.22) based on assume Gaussian distribution and significant difference in parametric test ($p < 0.05$). In tumors with high PRL R expression, low statistical significant difference VEGF positive cells number have been found to compare with PRL R low expression group. According to obtained data, we can conclude that in adenocarcinoma III grade, mammary cells may lose the ability to code PRL R which is correlated with high VEGF expression pattern.

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Increased stillborn rate in a free farrowing system – a case report

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Nowadays, the target level of stillborn piglets ranges between 5 to 7% in hyper-prolific sows. Recently, a herd examination was conducted in a Swiss piglet-producing herd with 112 sows that showed an increased stillborn rate of 8.7%. The general physical examination of the sows before birth revealed no abnormalities. The birth process of ten sows was analysed for the birth management, the total duration of birth and the duration of piglet expulsion. In addition, material from stillborn and weak-born piglets was taken for further examination. All sows received a routinely intramuscular treatment of 35 μ g carbetocin during parturition, which led to a prolonged piglet-piglet interval directly after application due to muscular spasm of the uterus, high losses of colostrum and a high amount of weak and stillborn piglets. Five heart samples of stillborn piglets were tested for porcine circovirus type 2 employing histology and qPCR. Furthermore, serum samples of all piglets of one litter with a mummified piglet were checked for antibodies against porcine parvovirus and serum of ten weak-born piglets were tested for porcine reproductive and respiratory syndrome virus using a PCR. Hence, all test revealed no findings, these pathogens could be ruled out. In this case, the use of carbetocin during parturition led to severe undesirable side effects. After stopping the regular treatment with carbetocin and improving the birth management, the still born rate decreased to 4.6%. In conclusion, monitoring during the farrowing process with selective measures and no prophylactic use of carbetocin enhance the birth process and thereby piglets' survival.

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Quantitative measurement of udder edema in dairy cows using ultrasound to control the success of a diuretic treatment with furosemide

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The aim of this study was to record the course of periparturient udder edema with ultrasonography in dairy cows and to investigate the diuretic effect of a treatment with furosemide. For this purpose, initially a pressure sensor was developed for the ultrasound probe, which ensured the generation of repeatable and comparable data under similar pressure conditions. In 10 cows, ultrasonographic measurements (UM) were performed daily at four locations of each udder quarter, beginning 14 days (d) ante partum (a.p.) until 14 d post partum (p.p.). Furthermore, free oestrogens (E) in plasma, sodium (SO) and potassium (PO) in saliva and quarter milk samples (QMS) were analyzed for their influence on the degree of severity of the edema. Another 50 cows were randomly divided into two groups. The experimental group ($n = 25$) received 10 ml Dimazon® (500 mg furosemide) and the control group ($n = 25$) 10 ml 0.9% NaCl intramuscularly on Days 0, 1 and 2 p.p.. From 21 d a.p. until 21 d p.p. 15 UM were performed in three-day intervals, measuring the base of the teats. Furthermore, QMS were collected on Days 0, 7 and 14 p.p.. No association among plasma E, saliva SO and PO, occurrence of non-clinical mastitis or latent infections and severity of the udder edema could be found. The average thickness of the udder edema between the treatment groups did not differ significantly. In conclusion, a method for UM of udder edema was established. The base of the teat was a suitable location to monitor the characteristic temporal course of udder edema. Treatment with furosemide did not provoke a measurable, positive effect on the severity of udder edema.

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Collection technique for intrauterine fluid samples in mares

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A Salivette® (SAL, Sarstedt, Germany) is a cotton-based absorbent (1 $\times$ 2 cm) used for saliva collection in horses. It is a single-use device connected to a centrifugation tube. We aimed to develop a minimal-invasive method to collect intrauterine fluid (IUF) from mares with this device. The reproductive tracts of participating mares ($n = 12$, 4–15 y) were

examined prior to and 24 hr after collection. To assure removal a suture (Vicryl, Ethicon, Germany) was placed on the SAL. After aseptic preparation the SAL was grasped with a uterine biopsy forceps. The suture end was fixed by the free hand; the construct was covered with a cut-open rectal sleeve and guided into the uterus. After 10 min the SAL was removed and IUF was recovered by centrifugation. This procedure was repeated 1, 2, 4, 6 and 24 hr after the first collection. Volume recovered and appearance of IUF was recorded. Mares were normal during clinical and gynecological exams. Two mares developed IUF accumulation and increased neutrophil count 24 hr after collections. Two mares showed a mixed bacterial flora before and a different mare mixed low-grade bacterial growth after collections. Recovered IUF volume per SAL was between 0–1.5 ml (mean: 0.42, median: 0.3, SD: 0.45). Colour varied between clear (14.1%), yellow- (49.3%) and red-tinged (36.6%). Consistency was more often aqueous (78.9%) than viscous (21.1%). Differences in volume recovery were noticed. This was independent from age ($r = -0.42$). Time between collections was positively correlated to volume recovery ($r = 0.86$). The SAL is a safe and minimal-invasive method for IUF collection in mares.

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Low abundance of regulatory T lymphocytes in the endometrium of oestrous and early-pregnant mares

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Regulatory T lymphocytes (Tregs) are involved in maternal tolerance of pregnancy in different species. In the horse, low levels of Tregs in peripheral blood have been associated with early pregnancy loss. In the present study we investigated the presence of Tregs in the endometrium of oestrous and early pregnant mares. A breeding soundness examination was performed before start of the projects. In project 1, the uterus of oestrous mares (presence of endometrial oedema and preovulatory follicle > 35 mm; $n = 12$) was inoculated with PBS (20 ml; control), raw semen (20 ml) or spermatozoa-free seminal plasma (20 ml). After 24 h, an endometrial biopsy was collected. In project 2, biopsies were obtained from the endometrium of the pregnant (P; adjacent to the conceptus) and non-pregnant (NP) uterine horn of mares at day 16 ($n = 3$) and day 30 ($n = 3$) of pregnancy. Endometrial biopsies were assessed for Tregs (FoxP3+ cells, antibody Foxp3-PE, eBioscience, USA) by immunohistochemistry. Values are means $\pm$ standard error of mean. In project 1, the number of Tregs in the endometrium of oestrous mares was not influenced ($p > 0.05$) by treatment (control 73 ± 21 , semen 173 ± 62 , seminal plasma 128 ± 35 Tregs/cm²). In project 2, the number of Tregs in the endometrium of early pregnant mares

did not change from day 16 to 30 of pregnancy ($p > 0.05$) and was similar ($p > 0.05$) in the uterine horns (day 16: $P 43 \pm 15$, NP 100 ± 9 ; day 30: $P 180 \pm 99$, NP 218 ± 171 Tregs/cm²). Results demonstrate a very low abundance of Tregs in the endometrium of oestrous and early pregnant mares which was neither affected by insemination nor the presence of a conceptus.

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Membrane-bound steroid hormone-receptors and their expression pattern in testis tissue of humans and different domesticated species

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Steroid hormones not only act via classical nuclear receptors but can additionally bind to membrane-bound receptors resulting in a more rapid cell answer and non-genomic effects. We aimed to unravel the estrogen and androgen actions by analyzing the G protein-coupled estrogen receptor-1 (GPER-1), ZIP-9, a member of the Zinc-transporting-protein family, and G protein coupled receptor C6A (GPRC6A) as well as a novel possible significance of progestins in the testis by analysing the progesterone receptor α (PAQR7). RT-PCR revealed the expression of all genes in testicular tissue of humans ($n = 5$) and additionally in a panel of domesticated species including bull, stallion, boar, and dog ($n = 2$). We performed immunohistochemical analysis (IHC) in human testis biopsies ($n = 5$) and exemplarily in two samples of each species. While we were able to detect a common expression pattern for ZIP-9, GPRC6A, and PAQR7 in all examined species, GPER-1 showed an additional species-dependent localization in Sertoli cells (SCs) of the boar and haploid germ cells (GCs) in all animals but not in the human. The only testis-specific cell type immuno-positive for GPER-1 in the human were peritubular myoid cells (PTMZ). GPRC6A signal was located in GCs, PAQR7 in SCs and ZIP-9 was detected in GCs and Leydig cells (LCs). The expression patterns suggest a common (or an overall) biological function of these membrane-associated receptors in spermatogenesis, while there could be an additional function for GPER-1 in animal haploid GCs or boar SCs in contrast to men.

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Expectations of Scandinavian veterinarians and dog owners about effects of neutralisation

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Although neutering of pets is common, recent data show that it might have a significant negative impact on canine health, e.g. by increasing types of various cancers. In spite of a significant increase in knowledge about side effects, the expertise and expectations of veterinarians (V) and dog owners (DO) have to be investigated. To obtain data from V and DO in Scandinavia, 2 separate online questionnaires were used. The results were compared to the literature. A total of 374 V and 3,449 DO completed the questionnaires. Neutering was considered to be associated with several positive effects: reduced risk for mammary tumours (♀ : V: 93%, DO: 63%) and pseudopregnancy in bitches (♀ : DO: 57%), reduced risk for benign prostatic hyperplasia in male dogs (♂ : V: 88%, DO: 42%), reduced aggression against other dogs (♂ : V: 83%, DO: 60%), reduced urine marking (♂ : V: 89%, DO: 61%), reduced tendency for hyperactivity (♂ : V: 68%, DO: 51%), for roaming (♂ : V: 87%, DO: 64%; ♀ : V: 64%, DO: 44%), for hypersexuality (♂ : V: 95%, DO: 61%; ♀ : V: 81%, DO: 40%) and increased life expectancy (♀ : V: 45%). Expected negative effects of neutering were increased appetite (♂ : V: 91%, DO: 76%; ♀ : V: 90%, DO: 76%), increased risk for overweight (♂ : DO: 86%, ♀ : DO: 84%), for coat changes (♂ : V: 84%, DO: 63%; ♀ : V: 87%, DO: 65%), urinary incontinence (♀ : V: 95%; DO: 36%) and hypothyroidism (♂ : DO: 27%; ♀ : DO: 28%). In conclusion, a certain discrepancy between participants' expectations and the literature was identified with a tendency to underestimate potential negative effects.

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Collection of uterine secretion samples and their diagnostic value for subclinical endometritis in dairy cows

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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. A special device including among others a highly absorbent Merocel®-swab is developed to perform consecutive collection of cytological, histological, bacteriological and uterine secretion samples. 110 cows at a state farm herd between 45 and 60 days *post partum* are enrolled in this study. Information concerning stage of oestrous cycle, reproductive performance and uterine health is obtained by anamnesis, vaginal examination, plasma-progesterone determination, transrectal palpation and ultrasonography. The concentrations of pro- and anti-inflammatory mediators in the uterine secretion samples is determined using AlphaLISA®-technology. The new sampling tool shows high practicability under farm conditions and produces samples of good quality. Clinical, histopathological and cytological findings allow the assignment of the sampled cows to one of three groups “no endometritis”, “subclinical endometritis” and “clinical endometritis”. In uterine secretions the concentrations of immunomodulatory proteins reveal the following ranges: IL1 β 1–2731 pg/ml, IL6 0.0056–20.1 ng/ml, IL8 2.1–8978.8 pg/ml, IL10 1–798 pg/ml, IL17A 3–3597.7 pg/ml. To assess the diagnostic value of uterine secretion samples for subclinical endometritis statistical analysis will be conducted. (Supported by FBF.)

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Impact of storing canine testis-epididymis complexes overnight at 4°C on epididymal sperm quality before and after cryopreservation

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Freezing epididymal sperm is a way to preserve the genetic material of dogs with high emotional or genetic value that die suddenly or must be castrated for therapeutic reasons. It may however be necessary to ship epididymides or isolated sperm to a laboratory equipped for semen freezing. We evaluated whether overnight storage of testis-epididymis complexes submerged in 0.9% saline at 4°C is suited for preserving sperm quality. Both testis-epididymis complexes were obtained from healthy dogs (n = 7). One was directly processed for sperm isolation and freezing, the other was stored overnight at 4°C. Number of sperm recovered was not influenced by storage, and correlated with testis weight (r = 0.79) and volume (r = 0.78, both p < 0.05). Storage had no effect on viability (direct: 85.3 ± 9.4%, overnight: 85.3 ± 7.1%) or CASA total motility (65.6 ± 18.6%, 62.0 ± 14.7%) before freezing (p > 0.05), but increased the incidence of sperm with coiled tails (4.3 ± 4.8% vs 34.2 ± 18.4%; p < 0.05), probably due to an altered intra-epididymal

milieu. Organ storage had no impact on post-thaw sperm quality. Freezing and thawing reduced motility (to 20.6 ± 12.8%) and viability (to 60.4 ± 15.0%; both p < 0.05, n = 14), and increased the slow, linear motile sperm subpopulation (before 3.9%; after 42.7%) as determined by cluster analysis. Sperm isolated directly or from overnight stored organs did not differ in %DFI (4.2 ± 1.3% vs 4.8 ± 1.0%; p > 0.05). In conclusion, storing canine testis-epididymis complexes in 0.9% saline at 4°C was suboptimal for preserving epididymal sperm morphology. Isolating epididymal sperm on site and shipment in semen extender may be an alternative.

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Immunohistochemical examination of DMRTB1 in human testis with normal spermatogenesis and different testicular disorders

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The transcription factor DMRTB1 plays a pivotal role in coordinating transition between mitosis and meiosis in murine germ cells (GC). No reliable data are available for human testis. Thus, the present study aims to examine the testicular expression pattern of DMRTB1 in men showing normal (nsp) and impaired spermatogenesis. Immunohistochemistry was performed using 54 human testicular biopsy specimens and a commercial rabbit polyclonal Anti-DMRTB1 primary antibody. A strong immunoreactivity (IR) was detectable in a subset of spermatogonia (Spg) in patients with nsp. Some spermatocytes (Spc) showed a weak immunostaining and adjacent Sertoli cells (SC) were immunonegative. In patients with spermatogenic arrest at Spg stage, an altered staining pattern was found. No IR was detected in SC in Sertoli cell-only (SCO) syndrome. In GC neoplasia in situ (GCNIS) tubules, pre-invasive tumor cells were immunonegative, and seminoma cells also showed no immunostaining. According to previous findings in mice, it seems reasonable that DMRTB1 is expressed in these normal GC populations and that it probably plays a similar role in men. Moreover, absence of DMRTB1 in GCNIS cells and tumor cells might be associated with uncontrolled neoplastic cell proliferation and progression into invasive GC tumors. Further research is required to elucidate e.g. the function of DMRTB1 in Spc and to characterize the immunopositive Spg population in nsp or in spermatogenic arrests.

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Ultrastructure and immunohistochemistry of fetal macrophages and maternal myofibroblasts in the bovine placenta at parturition

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In the present study we use transmission electron microscopy (TEM) and immunohistochemistry (IHC) to characterise two cell types in the bovine term placenta: fetal stromal macrophages (Hofbauer cells) and maternal stromal myofibroblasts. TEM was carried out on specimen from 5 term placentae and IHC on paraffin sections from 4 term placentae. In the stroma of fetal villi TEM revealed the presence of one cell type with a highly vacuolated cytoplasm and abundant lysosomes. The cells frequently showed several processes. In IHC two antibodies (anti-LAMP1, a lysosome marker, and CD68 clone EBM11) bound to this cell type. In the maternal stroma branched cells with contractile filaments, dense bodies and adhesion plaques were identified by TEM. These cells were stained with alpha smooth muscle actin (α -SMA)-antibody. Our findings reveal new information about these two cell types: 1) The macrophages in the fetal stroma morphologically resemble fetal macrophages in the human placenta (Hofbauer cells). Hofbauer cells are M2-polarised macrophages and are involved in regulatory processes and defense against placental infections. The functional roles of bovine Hofbauer cells need to be investigated. 2) The myofibroblasts in the maternal stroma are likely to be involved in the postpartal involution of the caruncle and possibly in the release of fetal membranes at parturition.

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Calves of genetically selected heifers differ in blood cell composition and serum IgG1/IgG2 concentrations

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The aim of the study was to compare immunoglobulin G serum (IgG) concentrations and blood leukocyte subset composition of newborn calves from Holstein Friesian heifers genetically selected for favorable (Q) or unfavorable (q) udder health. Calves (n = 31, 16Q/15q) were fed colostrum from their dams (3 liters within 3 hours after parturi-

tion). Colostrum of Q and q heifers did not differ in IgG1 or IgG2 content. From day 2 onwards, all calves received milk replacer. In addition, half of the calves were supplemented with a colostrum preparation (0.5% colostrum powder B.I.O.Ig). Blood samples were collected immediately after birth (before colostrum uptake, P01) and twice per week until day 21 (P02–P08). Health condition was determined daily (score 1–11). Q calves appeared to be significant healthier than q calves (score ≥ 2.5 ; $p = 0.0092$). At P01, Q calves displayed significant more CD4⁺ T-cells ($p = 0.0104$) and a significantly higher CD4⁺/CD8⁺ T-cell ratio ($p = 0.014$) compared to q calves. At P04, Q calves showed higher amounts of intermediate monocytes ($p = 0.014$). Between P02 and P08, blood serum of Q calves showed higher levels of IgG1 (P02: $p < 0.01$) as well as higher IgG2 levels (P02, P03: $p \leq 0.05$). Supplementation with colostrum preparation significantly enhanced IgG1 and IgG2 serum levels selectively in Q calves. Thus, calves with a different genetic background differ in the development of their blood immune cell repertoire. Interestingly, the calves' genetic background also influences the immunomodulatory impact of a colostrum supplement.

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Impact of short-term protein supplementation on estrus, ovarian activity and blood metabolites in Ossimi ewes synchronized with PGF_{2α} analog (Cloprostenol)

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The objective of this study was to elucidate the effects of short-term high-protein diet supplementation on ovarian activity and metabolic status in Ossimi ewes synchronized with PGF_{2α} analog. Fourteen Ossimi ewes were divided into high protein (HPG; $n = 7$) and normal protein (NPG; $n = 7$) groups. Estrous was synchronized using double doses PGF_{2α} with 10 days apart. Five days before the second dose of PGF_{2α}, a high-protein diet 20% crude protein (CP 2.1 fold of maintenance) was fed to HPG for five days while NPG was offered maintenance diet throughout the experiment. Ovarian activity, progesterone, estradiol-17 β and blood metabolites level were assessed daily. Our results revealed that the duration of estrus period was significantly longer in HPG than in NPG (20.8 ± 3.2 vs 14.1 ± 3.4 h, $p < 0.05$). There was a significantly higher ovulation rate in HPG than that in NPG (HEG 2.4 ± 0.3 vs NEG 1.4 ± 0.2 , $p < 0.05$). Ovulation time took place

significantly longer with larger ovulatory follicles diameter in HEG group ($p < 0.05$). High protein diet had a significant effect on the number of the recruited follicles, and on the growth rate/day (HEG 1.2 ± 0.01 vs NEG 0.8 ± 0.02 mm) and diameter of the ovulatory follicle (HEG 6.5 ± 0.2 vs NEG 5.3 ± 0.2 mm, $p < 0.05$). A significant ($p < 0.05$) higher level of estradiol-17 β , total protein, albumin, total cholesterol and glucose was noticed in HPG than in NPG ewes. Serum concentration of P4 was not significant different between groups. We conclude that a short-term high-protein supplementation improves estrus expression, ovarian activity and metabolic status in PGF_{2α} analog synchronized Ossimi ewes.

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A preliminary study on the relationship between the cycle stages and vaginal temperature as well as steroid hormone concentrations in female camels (*Camelus dromedarius*)

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The current study aimed to investigate the relationship between vaginal temperature and stage of the estrous cycle as well as steroid hormones concentrations in camels. Three apparently healthy, non-pregnant and non-lactating dromedary camels (8–12 years) were inserted with blank CIDR attached with intravaginal data logger for 53 successive days. Estrous behavior was evaluated using active and virile male camels. Blood samples were collected and sera were used to measure estradiol and progesterone. Results revealed that mean vaginal temperature recorded at dawn (6.00h; $p = 0.04$) and noon (12.00h; $p = 0.05$) varied significantly between days of CIDR insertion and was higher than that recorded at dusk (18.00h) and mid night (00.00h). Vaginal temperature correlated significantly with ambient temperature ($r = 0.29$; $p = 0.0001$). Vaginal temperature increased markedly at estrus but high progesterone was detected along the experiment. Mean daily vaginal temperature significantly correlated with estradiol concentration ($r = 0.63$; $p = 0.001$), while there was a low positive correlation with progesterone concentration ($r = 0.19$, $p < 0.05$). In conclusion, vaginal temperature measured with data logger increased during peaks of estrogen levels and could also be a reliable method for the prediction of estrus in female camels. Further investigations are recommended to get a deeper insight.

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Relationship between movement patterns of sperm and flow cytometric parameters in frozen-thawed bull semen

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It was previously demonstrated that motile sperm can be grouped in specific clusters based on their kinematic characteristics. This study aimed to assess the correlations between the movement patterns of frozen-thawed bull sperm and other quality indicators as determined by flow cytometry. Frozen-thawed samples of 47 ejaculates from three Simmental bulls were analyzed by CASA (SpermVision 3.8) for kinematic parameters and a two-step cluster analysis was used to identify four motile sperm subpopulations. Additionally, two flow cytometric assays (CytoFLEX, Beckman Coulter) were performed: (1) sperm viability, determined using the FITC-PNA/PI staining and (2) the percentage of sperm with intact plasma membrane and acrosome, low levels of calcium and high esterase and mitochondrial activity, determined using a Multicolor assay with five fluorochromes (PI; Peanut Lectin; Cell-Trace™ Calcein Violet AM; MitoProbe™ DiIC1; Fluo-4 AM). The two flow cytometric parameters correlated positively with the proportion of sperm showing high velocity and above average linearity ($r = 0.55$, $p < 0.01$ and $r = 0.37$, $p < 0.05$, respectively) and negatively with the proportion of sperm showing slow and non-linear movements ($r = -0.34$, $p < 0.05$ and $r = -0.51$, $p < 0.01$, respectively). The flow cytometric parameters were rather correlated with these two motile sperm subpopulations than with the percentage of motile sperm ($r = 0.37$, $p < 0.05$ and $r = 0.27$, $p > 0.05$, respectively). Moreover, the sperm subpopulation characterized by high velocity and above average linearity showed a positive correlation with the potential of semen samples to maintain sperm motility during three hours of incubation at 38°C ($r = 0.67$, $p < 0.01$). These preliminary results suggest that the movement patterns of bull sperm are related to other sperm characteristics important for fertility of bulls. Further examinations will try to determine which subgroups of motile sperm are most important within ejaculates.

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Deciphering the immune cell composition in the adult mouse testis

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Introduction Mammalian testis is an immune privileged organ. In the adult murine testis resident macrophages have been identified as the predominant leukocyte. Comprehensive knowledge of the leukocyte composition is lacking. This study defines the immune cell composition in adult mouse testis.

Methodology Flow cytometry and fluorescence studies were performed on the CX3CR1-GFP mouse model. Interstitial cells were collected from mechanically-dissociated testes. Two antibody panels distinguished leukocyte subpopulations of myeloid [F4/80 (macrophages) and CD11c (dendritic cells)] and lymphoid [T cells (CD3) and NK cells (NK1.1)] lineages. Cells were analysed using a BD LSRII flow cytometer in conjunction with matched isotype and fluorescence-minus-one controls.

Results Heterogeneous macrophage populations based on differential expression of surface markers for CX3CR1, F4/80 and CD11c were identified. Macrophages accounted for 80% of leukocytes. A unique myeloid population co-expressing CD11c+F4/80+ (4.3%) was identified for the first time. The remaining population was of lymphoid lineage and consisted of a rare CD3+NK1.1+ (14.4%) subset.

Conclusion Phenotypic variants of leukocyte subsets may reflect functional differences warranting further analyses of functional markers of these sub-populations in pre and pubertal testes. (Supported by DFG IRTG „Molecular Pathogenesis of Male Reproductive Disorders“, Project P2 (GRK 1871/2).)

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Is resistance against oxidative stress in boar ejaculates related to their fertilizing capacity?

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Sperm are under oxidative stress (OS) during processing, storage and in the female genital tract after insemination, e. g. by reactive oxygen species released from immune cells. The capacity of seminal fluid (SF) to reduce free radicals represents one protective mechanism against oxidative stress and was measured by

electron spin resonance spectroscopy in 47 ejaculates (breed: Piétrain). Besides an extended sperm quality analysis, lysophosphatidylcholines (LPC), final oxidation products of the most abundant lipids in sperm, were quantified by MALDI-TOF mass spectrometry. According to fertility outcome the ejaculates were classified in a group of high or a group of lower fertility (farrowing rate/mean $\pm$ SD of litter size/ $n_{\text{ejaculates}}/n_{\text{sows}}$: 100%/16.3 $\pm$ 0.7/25/209 and < 87.6%/13.9 $\pm$ 2.4/22/233, respectively). At least 5 sows (breed: German Landrace) were inseminated per ejaculate. The high fertility group had a lower DNA fragmentation index, more sperm with intact membranes and a lower SF protein concentration. No significant differences were found between the high and lower fertility group regarding the radical reduction capacity in SF or LPC accumulation in sperm. The latter was usually very low (mean $\pm$ SD for high and lower fertility group: 0.5 $\pm$ 2.1 and 2.0 $\pm$ 3.7, respectively). Even if a low radical reduction capacity of SF and an accumulation of LPC bear a higher risk of reducing the reproductive outcome, both parameters were not predictive for the fertilizing potential of an ejaculate.

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Preovulatory aglepristone treatment does not inhibit ovulation but lowers LH secretion in the bitch

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The exact involvement of the preovulatory progesterone increase in the regulation of ovulation is poorly understood. We used an experimental model with temporary elimination of endogenous progesterone action by aglepristone treatment to check if the preovulatory progesterone increase might be related to occurrence of ovulation and LH release in spontaneously cycling bitches. Seven bitches (group 1) were treated with aglepristone (Alzine, Virbac) at the recommended dose of 10 mg/kg b.w. 2 times, 24 hours apart during proestrus at a progesterone level of 0.5 ng/ml. Seven bitches (group 2 controls) were injected with placebo according to the same protocol. Progress of estrus cycle and ovulation were monitored clinically by vaginoscopy, vaginal cytology and endocrinologically by progesterone measurement (RIA) every day. In peripheral blood samples obtained every 8 hours from late proestrus to late estrus LH was measured by means of EIA. Ovulation occurred in all bitches of both groups. In the aglepristone treated bitches, mean LH release was significantly lower than in the control group ($p < 0.05$) combined with a slightly delayed (2–3 days) ovulation. We conclude that the preovulatory progesterone rise is in part involved in stimulation of LH surge needed for the induction of ovulation.

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Apparent Diffusion Coefficients in Magnetic Resonance Imaging and Prostate Imaging Reporting and Data System score in the canine prostate gland

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Pathological alterations of canine prostate gland include benign prostatic hyperplasia, intraprostatic cysts and abscesses, acute and chronic inflammation and neoplasia. Adenocarcinoma, transitional cell carcinoma and undifferentiated carcinoma are most frequently diagnosed histologically. Dogs are the only nonhuman mammals that develop spontaneous prostatic cancer, which shares many features with men. Prostate Imaging Reporting and Data System (PIRADS) is a scoring system used for evaluating the prostate cancer in men. It is based on a scale from 1 to 5, with 1 standing for „most probably benign“ and 5 for „highly suspicious of malignancy“. The Apparent Diffusion Coefficient (ADC) is measuring the diffusion of water molecules within tissue. It is calculated using diffusion weighted imaging in Magnetic Resonance Imaging (MRI), which is very useful for identifying prostatic tumors. The aim of this study was to compare PIRADS scores with ADC values. The study was conducted in 8 dogs. We observed that the maximal PIRADS was positively correlated with the ADC value. The ADC values range from 8676 to 1834 with $R^2 = 0.435$. The PIRADS scoring system reflects the degree of pathological changes in the canine prostate gland. Its implementation in veterinary medicine will be valuable for diagnosing prostatic cancer in dogs.

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Association between cortisol/DHEA and embryonic mortality in dairy cows

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It is well-known that stress is associated with a lower fertility rate in different species. Mice with higher corticosterone concentrations during early pregnancy show an impaired

embryonic development [Burkus et al. 2015]. However, less is known whether embryonic mortality (EM) after artificial insemination (AI) may also be associated to stress in dairy cows. The aim of the present study (approval number 2347-32-2016) was to determine the association between cortisol (C) and a potential chronic stress marker (dehydroepiandrosterone; DHEA), and late EM under field conditions. DHEA is expected to be lower in chronic stressed individuals. Therefore, in total 86 pluriparous cows were examined. On day (d) 32 ± 3 after AI, pregnancy was diagnosed by using ultrasonography, and the cows were allocated to the following groups: (1) pregnant (p, n = 40), (2) not pregnant (np, n = 40) and (3) EM (n = 6). EM was defined if a control examination on d 46 ± 3 after AI revealed a negative result in a previous pregnant animal. Blood samples were taken on day 38 ± 3 post partum, and on d 1, d 15/16 as well as on d 32 ± 3 after AI. Cortisol and DHEA were measured by immunoassays and the C/DHEA ratio was calculated. The C (p = 0.090) and DHEA (p = 0.091) concentration tended to be higher in EM (16.0 ± 5.5 and 0.7 ± 0.2 ng/ml) compared to p (8.7 ± 1.4 and 0.5 ± 0.04 ng/ml). Moreover, cortisol also tended to be higher in cows with EM than in np animals (9.3 ± 1.2 ng/ml; p = 0.76) on d 1 after AI. In conclusion, these data suggest that an acute stress response on d 1 after AI may be associated with EM. (We acknowledge the support of the Saxon State Office for environment, agriculture and geology, Dresden Pillnitz, Germany.)

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Evaluation of a wireless pulse oximeter for measuring the arterial oxygen saturation and pulse frequency in Holstein Friesian calves

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Pulse oximetry is a well-established technique in human and companion animal medicine. In the farm animal sector, it could be a useful tool for the detection of critical conditions of the oxygen supply and the cardiovascular system of the patient, in particular calves. The objective of this study was to evaluate the accuracy of the Radius-7 Wearable Pulse CO-Oximeter (Masimo Corporation, Irvine, USA) for monitoring the vital parameters of Holstein Friesian calves. For this, the sensor of the pulse oximeter was placed in the interdigital space of the calf's front leg. The arterial oxygen saturation (SO₂) of 40 newborn calves was measured and compared with the corresponding results from a portable blood gas analyzer (VetScan iStat1, Abaxis Inc., Union City, USA). The blood sample was taken from the medial intermediate branch of the caudal auricular artery. The pulse rate was measured on 10 calves between 0 to 7 days of age with the pulse oximeter and a heart rate belt (Polar Equine Belt, Polar Electro Oy,

Kempele, Finland) simultaneously and their level of agreement was evaluated. Spearman correlation coefficient was 93.8% for the SO₂ between the pulse oximeter and the blood gas analyzer and 97.7% for the pulse rate between the pulse oximeter and the heart rate belt. The pulse oximeter overestimated the SO₂ by $2.95 \pm 6.39\%$ and underestimated the pulse rate by 0.41 ± 3.18 bpm compared with the corresponding reference methods. This pulse oximeter is considered to be suitable in field for continuous monitoring of SO₂ and pulse of Holstein Friesian calves.

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Analysis of genital cancer cases in eastern region of Turkey: experiences from a university hospital

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The aim of this study is to assess the incidence, types, presentation and treatment outcomes of genital cancers of women live in eastern region of Turkey including five cities. The medical records of women who referred to Van Yüzüncü Yıl University (Turkey), Medical Faculty, obstetric and gynecology department between November 2014 and June 2017 were retrieved and patients treated for genital cancers were reviewed. Retrospective data analysis method was used. The clinical, demographic data and oncologic outcomes of the patients were included and analysed. During study period, 124 cases with genital cancers were detected from medical records. The most common seen cancer was endometrial cancer (75/124, 60%). The second most common type was ovarian cancer (24/124, 19%). The other types were cervical carcinoma (11/124, 8%) and vulvar cancer (3/124, 2%). Among endometrial cancer cases, the most common pathology was endometrioid type endometrial carcinoma which consists of 60 cases (80%). Serous papillary type ovarian carcinoma was the mostly frequently seen pathologic type in ovarian cancer (66%). The mean age of all women with genital cancer was 48 ± 5.8 years. The mean gravidity and parity of all cases were 4.1 ± 1.6 and 2.4 ± 1.1 , respectively. The mean age of ovarian cancer cases was significantly higher than endometrial cancer patients (61 ± 5.6 vs 49 ± 4.6 , p < 0.05). The majority of genital cancer cases treated with surgical option (112/124, 90%). Chemotherapy and radiotherapy was applied to patients with advanced stage disease either preoperatively or postoperatively. As a result endometrial cancer is the most common type in our region as consistent with literature. Ovarian cancer should be suspected in women with presenting symptoms because of being diagnosed at advanced stage. Surgical therapy remains the cornerstone for treatment of genital cancer in

eastern region of our country as similar to the literature data.

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Preliminary evaluation of CA 15-3 biomarker in different histopathological types of canine mammary tumours

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Aim Mammary tumours are the most frequent diagnosed neoplasias in female dogs [Benavente et al. J Vet Adv 2016; 6: 1291–300]. Therefore, it represents a serious clinical problem. The aim of the study was to evaluate the expression of CA 15-3 (MUC 1) in different histopathological types of canine mammary tumors as it seems to be a good biomarker of neoplastic process.

Material and Methods 70 canine mammary tumours and serum samples were collected during mastectomy in veterinary clinics in Poland. Mammary tumors were evaluated histopathologically. CA15-3 expression was preliminarily evaluated by immunohistochemistry using MUC1 as secondary antibody (monoclonal, NBP2 45838, Novus Biologicals) in 20 samples. Staining was done using Dako EnVision™ + System/HRP, Mo (DAB+). Microscopic slides were further evaluated using Tissuegnostics computer program, Histoquest module.

Results The most frequent malignant tumor was simple carcinoma (29%), carcinoma arising in benign mixed tumor (20%) and carcinoma complex (17%). Adenoma complex (17%) and benign mixed tumor (13%) were the most frequent observed benign neoplasias. Evident CA 15-3 expression was seen in some of the immunohistochemically stained samples. A stronger expression (74% of all cells) was seen in simple carcinoma than in benign mixed tumor (47%).

Conclusion Tumor biomarkers, like CA 15-3 can facilitate an early diagnosis of mammary neoplasia. Nevertheless, further studies concerning CA 15-3 expression in canine mammary tumors must be done.

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Assessment of spermatogenesis in harbour porpoises (*Phocoena phocoena*) from the North and Baltic Seas

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Male germ cells (GC) are arranged within the seminiferous epithelium in a set of well-defined cellular associations called stages. The identification of these stages can be used to assess the developmental profile of gene expression during spermatogenesis and to identify defects in spermatogenesis arising in pathological conditions. Detailed knowledge on spermatogenesis in adult harbour porpoises (*Phocoena phocoena*) is restricted to a few studies. Though the seminiferous epithelium seems to comprise similar GC populations and somatic Sertoli cells like terrestrial wildlife species, spermatogenesis is additionally known to proceed in a seasonal pattern. No information is available about the number of stages in the porpoise testis. For that purpose, testes of 115 harbour porpoises from German and Dutch waters were collected at different time points of the year and histologically analyzed using HE staining. The selected sections were examined using the Periodic acid Schiff (PAS) reaction to assess spermatogenic stages. In order to complete staging and to detect spermatids, protamine 1 mRNA in-situ-hybridization was performed. Spermatogenesis in the high-mating season (July) is found to proceed in eight stages showing a multi-stage arrangement within one tubular cross section. An increasing number of missing GC generations from August to September has been encountered. Our data provide a detailed staging of spermatogenesis in harbour porpoises that might be useful for the detection of possible influences of endocrine disruptors on male reproductive biology in upcoming studies.

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Uterine torsion in cattle: treatment, risk of injury for the cow and prognosis for the calf

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Uterine torsion is a common cause of bovine dystocia. The aim of the study was to investigate factors, which are important to predict neonatal survival and risk of injury to the dam. The study presents 114 cases of uterine torsion documented under field conditions. The cows were examined during delivery, and 2 h post partum. The grade the dam was prepared for parturition had a significant influence on the survival rate of the calf ($p = 0.006$): In cases of good preparation, neonatal mortality was 14.85%, in cases of insufficient preparation it rose to 58.33%. When uterine torsion lasted longer than 12 h, only 34.78% of the calves survived, while in cases with durations shorter than 6 h and between 6 and 12 h, 85.71% and 92.21% of the calves survived, respectively ($p < 0.001$). In 82.46% of the cas-

es treatment was made by manual rotation of the fetus and uterus per vaginam, in 17.54% by rolling the animal with the application of a plank. No significant differences were found between the different treatment types regarding neonatal mortality (4.71% vs 18.18%; $p = 0.139$) or lacerations of the dam (31.9% vs 42.1%; $p = 0.391$). A delayed extraction of the calf after successful retorsion is an option in cases of insufficient dilatation of the cervix even if there is a significantly higher risk for lacerations of the dam (57.15%) compared to an immediate extraction (26.75%; $p = 0.018$). In conclusion, the quality of birth preparation in the cow with uterine torsion as well as the duration until therapy takes place, can be used as prognostic factors for calf survival. Therefore, proper periparturient monitoring and early intervention are of crucial importance in cases of uterine torsion in cattle.

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Effect of a single acyline treatment on canine spermatogenesis

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The use of GnRH antagonists is discussed as a possible non-surgical approach to castration of male dogs. Information about histological effects on spermatogenesis is, however, missing. Therefore, we investigated the effect of a single GnRH antagonist treatment on spermatogenesis in canine testes. Sexually mature, healthy male dogs ($n = 4$) were treated subcutaneously with a single injection of the GnRH antagonist acyline (330 µg/kg) and surgically castrated two weeks later. Five untreated normospermic dogs served as controls. From each dog, 200 cross-sections of approximately round tubuli seminiferi contorti were evaluated for whether there was spermatogenic arrest or undisturbed spermatogenesis. In case of full spermatogenesis being present, the different tubules were categorized in stages (Stage I–VIII). Additionally, the area of 100 approximately round tubules from each dog was determined. The investigators were blinded to the group the dogs belong to. Histological changes in the tubular structure with a disruption of the normal cellular distribution of the testicular tissue was observed in all treated dogs with occurrence of spermatogenic arrest being significantly higher at the level of round ($p = 0.0199$) and elongating spermatids ($p = 0.0108$) compared to the control group. Percentage distribution of spermatogenic stages among seminiferous tubules differed significantly for all stages (from $p = 0.0160$ to $p = 0.0179$), except stage VIII. Furthermore, the tubular area was significantly reduced in treated compared to control dogs ($p < 0.001$).

The histological changes in the tubular structure of the testicular tissue indicate that a single acyline treatment has a negative impact on canine spermatogenesis. (The authors thank the National Institutes of Health, USA for provision of acyline to CG.)

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Expression of androgen receptor and integral membrane proteins in canine tubular tissue at down-regulation and during restart of spermatogenesis

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Initiation and maintenance of spermatogenesis requires androgens, in particular testosterone, and FSH. Integral membrane proteins contribute essentially to the formation of the blood-testis barrier (BTB) and are therefore crucial for normal spermatogenesis. Recently, we investigated BTB protein and androgen receptor (AR) expression in whole testicular homogenates during GnRH-slow release implant mediated downregulation and subsequent restart of spermatogenesis following implant removal after 5 months (week 0). In the present study, RNA from tubular tissue only (500 round tubules/sample) of the respective animals castrated in week 0 ($n = 3$), 3 ($n = 3$), 6 ($n = 4$) and 12 ($n = 3$) was extracted and RT-qPCR was performed using primers against Ocln, Cldn-3, -11, Cx43 and AR. Tissue from 4 untreated dogs served as controls. Whereas no significant differences in mRNA expression of Cldn-3 and AR could be identified in tubular tissue between the downregulated testes, the different stages of restart of spermatogenesis and CG, relative gene expression for Cldn-11 ($p = 0.0213$), Cx43 ($p = 0.0113$) and Ocln ($p = 0.0030$) differed significantly between groups. The highest ratio for Cx43 and Cldn-11 was obtained in week 0. For Ocln, the relative mRNA expression was highest in weeks 6 and 12. The unchanged AR expression in tubular tissue at downregulation allows for a rapid responsiveness to androgens during restart of spermatogenesis. Whereas Cx43 and Cldn-11 seems to be upregulated during downregulation, results for Ocln indicate a rebound effect at the time when spermatogenesis is nearly re-established.

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Anti-Müllerian hormone concentration in dogs with unilateral cryptorchidism

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The Anti-Muellerian hormone (AMH) is a glycoprotein, which is mainly responsible for the sexual differentiation in the fetal period. Undifferentiated gonads (prepubertal, atrophic) produce higher amounts of AMH. However, studies did not show significantly higher serum AMH concentrations in dogs with bilateral and stallions with unilateral cryptorchidism compared to healthy individuals [Gharagozlou et al. Vet Rec 2014; 175: 460; Claes et al. Theriogenology 2013; 79: 1229–35]. Aim of this study was to evaluate the serum AMH concentrations in dogs with unilateral cryptorchidism compared to healthy dogs. Peripheral blood samples of eight dogs with unilateral cryptorchidism (age: 12 months to 3 years) and eight dogs with scrotal testicles (age: 6 months to 10 years) were taken prior to routine castration. Serum AMH was determined using a chemiluminescence immune assay validated for dogs (Laboklin, Bad Kissingen, Germany). In cryptorchid patients the serum AMH concentrations were significantly higher (≥ 23 ng/ml, $p \leq 0.001$) compared to the group with normal testicular descent (median: 5625 ng/ml). Pathohistological examinations of 6 out of the 8 removed cryptorchid testicles have been performed. None of them showed signs of neoplasia. In conclusion, serum AMH concentrations show significantly higher values in unilateral cryptorchid dogs than in normal dogs. After all, further studies have to be performed to understand the variations of AMH in the different forms of cryptorchidism.

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Autocrine effects of the bovine trophoblast cell product interferon tau *in vitro*

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In ruminants trophoblast cell products, namely the type I interferon tau (IFN τ), are crucial for maternal recognition, uterine receptivity and ultimately pregnancy establishment. Since the investigation of these effects *in vivo* is challenging, an *in vitro* model of bovine trophoblast cells was used to study autocrine influences of IFN τ . Based on a dose-dependent stimulation assay (10, 100, 1000 ng/ml IFN τ) Western blot analysis and RT-PCR was used to examine alterations in protein- and mRNA expression of type I interferon associated receptors (IFNAR1, -2) and related cell signalling pathways (JAK-STAT). Changes in metabolism as well as directed and undirected motion (Motility & Chemotaxis) were examined by MTT-Assay, live cell imaging and agarose-spot-assay. All *in vitro* experiments were carried out in triplicates. Possibly related structural alterations of the cytoskeleton (Ezrin & Cytokeratin [CK18]) in peri-/post-implantation bovine embryos (gestation days 20 and 39) were investigated by immunohistochemistry (IHC). The results revealed distinct and highly significant impacts on the signalling pathways by activating especially non-classical interferon associated pathways

(MAPK42/44, proteinkinase B). Furthermore, significant increases in metabolism, motility and chemotaxis were demonstrated which were to a large extent dose-dependent. IHC of the bovine embryos showed an altered expression of Ezrin and CK18 in contact areas specifically during implantation. In summary, autocrine effects of IFN τ on motility, chemotaxis, corresponding signalling pathways and the metabolism of bovine trophoblast cells *in vitro* could be shown. These data together with the observed depolarization of bovine trophoblast *in vivo* indicate additional so far unknown physiological roles.

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Temperature profiles and boar sperm quality after different adaptation regimes of semen doses at 22°C

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A well-controlled cooling regime during semen processing is decisive for the quality of liquid preserved boar spermatozoa. So far, practical recommendations for the cooling velocity after filling of semen doses until the beginning of storage at the desired temperature is missing. The aim of this study was to monitor variations in cooling regimes of freshly filled semen doses under practical conditions and to determine the influence of a prolonged holding period at 22°C (RT) on the quality of stored semen. Temperature profiles of freshly processed semen doses were recorded (Mikromec® multisens, Technetics) considering their position in open package boxes and an adaptation phase at 22°C (RT). Starting from 28°C and depending on their position in the box, cooling rates of semen doses directly (0 h RT) subjected to the storage cabinet at 17°C varied between 2.0 and 3.2°C/h. Tubes in outer and middle positions reached 17°C after 8 h and 19 h, respectively. Sperm quality of semen doses ($n = 9$ boars) in middle positions of packages containing 35 semen tubes (90 ml) was then compared with and without exposure to RT for 6 h before storage at 17°C for 96 h. The adaptation phase at RT did not influence motility assessed with CASA (AndroVision®, Minitube Tiefenbach, Germany), membrane integrity (propidium iodide and FITC-PNA negative sperm) and mitochondria membrane potential assessed in flow cytometry ($p > 0.05$). In conclusion, cooling rates of semen doses varies widely within package units. Semen doses can be immediately stored at 17°C without loss of sperm quality if packed in insulating packages.

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LED light photostimulation does not improve the quality of liquid preserved boar spermatozoa

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Recent reports indicate that exposure of liquid-stored boar semen to red LED-based light might improve sperm quality and reproductive performance in sow herds. The effect is thought to be linked with a light-induced activation of sperm mitochondria. The aim of this study was to examine the effects of photostimulation on sperm quality and function in boar semen stored at 17° and 5°C, respectively. Semen doses (85 $\pm$ 1 ml) were prepared containing 1.8×10^9 (Experiment 1, $n = 10$ boars) or 2.0×10^9 sperm (Experiment 2, $n = 6$ boars) in Beltsville Thawing Solution. In Exp. 1, semen doses were stored at 17°C for 24 h and then exposed to red LED based light in an air refrigerated chamber (maXipig®, GenIUL, S.A., Barcelona) according to the manufacturer's recommendation. In Exp. 2, semen doses were stored hypothermally at 5°C up to 144 h and then subjected to the LED-based photostimulation. In both experiments, sperm kinematics assessed with the CASA system AndroVision® (Minitüb, Tiefenbach) revealed no difference ($p > 0.05$) between light-treated samples and their controls. Accordingly, flow cytometry data did not differ in the percentages of sperm with intact plasma membrane and acrosome (propidium iodide and FITC-PNA negative) and in the proportion of sperm with high mitochondrial membrane potential as assessed with JC-1 or mitochondrial activity as measured by Rhodamine 123. In conclusion, photostimulation with maXipig® gives no advantage to the quality of liquid preserved boar spermatozoa, even not when sperm quality is challenged by hypothermic storage and subsequent thermal stress.

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Genital blood flow differs between pregnant and non-pregnant heifers during the first weeks after artificial insemination and embryo transfer

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Objectives of this study were to compare the impact of pregnancy on genital blood flow in dairy heifers after artificial insemination (AI) and embryo transfer (ET), and to evaluate color Doppler sonography as a possible tool for early pregnancy diagnosis. Heifers were artificially inseminated in estrus ($n =$

20; Day 1 = ovulation) or received an embryo (IETS class I) 6 to 7 days after ovulation ($n = 35$). Sonography and blood sampling were performed on Days 7/8, 9/10, 11/12, 14/15, 16/17, 18/19, and 21/22 to determine luteal size (LTA) and blood flow (LBF), time-averaged maximum velocity (TAMV) and pulsatility index (PI) in uterine artery perfusion, and plasma progesterone (P4) levels. Pregnancy diagnosis on Day 25 revealed 11 and 17 pregnant heifers after AI and ET, respectively. After AI, LBF and P4 were lower ($p < 0.05$) in non-pregnant compared to pregnant heifers on Days 18/19 and 21/22, whereas LTA was not decreased in non-pregnant heifers before Days 21/22. Furthermore, TAMV was lower ($p < 0.05$) in non-pregnant heifers on Days 14/15. After ET, LBF and P4 were lower ($p < 0.05$) in non-pregnant compared to pregnant heifers on Days 16/17 to 21/22, whereas the time of decrease in LTA did not differ from heifers after AI. Interestingly, TAMV was higher ($p < 0.05$) in non-pregnant heifers on Days 14/15, and PI was higher ($p < 0.05$) in pregnant heifers on Days 21/22. Coefficients of variation ranged from 20 to 118%. In conclusion, genital blood flow differs between pregnant and non-pregnant heifers during the first 3 and 2 weeks after AI and ET, respectively, but is not suitable for early pregnancy diagnosis due to high inter-individual variability.

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Predicting the fertility of sex-sorted sperm through the multi-color flow cytometric assessment of conventional cryopreserved non-sorted semen in the bull

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Our study aimed to evaluate the functional parameters of conventional frozen semen as a fertility marker of sex-sorted semen in the bull. Thus, 116 frozen ejaculates of 30 bulls were examined at 0 (0 h) and 3 hours of post-thaw incubation (3 h). A five-colour flow cytometric panel including calcein violet AM, propidium iodide, R-phycoerythrin-conjugated peanut agglutinin, Fluo-4 AM and Mitoprobe™ DiIC1(5) was employed to simultaneously quantify sperm with high esterase activity (C_{pos}), intact plasma membrane (PI_{neg}), intact acrosome (PNA_{neg}), low intracellular Ca^{+2} levels (F_{neg}) and high mitochondrial membrane potential (M_{pos}). The relative difference between the non-return rates of each bull after AI with conventional semen and sex-sorted semen (Δ_{NRR} , %) was computed; mean Δ_{NRR} was $-10.6\% \pm 10.3\%$ (range -31.4% , 13.1%). Bulls were classified as having low, moderate, good or high performance at sex-sorting based on the 25th, 50th and 75th percentiles of Δ_{NRR} . The relation between bull performance after sex-sorting and the functional parameters of conventional sperm was explored with multivariate analysis of variance. At 3 h, C_{pos} , PI_{neg} , PNA_{neg} , F_{neg} , M_{pos} sperm

in high performing bulls ($39.4\% \pm 13.5\%$) was higher than in bulls with good ($26.7\% \pm 8.0\%$), moderate ($23.9\% \pm 6.1\%$) and low ($22.4\% \pm 10.9\%$) performance ($p < 0.001$ in all cases); a similar but not statistically significant trend was observed at 0 h. Values of PI_{neg} , PNA_{neg} , PI_{neg} , F_{neg} and C_{pos} , M_{pos} sperm at 0 h and 3 h did not differ across groups. Concluding, multi-colour flow cytometric assessment of conventional semen could provide useful information on the fertilizing potential of sperm after sex-sorting in the bull.

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Population structure of breeding warmblood mares in Poland in relation to results of stationary and field performance tests

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The effect of inbreeding on performance traits is still not clear especially in Polish Warmblood mares population. For inbred individuals, impaired fertility, reduced vitality, weaker growth and worse performance may appear, based on the negative effect on conformation traits and reproduction results. In Polish warmblood mares population repeated use of the same studs for breeding were common due to breeders preferences and explicitly trends in horses selection. The aim of the study was to compare inbreeding coefficient obtained from mares participating in stationary with field performance tests. The inbreeding coefficient were estimated based on the pedigree data of 2105 mares in years 2002–2015 and analyzed using INBREED procedure of the SAS package and self-written software. The group included mares of the following breeds: Polish Warmblood (sp) ($n = 337$ in stationary and $n = 109$ in field tests), Malopolska breed (mlp) ($n = 272$ in stationary and $n = 60$ in field tests) and Wielkopolska breed (wlkp) ($n = 390$ in stationary and $n = 345$ in field tests). We demonstrated higher percentage of inbred mares in each breed participant in stationary (sp: 55.25%; mlp: 70.96%, wlkp: 50.00%) in contrary to field (sp: 25.47%; mlp: 26.67%; wlkp: 27.25%) tests. However, the inbreeding coefficient (Mean $\pm$ SEM) was significantly lower for mares in stationary than in field tests for all examined breeds: sp: 0.89 ± 0.08 and 1.39 ± 0.21 with $p < 0.0001$; 1.19 ± 0.15 and 3.13 ± 1.04 with $p = 0.0003$; wlkp: 1.06 ± 0.16 and 1.36 ± 0.21 with $p = 0.0004$. The highest inbreeding coefficient was stated in mlp mares participating in field performance tests. Due to breeders preferences both horses' selection and choice of performance

test seem to be related, in favour of field test which is easier for the mare. The inbreeding coefficients, as a helpful tool, may be used in improvement of Polish warmblood horses breeding management and testing, especially for Malopolska breed.

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Expression of Claudin-11 in canine testis showing normal and impaired spermatogenesis

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Tight junctions between adjacent adult Sertoli cells form the blood-testis barrier (BTB). The BTB consists of different proteins, including claudin-11 (Cldn11). As only few investigations have been conducted on the canine BTB, the aim of the present study was to assess the expression pattern of Cldn11. Seminiferous tubules of dog testes both with normal spermatogenesis (NSP) and impaired spermatogenesis and neoplasia (like seminoma) were examined. Immunohistochemistry was performed using a specific commercial antibody against Cldn11. To confirm collected data, qualitative Western blot analyses (WB) and RT-PCR were implemented. In tubules showing NSP, immunohistochemical detection revealed a typical circumferential seal at the supposed BTB localization. Staining of tubules with impaired spermatogenesis including maturation arrest at different levels and Sertoli cell only syndrome appeared diffuse, cytoplasmic and more intense corresponding to the findings described in human testes. In seminoma, Cldn11 staining is dislocated and weak, presenting only residuals of Cldn11 protein. WB and RT-PCR for Cldn11 using testicular tissue from dogs with NSP revealed specific bands, confirming its identification on protein- and mRNA-level, respectively. For the first time, the expression pattern of Cldn11 protein in canine testis showing NSP and impaired spermatogenesis could be demonstrated, with proof given by WB. Since altered Cldn11 levels could be part of adaptive mechanisms to modify BTB integrity, further functional investigations to characterize the canine BTB have to be conducted.

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Treatment of postpartum anestrus in Egyptian buffalo (*Bubalus bubalis*) using herbal drugs

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A polyherbal veterinary formulation, Exapar was used to study its effect on the induction of postpartum estrus and subsequent conception in buffaloes. Janova is a polyherbal preparation scientifically formulated to mimic the action of gonadotrophins and synchronise the release of physiological hormones for inducing ovulatory oestrus. The study was carried out on 120 clinically healthy buffalo cows having prolonged post-parturient anoestrus more than 4 months. Comprise of Exapar (E); 100 ml for 5 days followed by Janova (J) 3 capsules/day for 6 days (days 1–2, 6–7 and 17–18). Transrectal ultrasonography was done for each buffalo cow during the post-partum period. Day of ovulation (DO) was recorded. Milk progesterone concentrations were measured from samples collected every four day start from 0 till 32 days after treatment. The total response for treatment with Exapar and Janova was recorded in 50/76 (65.79% of the anestrus buffalo-cows). Only small number of treated and responded buffalo-cows showed estrus at fifth day from the beginning treatment (9/50, 18%). The majority of buffalo-cows responded at eighteenth day of drug administration (29/50, 58%). The rest of responded buffalo-cows came in estrus somewhat late at the 32nd day from the start of medicament application (12/50, 24%). In conclusion, combined treatment of Exapar and Janova is highly effective for inducing the heat in the post partum anoestrus buffaloes.

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Precolostrum pH in late pregnant Shetland pony and Haflinger mares

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Supervision of parturition is recommended to avoid fetal loss due to dystocia which has an increased incidence in small pony breeds. However, horses only show week signs of impending parturition. It has been suggested that daily pH measurements of the mammary gland secretion can help to predict foaling with most mares giving birth within 24 hours at pH < 7. We hypothesised that precolostrum pH can predicted the day of parturition, but pH characteristics differ among Shetland pony and Haflinger mares. To test this hypothesis, pH in precolostrum was determined daily at 8:00 am during the last 7 days before foaling in Shetland pony (n = 11; age 10.7 ± 1.8) and Haflinger (n = 15; age 9.2 ± 0.9) mares with pH indicator strips MColorpHast (ph 5.0–10.0). In both groups, pH in precolostrum decreased during the last week before foaling (p < 0.001; 7 days before foaling: Shetland pony 7.6 ± 0.1; Haflinger 7.3 ± 0.1; day of foaling: Shetland pony 6.8 ± 0.2; Haflinger 6.1 ± 0.1). During the last two days before parturition differences between groups were found with higher precolostrum

pH in Shetland pony mares (p < 0.01). Within 24 hours before foaling, 100% of Haflinger mares showed precolostrum pH < 7 but this cut of value was already reached 72 and 48 hours before parturition in 50% and 75 of mares, respectively. In Shetland pony mares 24 hours before foaling, only 54.6% showed pH < 7. In conclusion, precolostrum pH decreases during the last week before foaling in all mares but the reliability for prediction of the onset of parturition within the next 24 hours differs with breed.

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Cows suffering from embryonic/early fetal mortality show higher IGF-binding protein-4 concentrations

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Insulin-like growth factor 1 (IGF-1) and binding proteins (IGFBPs) are essential for fetal growth and development. In humans, it is well-known that proteolytic cleavage of IGFBP4 occurs in order to release free IGF for respective action at the tissue level. The aim was to proof if IGFBPs serum concentrations are associated with embryonic/early fetal mortality. Holstein Friesian cows (n = 500) were examined four times after artificial insemination (AZ 2347-29-2014). On day 24–27 and 34–37 in all animals and on day 54–57 in pregnant cows pregnancy status was checked and blood samples were taken (IGF-1 and IGFBP2, 3, 4). In total 203 cows were pregnant and 284 were not pregnant. Two groups of pregnancy loss were defined: pregnancy loss between day 24/27 and 34/37 = late embryonic mortality (em, n = 7) and between day 34/37 and 54/57 = late embryonic mortality and early fetal mortality (em/fm, n = 8). In order to analyze a balanced subset pregnant (n = 30) and non-pregnant (n = 20) cows were selected for analyses. Interestingly, IGFBP2 concentrations were significantly higher in em and em/fm animals compared to np and p ones and IGFBP4 was highest in em/fm cows compared to em, p and np counterparts (p < 0.001). Distinct adaptations within the somatotrophic axis during early pregnancy were detected. Both IGFBP2 and IGFBP4 were higher in cows developing embryonic or early fetal mortality. Interestingly, these IGFBP concentrations were already higher on the day of insemination and therefore may on the one hand serve as a biomarker and on the other hand may have a causative relationship to pregnancy loss. (The authors thank "Förderverein Bioökonomieforschung e. V. [FBF]" for financial support.)

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Caesarean section and uterine torsion in Huacaya alpacas: two case reports of successful obstetrical intervention

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Increasing numbers of South American camelids in Europe pose a new challenge for veterinarians. Among the relatively rare obstetrical cases, foetal mal-positioning and uterine torsion are the most likely causes of dystocia. We report two obstetric cases from our clinic.

Case 1 A 2-year-old, nulliparous female Huacaya alpaca was presented in labour > 12 h with high grade vulvar swelling. During vaginal examination the foetus was suspected to be in breech position with hiplock, since only the tail could be palpated through the insufficiently dilated cervix. Caesarean section in the left flank was performed under local anaesthesia und sedation. A dead female cria was delivered in breech position with hiplock. Foetal membranes were carefully removed during surgery. The patient received antibiotic and anti-inflammatory treatment for one week and recovered without further complications.

Case 2 An 8-year-old, multiparous pregnant Huacaya alpaca was presented with intermittent colic > 12 h. The initial vaginal examination after transport did not reveal any sings of parturition or uterine torsion. Transabdominal ultrasound confirmed a life foetus. Two hours later, the animal showed colic and right side uterine torsion was diagnosed by vaginal examination. By placing the patient on its right side and carefully rolling her with simultaneous transabdominal fixation of the gravid uterus, the torsion was resolved and did not recur until the partus of a live-born male cria. If foetal malposition in alpacas cannot be corrected, caesarean section is a well-tolerated method. Uterine torsion can often be resolved by rolling.

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Time-lapse imaging unveils distinctive contractile patterns in rat and human seminiferous tubules

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In the testis, transport of spermatozoa relies on the activity of peritubular smooth muscle cells which surround the seminiferous tubules. To assess and characterize differences in contractility and sperm transport in rat (n = 84) and man (n = 22), we used a time-lapse imaging approach combined with Fourier analysis. In rat seminiferous tubules we observed a pattern of spontaneous, irregular and undulating wall movements which was

further characterized by Fourier analysis. Wall movements were tracked over time, yielding an irregular curve. Fourier analysis allows to decompose a given irregular curve into a characteristic frequency spectrum of sine curves which contribute to the original curve. This technique revealed several specific contraction patterns that can be correlated to certain spermatogenic stages. In contrast, in human seminiferous tubules where spermatogenic stages are arranged in a spiral manner, no undulating wall movements were observed. Pursuing time-lapse imaging over several hours, very slow spontaneous peristaltic contractions were seen in human samples. Interestingly, long-term time-lapse imaging of rat seminiferous tubules unveiled an additional slow contractile activity that was superimposed onto the undulating wall movements. These slow contractions also occurred spontaneously with a rhythmic pattern; they showed clear diameter changes of the tubule which propel intraluminal spermatozoa through the seminiferous tubule. Time-lapse imaging represents a valuable tool to visualize and characterize smooth muscle cell function in tissues under near-physiological conditions.

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Myoepithelial and myofibroblastic differentiations – new insights into the pathogenesis of equine endometrosis?

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Equine endometrosis is a fertility reducing disease of undetermined pathogenesis. Affected glands are surrounded by stromal cells (periglandular fibrosis). Myoepithelial cells and myofibroblasts play a key role for fibrosis in other organs. Aim of this study is to examine the expression of myoepithelial and myofibroblastic markers in endometrial tissue with different degrees of endometrosis by comparing healthy and endometrotic glands. Immunohistochemistry for vimentin, desmin, α -smooth muscle actin (SMA), calponin and glial fibrillary acidic protein (GFAP) is performed. Epithelial cells of fibrotic glands express more frequently calponin than those of healthy glands. A positive reaction of stromal cells for SMA, vimentin and desmin is more commonly detected in areas of periglandular fibrosis than in the adjacent stroma; the expression of desmin rises with an increasing severity of endometrosis. GFAP is observed in epithelial or stromal cells of a few fibrotic glands. The described alterations occur even in glands with early fibrosis. No differences exist between single or nested endometrotic glands. These findings indicate that glandular epithelia and stromal cells can differentiate into myoepithelial cells and myofibroblasts, respectively, and that this process likely represents an early event in the pathogenesis of endometrosis. Endometrosis is regarded as an irreversible condition. Our results suggest that prevention or reversal of myoepithe-

lial and myofibroblastic differentiation could possibly influence the development or the degree of endometrosis. In this regard, it has been shown that stem cell infusion can influence cellular differentiation.

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Superovulation followed by ovum-pick-up (OPU) in common marmosets (*Callithrix jacchus*) in compare to rhesus macaques (*Macaca mulatta*)

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Oocytes are an invaluable tool in biomedical research, as they can be used to generate different types of stem cells, e.g. parthenogenetic stem cell lines, developed from unfertilized oocytes or embryonic stem cells (ESC) derived from blastocysts after in vitro fertilization. These stem cells can be used for cell replacement therapies. Adult female common marmosets (*Callithrix jacchus*) and rhesus monkeys (*Macaca mulatta*) were hormonally super-ovulated with follicle stimulating hormone (FSH) and human choriongonadotropine (hCG). In the common marmoset, a species without any visible sign of estrus, the monthly cycle is monitored by progesterone levels in the blood and the ovarian stimulation is preceded by the application of a prostaglandin (PGF) in a progesterone peak. The ovum pick-up (OPU) was performed via laparotomy and puncture of the follicles. In contrast, female rhesus macaques show estrus by a menstrual bleeding and red skin swelling of the anogenital region. Here, the stimulation started within 4 days after the initiation of menstruation. Puncture of the follicles was performed in a minimal-invasive way through the abdominal wall under ultrasound control. In both species, we super-ovulated 5 females each twice and were able to obtain 73 oocytes from the rhesus macaques and 84 from the common marmosets with huge differences in the number of oocytes/animal and in the quality of stimulation. Although the protocols require refinement, OPU is in both species a reasonable and promising option to obtain oocytes for research.

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Effects of different concentrations of epidermal growth factor (EGF) with DMSO on maturation rate of camel oocytes vitrified at germinal vesicle (GV) stage

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The aim of the work was to elucidate the effects of different concentrations of epidermal

growth factor on vitrified camel oocytes. Dromedary camel ovaries (n = 1350) were collected from El-Bassatein Slaughter House in Cairo, during the period between September 2016 to May 2017. These ovaries were used for the study of IVM and vitrification experiments. They were collected within 15-30 minutes post-slaughter and placed in thermo-flask containing sterile physiological saline and transported within 1h to the IVF lab., where oocyte were recovered using an 18-gauge needle attached to a 10-ml syringe containing 1 ml tissue culture medium-199 (TCM-199) from 2–8 mm follicles. The recovered oocytes were examined and selected under stereomicroscope. COCs were exposed to (HM) for 1 min, then transferred to equilibration solution (HM+ EG 20% (v/v) + DMSO 20% (v/v)) for 4 min, subsequently COCs transferred to vitrification solution (HM+ DMSO 40% (v/v) + 5 ng/ml, 10 ng/ml or 20 ng/ml EGF) for 30 sec and vitrified by 0.25 ml straw. COCs were exposed to (HM) for 1 min, then transferred to equilibration solution (HM+ EG 20% (v/v) + DMSO 20% (v/v)) for 4 min, subsequently COCs transferred to vitrification solution (HM+ EG 40% (v/v) + DMSO 40% (v/v) + 5 ng/ml, 10 ng/ml or 20 ng/ml EGF) for 30 sec and vitrified by 0.25 ml straw. The results revealed that the maturation rate of the oocytes obtained from using 20 ng/ml of EGF with EG 40% + DMSO 40% and EG 40% was significantly higher than that resulted using 20 ng/ml of EGF with DMSO 40% (n = 32/47 [68.76%], n = 27/42 [63.07%] and n = 21/47 [45.36%], respectively). It could be concluded that vitrification of immature camel oocytes by using 40% EG + 40% DMSO for 4 min equilibration are suitable methods to limit drawbacks of vitrification methods.

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Assessment of human sperm morphology by Computer Assisted Sperm Analysis (CASA) and two new methods using high optical magnification

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Morphological defects of sperm are one of the most important factors determining male infertility. In this study sperm morphology assessment according to WHO 2010 by CASA (SCA Microptics, Spain) was compared with different new methods: fixed preparations and preparations by Trumorph® system (Proiser R+D, Paterna, Spain) both performed at a magnification of 6600X. Additionally, we have compared the number and size of vacuoles within heads according to Vanderzwalmen et al. [Reprod Biomed Online 2008; 17: 617–27], using Motile Sperm Organelle Morphology Examination (MSOME) and

modified the Trumorph system, respectively. Fifty ejaculates were analyzed. Using the CASA system $43.3 \pm 15.6\%$ of spermatozoa showed normal head morphology in comparison with $12.9 \pm 6.4\%$ detected by Trumorph ($p \leq 0.01$) and $5.5 \pm 3.9\%$ in fixed preparations ($p \leq 0.01$). Furthermore, a strong correlation between MSOME and Trumorph was observed when identifying the classes of vacuoles (I $r = 0.904$, II $r = 0.856$, III $r = 0.625$ and IV $r = 0.716$). The divergences between different methods in the morphology values are related to the use of more sophisticated techniques and the evaluation of morphological defects in different populations of spermatozoa. Both techniques, MSOME and Trumorph, permit the morphological examination in the population of live spermatozoa, giving more accurate information about fertility.

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Flow cytometric assessments of bacterial counts in native boar ejaculates to determine the intra-boar variability

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The aim of the study was to investigate the variability of bacterial counts in native boar semen with focus on the individual variance among different boars at an AI-station. Nineteen boars routinely used for semen collection of different age and different breed were selected and ejaculates collected repeatedly in a weekly interval over a period of 5 weeks by the gloved-hand technique. Right after collection an aliquot of each ejaculate was diluted 1:10 in Tyrode solution without antibiotics to prevent agglutination. Then samples were stained with SYBR Green I and Propidium Iodide and measured after 15 min of incubation by flow cytometry to assess the number of live bacteria in the semen. The median bacterial count of all samples was 40,000 bacteria/ml, with a minimum of 2,000 bacteria/ml and a maximum of 285,000 bacteria/ml. Median bacterial counts of the different ejaculates of the same boar varied between 4,000 bacteria/ml and 138,000 bacteria/ml among all individuals. Performing a variance component analysis displayed that 24% of the variance in bacterial counts were attributed to the variability between different boars, while 9% of the variance were attributed to the variability within one boar. In conclusion, the level of bacterial count in native boar semen is only partly influenced by the individual animal. There is a tendency of the single individual to either lower or higher bacterial counts in their ejaculates, though regarding the formation of the total bacterial count various environmental components have to be taken in account as well.

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Diffusion of different cryopreservation solution components into ovarian tissue studied by ATR-FTIR

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Cryopreservation of ovarian cortex tissues is done using complex mixtures of cryoprotective agents (CPAs) to reduce the damaging effects of freezing. However, care should be taken to avoid toxic effects of CPAs themselves, particularly for high concentrations as used for vitrification. In order to rationally design cryopreservation strategies for ovarian tissues, it is important to precisely determine permeation kinetics of the protectants that are used to ensure maximum permeation and homogeneous distribution, while minimizing the exposure time and toxicity effects. In this study, we have used an attenuated total reflection - Fourier transform infrared spectroscopy (ATR-FTIR) setup to simultaneously monitor diffusion of multiple components in a mixture into ovarian tissues. Diffusion studies were done with sucrose, dimethyl sulfoxide, glycerol, ethylene glycol, and propylene glycol as well as with mixtures of these compounds. To assess diffusion kinetics of different solutes in mixtures by ATR-FTIR, the increase in solute specific infrared absorbance bands was monitored during diffusion through the tissue. The relative increase in band area was assumed to be proportional to the CPA concentration in the tissue and plotted versus the diffusion time. For comparison, diffusion studies using osmometer measurements were done; by measuring the increase in osmolality of a saline solution in which tissue loaded with CPAs for a defined period was equilibrated. Diffusion equations based on Fick's second law of diffusion were used to fit experimental data and to derive diffusion coefficients.

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Membrane permeability of porcine oocytes to water and cryoprotective agents

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With vitrification, oocytes are exposed to high concentrations of permeating cryoprotective agents (CPAs) followed by rapid cooling. CPA addition and removal is typically done step-wise to avoid osmotic damage caused by cell volume changes. In order to predict cell volume changes, osmotic tolerance limits and

plasma membrane permeability properties need to be experimentally determined. In this study, volume responses of immature and in vitro matured porcine oocytes were recorded upon addition of ethylene glycol (EG), dimethyl sulfoxide (DMSO), polypropylene glycol (PG), and sucrose (SUC). The two parameter formalism was used to fit volume versus time plots and derive the membrane permeability to water (L_p) and the various CPAs (Ps). It was found that L_p differs in the presence of the different CPAs, particularly for matured oocytes, and is strongly reduced in the presence of SUC. Ps increased in the order $EG < DMSO < PG$, and values were found to be lower for immature oocytes. As expected, membrane permeability for the membrane impermeable SUC was negligible. The obtained insights can be used to rationally design vitrification methods for porcine oocytes.

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The evidence of paracrine signaling in porcine cervix after mesenchymal stem cells transplantation into the muscle layer

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Bone marrow-derived mesenchymal stem cells (MSC) are a promising source for cell-based treatment in regenerative medicine. In recent study we revealed that MSC survived after transplantation into the muscle layer of porcine uterine cervix and demonstrated significantly higher proliferative potential than smooth muscle cells. We suggested that transplanted MSC probably stimulate the endogenous cells of muscle layer of uterine cervix in pig in paracrine signaling. One of the protein, which is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells is hepatocyte growth factor protein (HGF). It regulates cell growth, cell motility and morphogenesis in numerous cell and tissue types. The aim was to evidence the HGF expression after transplantation of MSC into the muscle layer of uterine cervix in sow. MSC was autotransplanted into the muscle layer of cervix of Polish Landrace sows ($n = 10$) after 3 weeks in vitro culturing with DID markers. After 28 days pigs were neutered, cervixes were collected, fixed and immunofluorescence (IF) labeled with anti-HGF antibodies. Then MSC and HGF were imaged using confocal and scanning microscopy (mean% $\pm$ SEM). Both cells positive and negative (endogenous cells)

for DID showed HGF staining in cytoplasm. The HGF expression was higher ($p = 0.047$) in transplantation places ($9.20\% \pm 2.38$) close to DID positive cells compared to no transplantation places ($3.47\% \pm 0.75$). However the colocalisation of MSC and HGF was low and included only $0.21\% \pm 0.12$ of double positive cells out of all the cells in the tissue. We suggest HGF is secreted by both MSC and endogenous cells. The paracrine signaling for cells proliferation of HGF secretion may be stimulated by MSC transplanted into muscle layer of porcine cervix.

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Correlation between basic biochemical and gasometric parameters and distribution of follicle size in Holstein Friesians (HF) cows

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Follicular size and oocyte quality is one of the key factors limiting female fertility. The development of the oocyte occurs in the ovarian follicle, the follicle components may have crucial impact effect on the oocyte's quality. The aim of this study was the assessment of a correlation between basic biochemical and gasometric parameters (acid-base balance - ABB) and the distribution of follicle size in HF cows. The material (follicular fluid, FF) was collected from 40 slaughtered cows (HF breed). The cows were assessed pre-slaughter by ultrasound examination of the reproduction tract. Follicles were divided into 2 groups, according to their size: I ($n = 16$) 5–10 mm (small FF); II ($n = 24$) 11–25 mm (dominant FF) diameter. Parameters such as pH, pCO₂ (mmHg), pO₂ (mmol/L), HCO₃-act (mmol/L), BEecf (mmol/L), ctCO₂ (mmol/L), Na⁺ (mmol/L), K⁺ (mmol/L), Ca⁺⁺ (mmol/L), Cl⁻ (mmol/L), AnGap (anion gap, mmol/L), Glu (mg/dL) were assessed in FF with a critical points analyzer Siemens RAPIDPoint 500. By applying logistic regression to the data, we identified four variables that contribute the most to the discrimination between follicles from I and II group. These were the concentrations of Na⁺, K⁺ and Cl⁻ ions and AnGap variable, which is a derivative of the former concentrations. The equivalent of R² parameter for logistic regression called McFadden R² index was estimated to be 0.51, indicating a strong correlation between such a simple parameters and follicle size. Even the slight differences in FF electrolytes could have a significant impact on the growth and quality of oocytes. This study represents a preliminary stage of describing the direct effect of basic biochemical and ABB changes in FF on oocyte development.

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Influence of intrauterine administration of *Lactobacillus buchneri* on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis

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Potential beneficial effects of lactic acid bacteria on the genital health of cows become of particular interest when considering the importance of an optimal uterine health status for the success of breeding in dairy farming. Therefore, the aim of the present study was to analyse the influence of an intrauterine administration of the *Lactobacillus buchneri* DSM 32407 on reproductive performance, uterine health status, endometrial mRNA expression of pro-inflammatory factors of cows with signs of subclinical endometritis (SCE). *L. buchneri* DSM 32407 ($n = 56$; [LAC]) or a placebo ($n = 60$; [PLA]) was administered on day 24–30 post partum to cows with signs of SCE and healthy cows, because detection of SCE could be only done after administration. Endometrial cytobrush samples of cows with SCE were taken before the administration and at three following weeks ($n = 16$ cows each for LAC/SCE and PLA/SCE). A higher proportion of cows of the LAC and LAC/SCE group was pregnant after the first service compared with the PLA and PLA/SCE group, respectively. The median days to conception for cows pregnant on day 200 pp were 90 days and 30 days shorter in the LAC/SCE ($p = 0.001$) and the LAC ($p = 0.035$) group compared with the PLA/SCE and the PLA group, respectively. Three weeks after the administration, the endometrial mRNA expression of *CXCL1/2*, *CXCL3*, *CXCR2*, *IL1B*, *IL8* and *PTPRC* was lower in the LAC/SCE group compared with the PLA/SCE group. These findings suggest that the presence of *L. buchneri* DSM 32407 contributes to a uterine environment that results in a better reproductive performance. (Study was supported by DFG [GA 1077/5-1].)

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In vitro efficacy of bovine antimicrobial peptides against major mastitis pathogens

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During bovine mastitis, a significant number of genes coding for antimicrobial peptides

(AMP) is being upregulated. However, their immunological role in battling intramammary bacterial infection still remains unclear. The aim of the study was to assess antimicrobial effects of assorted AMP, which were shown to be upregulated during clinical or subclinical mastitis. Lingual antimicrobial peptide (LAP), indolicidin and S100A9 were tested *in vitro* for their minimum inhibitory concentration (MIC) against bovine mastitis isolates of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus uberis* (*Sc. uberis*). Bacterial growth *in vitro* was assessed by optical density (600 nm) over 6 h and was validated by plate counts. Statistical analysis (Mann-Whitney-U-Test) was performed with the program R (Version 3.2.2.). The AMPs LAP (40 µg/mL) and indolicidin (40 µg/mL) significantly inhibited bacterial growth of all three tested pathogens ($p < 0.01$). However, bacteria were still able to multiply. The AMP S100A9 showed to be highly effective against *S. aureus* and *Sc. uberis* and totally inhibited bacterial growth in concentrations of 5 µg/mL. It did not show any inhibitory effect against *E. coli* *in vitro*. In conclusion, assorted bovine AMP display *in vitro* activity against mastitis pathogens. However, their antimicrobial effects may be pathogen-specific. In future, triggering the expression of endogenous AMP may be a tool to more effectively battle intramammary infections and to reduce the amount of antimicrobial drugs in the dairy industry.

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Analysis of expression patterns of selected genes and proteins in uterine endometrium during implantation in pigs

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Pig litter size is one of the most important economic traits affecting production efficiency in the pig industry. Studies have revealed that there are very different patterns of gene and protein expression in uterine endometrium at different stage of implantation, but molecular mechanisms are still poorly understood. We examined the level of expression of 6 genes (*NMB*, *S100A8*, *SELL*, *PPARG*, *RBP4*, *OPN*) in porcine endometria on days 12 and 16 of gestation. Eight pregnant Large Polish White (WPB) sows were slaughtered on days 12 ($n = 4$) and 16 ($n = 4$). The non-pregnant sows ($n = 4$) were slaughtered on day 12 after insemination. qRT-PCR showed that all 6 genes were differentially expressed at the two time points of implantation. The significantly higher level of the *NMB*, *S100A8* and *SELL* mRNA was observed on day 12 of gestation

vs. on day 16. The elevated *PPARG* and *RBP4* mRNA expression levels were observed in endometrium of sows both on days 12 and 16 of gestation. The highest level of the *OPN* mRNA was noticed on day 16 of pregnancy. Moreover, identification the differences between the transcriptome and proteome using 2D electrophoresis was made. Studies in pigs have shown that as many as 43% of the spots proteins showed differential expression between 12 and 16 of gestation. Further the mass spectrometry methods, MALDI-TOF/TOF will be used to precise qualitative analysis of the proteins spots. The results identified candidate genes that may play crucial role in the regulation of endometrium function during early pregnancy, and might to contribute to differences in litter size. (This work was supported by grant from NCN, Poland [No. 2013/09/N/NZ9/03135].)

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Determination of equine fetal sex in mid and advanced gestation by transabdominal two- and three-dimensional sonography

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This study aims to evaluate feasibility of transabdominal 3D tomographic ultrasound imaging (TUI) as an additional diagnostic tool for determination of equine fetal sex. Pregnancy checks were performed by transabdominal sonography on 386 Thoroughbred mares between 86 and 231 days of gestation. Time for examination of each mare was limited to a maximum of 3 minutes. Predicted fetal sex was compared to the sex at birth to determine accuracy of the methods. Fetuses that had gonads with a homogeneous echotexture (testicular tissue) and a thin central longitudinal echogenic line (mediastinum testis) were considered to be male. Fetuses presenting gonads with a central circular echogenic structure (cortex) surrounded by a hypoechogenic external ring (medulla) were considered to be female. Doppler ultrasonography was used to identify the vascularisation of pampiniform plexus and testicular vein in male fetuses and a circular vascularisation between the cortical and medullary layers of the ovary in females. In addition, 3D image volumes were analyzed using Tomographic Ultrasound Imaging (TUI) (4D View® Version 10.x, GE Healthcare, Austria). Sectional images of the gonads were evaluated due to their sex specific anatomical structures. The gender of the fetus could be determined in 297 cases (77%) within the three-minute examination time frame. For 94% of foals born predicted sonographic fetal sex was correct. 3D TUI imaging allowed a sex diagnosis in 18 cases where 2D sonography showed doubtful results. Transabdominal 3D TUI of

fetal gonads enables to increase accuracy of sex determination in mares during mid- and advanced gestation.

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Characterisation of the intra-uterine bacterial flora of dairy cows at the time of insemination

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Dairy cows with persistent clinical endometritis (CE) at the time of artificial insemination (AI) are often not inseminated and/or undergo an intrauterine antibiotic treatment. Based on the characterisation of intrauterine bacterial growth in cows with and without CE at the time of AI, the impact of bacterial infections as well as the indication of an antibiotic treatment at that specific time was assessed. On the day of AI, vaginal discharge was evaluated with the Metrichick device. Animals showing clear discharge were regarded as healthy (E0, n = 58) whereas those with flecks of pus were defined as cows with mild endometritis (E1, n = 64). Uterine samples were taken by the cytobrush technique. Bacteria were cultivated aerobically and identified by Fourier-transform infrared spectroscopy. The bacterial growth density was similar in both groups. Furthermore, no striking differences regarding the bacterial composition were detected. Most frequently detected bacteria in group E1 were representatives of the genera *Staphylococcus* (16%), *Bacillus* (12%), *Corynebacterium* (10%) and *Lysinibacillus* (10%). Most frequently detected isolates in group E0 were members of the genera *Bacillus* (25%), *Corynebacterium* (16%), *Micrococcus* (13%) and *Staphylococcus* (10%). Pathogenic bacteria, such as *T. pyogenes* and *E. coli*, were isolated rarely. Hence, the impact of bacterial infections at the time of AI ought to be subservient and questions the indication of an intrauterine antibiotic treatment in cows with mild endometritis at that time. More detailed analyses including fertility data are needed to confirm this assumption.

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Fertility parameters in German dairy herds – determined by herd size and milk yield?

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Milk production per cow has increased dramatically over the last 50 years. Furthermore, dairy herds become larger resulting in fewer employers per cow. The shift towards more productive cows and larger herds is associated

with a decrease in reproductive performance [Lucy, J Dairy Sci 2001; 84: 1277–93]. To elucidate the situation in Germany selected parameters, in particular conception rate, service rate, pregnancy rate, days to first insemination and days open, were studied using data of 148 herds. For statistical analysis the herds were categorized concerning milk yield: (1) < 30kg, (2) 30–35kg, and (3) > 35kg per cow and day; as well as concerning herd size: (1) < 200, (2) 200–400, (3) 400–1000 and (4) > 1000 milking cows. Conception rate did not reveal any statistical difference between the herds. That means in dairy farms at a larger size or with high milk yield the risk of an inseminated cow to become pregnant is the same as in small herds or in herds with low milk yield. Small herds (< 200 cows) had lower pregnancy rates than larger herds. The pregnancy rates of herds with different milk yield were not statistically different. Though, there was a trend that dairy farms with higher milk yield (> 30 kg) had higher pregnancy rates. Statistical differences and trends in pregnancy rates are due to higher service rate in larger herds as well as in herds with higher milk yield. Therefore, service rate can be mentioned as the key element for high reproductive performance in dairy herds. Poor fertility is not associated with high milk yield or large herd size but represents deficient farm management.

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The thecal glands in the ovary of the quail (*Coturnix japonica*) – an immunohistochemical and ultra-structural study

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The role of thecal glands in the ovary of birds is still controversially discussed. Using transmission electron microscopy and immunohistochemistry (immunohistochemical localisation of cyclooxygenase I and II (COX-1 and COX-2), prostaglandin receptors, estrogen receptor α and β (ER α and ER β), progesterone receptor (PR) and androgen receptor (AR) a detailed analysis of the thecal glands was performed. Our ultra-structural studies revealed that the thecal glands of the quail's ovary consists of two cells types, steroid producing cells (SPCs) and enclosing cells (ENCs). The SPCs are large, light cells containing a varying number of lipid droplets. The cytoplasm is characterized by a large amount of smooth endoplasmic reticulum. The enclosing cells are always located at the periphery of the gland. Some of the ENCs contained an abundant amount of microfilaments, but lipid droplets and dense bodies were rare. Within one gland, SPCs with strong immunostaining were interspersed between a usually larger number of moderately Cox-2 positive cells. A completely different staining pattern was observed for Cox-1, where the cytoplasm of the ENCs was distinctly immunopositive, whereas the SPCs stained only weakly. The thecal glands showed a distinct immunostaining for

ER β but only a weak to negative reaction for ER α , PR and AR. Our immunohistochemical and electron microscopic data support our hypothesis that thecal glands of the quail are involved in steroid hormone and prostaglandin synthesis. The prostaglandins secreted by the thecal glands probably contribute to the ovulation of the F1-follicle.

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Histological comparison of testicular biopsies and tissue samples from castrated testes in bull calves

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To date, the number of studies on the comparability of bovine testicular biopsies with tissue samples derived from bulls are limited. Hence, the aim of this evaluation was to investigate, if histological findings of biopsy samples of bull calves were comparable in quality and interpretability to those of their respective organ samples. Testes of five beef bull calves (2 crossbreed beef/1 and 10 months of age/and 3 Limousin/5 months of age) were obtained by routine castration. After taking 3 biopsy samples, the remaining testes were dissected. All samples were fixed in Bouin's solution, embedded in paraffin and stained with hematoxylin-eosin. For histological comparison, number of Sertoli cells (SC) per tubular cross section and of seminiferous tubules containing elongated spermatids as well as the outer diameter of the tubules. The latter increased with age in all calves, both in the biopsy and the organ samples. In four calves, the mean value of the SC number per tubular cross section (except for the 10-months old) and also the outer diameter (except for one of the 5-months old) from the biopsies and the dissected testis were comparable. Elongated spermatids were only found in the seminiferous tubules of the 10-month old bull calf. In conclusion, testicular biopsy might be a useful tool to obtain a representative overview of the testicular parenchyma, assuming that biopsies were collected from three different areas of the testis and possible alterations are evenly spread throughout the organ. To confirm these preliminary results, additional samples should be investigated. (This project received financial support through the Association for Bioeconomy Research (FBF).)

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Application of liposomes in sex sorting and cryopreservation of bovine semen

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Standardized phospholipid liposomes are promising candidates to replace egg yolk in semen extenders. In this study liposomes were used in catch fluid (2% liposomes) during sex sorting and during cryopreservation (20% liposomes) of sorted and unsorted sperm. In a thermos-tolerance test, compared to controls containing egg yolk, sperm quality was not diminished at 0 h and 6 h, respectively (i.e. motility: 91.6%, 86.0%; membrane integrity: 82.6%, 82.4%; morphology: 94.3%, 92.9%). Cryopreservation in extenders Sexcess[®] and Optixcell[®] containing liposomes was conducted with unsorted sperm and with spermatozoa that were sorted into the liposomes containing catch fluid. All samples were evaluated in a 6 hour lasting thermo-tolerance-test (n = 14). Sexcess[®] supplemented with egg yolk resulted in a higher post-thaw motility (56.6%, 43.8%, 37.4%), membrane integrity (52.0%, 49.1%, 50.1%) and morphology (87.1%, 78.7%, 69.3%) at 0 h, 3 h and 6 h respectively of sorted sperm cells compared to the supplementation with 20% liposomes (motility: 45.0%, 26.7%, 17.2%; membrane integrity: 30.0%, 21.2%, 28.9%; morphology: 75.3%, 68.1%, 61.4%) or Optixcell[®] (motility: 56.4%, 34.1%, 27.0%; membrane integrity: 33.6%, 23.6%, 25.7%; morphology: 75.3%, 67.0%, 60.1%). In case of unsorted semen, even at 6 h motility (57.9%, 44.2%, 50.2%), membrane integrity (64.9%, 38.9%, 47.2%), and morphology (85.5%, 83.1%, 83.2%) of the mentioned diluents had been on a higher quality level. In conclusion, with the chosen setup, liposomes may serve as egg yolk replacement in catch fluid during sorting but not as supplement in Sexcess[®] freezing extender.

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Analysing differences in the secretory capacity of individual bovine corpora lutea; a revised approach

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With the aim to better understand spontaneous variations in the secretory capacity of bovine corpora lutea (CL), daily changes in the peripheral blood progesterone concentration (P4) of Swiss Brown dairy cattle were evaluated according to a revised approach. Instead of considering P4 samples as discrete data in a time series (common method), they were regarded as components of a stepwise increasing total amount ("integral" method). The study included 360 luteal periods (oestrus cycles and early pregnancies; 144 animals) during which, in addition to daily blood sampling for RIA assessment, ovarian function continuously was monitored by transrectal palpation. Data analysis is based on boxplot technique and on the calculation of Pierson correlations. Apart from confirming and stressing previous findings, the "integral"

evaluation pointed out that: (1) the marked variability of P4 secretion much more is due to differing lasting trends than to incongruent transient fluctuations; (2) early rising dominant follicle waves seem to be rather accompanied than anticipated by a sustained increase in the median blood progesterone concentration; (3) the development of ovulatory follicles whose lifespan exceeds 6 days is influenced by the amount of P4 secreted by the regressing CL in this period. Evaluating the activity of bovine CLs according to the "integral" method proves to be a promising approach.

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Impact of transport stress on boar semen quality during long-term storage

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The shipping of semen doses to the sow farm is an important step that can influence boar semen quality. Unfortunately, no practice-oriented information is available related to general shipping conditions of boar semen until today. For this reason, a special mobile sensing app (TransportLog 1.0), utilizing built-in sensors of smartphones, has been programmed to capture temperature and vibration emissions during shipping of semen doses (QuickTip Flexitubes[®], Minitüb). Data were analyzed, transformed and used as standards for simulating vibration emissions by an orbital shaker (IKA MTS 4, Laborgeräte München) in a spermatology reference laboratory. Thirty ejaculates were collected randomly and diluted one-step isothermally in a split-sample procedure in a BTS extender (Minitüb). The sperm concentration was adjusted to 23.5×10^6 spermatozoa ml⁻¹. The filling volume was 85 ± 1 ml. Samples were stored for seven days at 17°C. A comparison of the two main storage positions (horizontally vs. vertically) showed no influence on semen quality. In contrast, temperature undulations for 6 h below 10°C after processing affected semen quality, especially progressively motile, acrosome-defect, mitochondrially active and membrane-intact spermatozoa. Temperature undulations between 10°C and 30°C showed no effect. Finally, we found that maximal vibration emissions with circular horizontal frequencies of 300 rpm for 6 h during shipping had a negative impact on pH value of the BTS extender and semen quality during long-term storage. This study leads to new recommendations for boar semen transport. (Supported by FBF Germany.)

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Histopathological and immuno-histochemical examination of equine penile squamous cell carcinomas for diagnosis and prognostic evaluation

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Squamous cell carcinomas (SCC) are the most common neoplasms of the male external genitalia in the horse. The aim of this study was to classify equine penile SCC by means of histopathological and immunohistochemical characteristics for a precise clinical staging. Ninety-five SCC were divided into carcinoma in situ ($n = 13$), microinvasive ($n = 11$) and deeply invasive carcinoma ($n = 71$). All invasive SCC were classified into malignancy grades according to the proposal of van den Top et al. (2008) and into histological growth patterns according to the human WHO classification [Cubilla et al. 2016]. Additional immunohistochemical studies (proliferation marker Ki-67) were performed. Twenty-nine percent of the invasive SCC are well (grade 1), 55% moderately (grade 2) and 16% poorly (grade 3) differentiated. Comparable to the findings in men, some growth patterns can be found in equine penile SCC, especially the warty (45%) and the usual type (40%). A prognostic assessment as described by van den Top et al. (2008) and by Cubilla et al. (2016) is also assumed for comparable equine neoplasia in this study. The Ki-67 indices in SCC are obviously higher than in non-neoplastic tissues. Furthermore, an association of proliferation rate and grade of differentiation has to be taken into consideration. The histopathological and immunohistochemical examination is not only necessary for a definitive diagnosis of masses of the equine penis, but could also be helpful to evaluate the clinical outcome of the affected male horses more accurately.

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In the epididymal duct, structural changes of the smooth muscle cell layer predominate over functional changes after long-term treatment with LPS

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In a mouse model of ascending epididymitis by uropathogenic *E. coli* (UPEC) we previously observed a reduced diameter of the epididymal duct three days after infection upstream of the infected area, putatively blocking further ascent of bacteria (Stammli et al. 2015). In the present study, we investigated whether long-term treatment (≥ 24 h) with lipopolysaccharide (LPS), used to mimic bacterial infection, results in (i) disturbed

contractile activity of the epididymal duct and/or (ii) structural changes of its smooth muscle cell (SMC) layer. A mouse model using i.p. LPS injection and organ cultures of the epididymis (24 h, 48 h, 72 h) were investigated. Time-lapse imaging using isolated parts of the epididymal duct did not show any differences in spontaneous contractile frequency between LPS- and vector-treated mice. Whether in this model effects on the duct diameter by noradrenaline, the main contracting agent of the epididymis [Mewe et al. 2007], are altered, is currently tested. In the LPS mouse model and in cultured epididymis, long-term treatment with LPS revealed an increase of collagen fibres in the SMC layer. In the culture model this was accompanied by an increase of SMA- and calponin-expressing SMCs surrounding a defective epithelium. In summary, data on long-term treatment with LPS suggest that structural changes of the epididymal duct SMC layer in response to bacterial infection might be more important than functional changes. Such remodeling could represent a defensive mechanism of the host explaining the reduced diameter of the epididymal duct previously found in a mouse model of ascending epididymitis.

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Investigation of the relationship between pregnancy losses and the backfat thickness in the dairy cattle: observations of the critical days 28–49 post insemination

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It is in limited studies reported that the reduction of the back fat thickness (BFT) may be risky for post-insemination embryo survival [López-Gatius et al. 2002; Silke et al. 2002]. However, a complete elucidation of the relationship between early pregnancy losses and change of the BFT post-insemination has still not achieved. For this reason, the role of the change of the BFT in the pathogenesis of the embryonic deaths, between days 28–35 and 42–49 post-insemination, constituted the objective of our study. A total of 520 pregnant animals (54 heifers and 466 lactating cows) from five dairy farms were included in the study. Conditions for inclusion in the study were conceiving via artificial insemination at a known and documented date, positive first pregnancy examination (day 28–35 post-insemination by ultrasound) and re-examination for pregnancy 14 days later (day 42–49 post-insemination by ultrasound). At the time of both pregnancy examinations, the BFT was measured via ultrasound. The following results were obtained: The pregnancy losses were 23 cows and 1 heifer. There was

no statistically significant difference in the BFT measured at two week intervals, both in pregnant (12.9 ± 3.7 mm and 13.1 ± 3.9 mm) and in animals with pregnancy losses (14.4 ± 3.6 mm and 13.5 ± 3.9 mm). So, in our study we can not shown that BFT loss is a risk factor for embryonic death.

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Sensitivity and specificity of different endometrial swabbing techniques and their invasiveness and practicability in stud farm practice

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The aim of the present study was to evaluate the accuracy, invasiveness and practicability of different endometrial swabbing techniques. Cultural results of uterine swabs taken manually, by speculum or iVetscope® were compared with regard to bacterial growth, sensitivity and specificity, signs of inflammation of the mucosa or insufflation of air and economic and time effort (time for sampling, manpower requirements, cleaning and disinfection time and required materials). After gynecological examination double-guarded swabs were taken from 88 mares in estrus manually ($n = 29$), by speculum and forceps ($n = 29$) or by iVetscope® ($n = 30$). Zero, 4, 24 and 48 hours after sampling a vaginoscopic examination via iVetscope® and a transrectal ultrasonography were performed to evaluate vaginal haemorrhage and purulent secretions as well as cervical and uterine fluid and air. Sampling was repeated after 48 hours. The three different sampling methods showed significant differences in bacterial growth, especially in the second sampling after 48 hours ($p < 0.05$). The sensitivity and specificity to detect an endometritis, assessed in relation to the cytological examination of a cytobrush, was 0.67 and 0.75 for manual, 0.25 and 0.96 for iVetscope® and 0.5 and 0.96 for speculum sampling. Evaluation of invasiveness with regard to signs of inflammation of the vaginal mucosa and insufflation of air into the cervix and uterus showed no differences between methods ($p > 0.05$). If swabs are taken manually, the effort for personal, material, cleaning and disinfection is the lowest compared to the other methods. The results strongly recommend endometrial swabbing by speculum and forceps or iVetscope®. Although, these instrumental techniques might be slightly more time and material consuming, the significantly reduced bacterial contamination underlines the relevance of the swabbing technique in equine practice.

Left flank ovariohysterectomy as the final surgical treatment of pseudopregnancy in goats – a case report

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Nowadays goats are not only farm animals but also kept as outdoor pets at modern eco type or agro tourism farms. Pseudopregnancy is one of the major causes of anestrus in goats during the reproductive season. It is characterized by the persistence of a corpus luteum in the absence of a (viable) conceptus in the uterus. The primary clinical feature is hydrometra (accumulation of fluid in the uterus) imitating the normal pregnancy development. A two and a half year old dairy goat was brought to the clinic with severe bilateral abdominal enlargement. The goat had never been naturally mated or artificially inseminated. A clinical examination was done and all the basic life parameters evaluated were normal. A large volume of fluid accumulated inside the uterus was confirmed by ultrasound examination. During the previous as well as the present breeding season, the goat was administered a single dose of prostaglandin F2 alpha and oxytocin; nevertheless the hydrometra reoccurred. Radical surgical treatment was demanded by the goat owner. A general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with endotracheal intubation following intramuscular premedication and intravenous induction was chosen in order to perform left flank ovariohysterectomy. During surgery a total amount of accumulated fluid of 15 liters was evacuated prior to removing uterus and ovaries. Postoperative, antibiotics in combination with non-steroidal anti-inflammatory drugs were administered. In conclusion, well-managed surgeries on reproductive tract in goats under general anaesthesia enable successful treatment of severe clinical cases like extreme types of hydrometra.

Indicators of structural ability of equine uterus to relaxation in relation to hormonal regulation

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Proper uterine contractility and relaxation are major factors affecting mare's fertility and uterine defense mechanisms. The contraction-relaxation process depends on the unaffected function of both active and passive structures of the uterine wall, which are connective tissue and smooth muscle cells (SMCs). Collagen, as the most abundant structural protein of the uterine extracellular matrix, takes part in the passive relaxation process. In this study, assessment of the collagen content (CC) of equine uterus during the follicular and the luteal phase was demonstrated. Fresh, whole thickness samples of corpus uteri were collected from 29 slaughtered mature mares with no signs of pathological alterations of the reproductive tract. The samples were stained with hematoxylin eosin (HE) and Masson's Trichrome Stain (MTS) and evaluated using light microscopy and scanning cytometry for CC and the number of SMCs (mean% ± SEM). Tunica mucosa formed folds with connective tissue underneath, demonstrating higher ($p = 0.047$) collagen content during the follicular ($49.62\% \pm 3.67$) than the luteal ($34.88\% \pm 3.28$) phase. In tunica muscularis neither CC nor the number of SMCs had changed during the oestrus cycle significantly, obtaining $49.71\% \pm 2.53$ and $39.50\% \pm 1.50$ in the follicular and $47.44\% \pm 8.15$ and $43.15\% \pm 5.83$ in the luteal phase, respectively. There were no significant differences in CC between tunica mucosa and tunica muscularis during the follicular phase; however, during the luteal phase CC was significantly higher in tunica muscularis than in tunica mucosa ($p < 0.05$). In conclusion, CC in the equine uterine body undergoes hormonal regulation and differs significantly in tunica mucosa due to the phase of the oestrus cycle. The ability of relaxation of tunica muscularis is comparable during estrus cycle, with still the same proportion of the number of SMCs.

Evaluation of a Rapid Visual Pregnancy Test for detection of Pregnancy-Associated-Glycoproteins (PAGs) in sheep – preliminary results

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The "Idexx Rapid Visual Pregnancy Test" is used for pregnancy diagnosis in cattle by testing for pregnancy associated glycoproteins (PAG). We evaluated its sensitivity and specificity in sheep, as well as its diagnostic accuracy in early pregnancy and post partum (p.p.). The test was performed with whole blood (WhB), plasma (P) and serum (S) of 163 mid- to late pregnant and 153 non-pregnant ewes. In addition, eleven pregnant ewes

were tested weekly from day 14 to 49 and monthly from day 60 to 149 of gestation. Ten ewes were followed up weekly from the date of lambing until day 63 p.p. The sensitivity in mid- to late pregnancy was 98.77% (WhB), 99.39% (P) and 99.39% (S), while the specificity was 94.12%, 76.47% and 93.46%, respectively. Earliest detection of pregnancy varied in the eleven ewes examined: They were all correctly identified as pregnant on day 42 (100%, WhB, P & S). Surprisingly, the test accuracy subsequently dropped to between 54.6% (WhB) and 63.6% (P & S) on day 49. All eleven ewes were again consistently identified as pregnant on day 60 (P) or on day 120 (S & WhB), respectively. Post partum, the test was consistently negative from day 42 p.p. onwards in eight ewes. The remaining two remained consistently positive until day 63 p.p. (last sample). The case history should therefore always be considered when interpreting the test results. The sensitivity of the Visual PAG Test is good in mid to late pregnancy and early detection of pregnancy is possible in individual animals. In some ewes the PAGs were however detectable for more than 63 days p.p.

Unusual hormonal profile in tomcat with fibroadenomatosis

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Introduction and description of a case In a two years old Main Coon tomcat after treatment with cyproterone acetate (Androcure, Bayer) due to behavioral disorders, a hyperplasia of mammary glands was noticed. It is known that fibroadenomatosis is induced by progesterone and is accompanied with its high serum concentration. The blood results of the patient revealed very low progesterone and high estrogens concentrations. Tumor of the testes and cryptorchidism were excluded. Partial mastectomy was carried out, leaving two mammary glands due to technical problem. Therapy with tamoxifen (Tamoxifen, Ebewe) was carried out and the mammary gland involution was seen. Materials and methods: A sample of changed mammary tissue was collected and standard histopathological examination was done.

Results Histopathological examination confirmed fibroadenomatosis. Blood results revealed high estrogens (121 pg/ml) and very low progesterone, below 0.2 ng/ml concentrations. After the mastectomy estrogens concentration was between 74 and 127 pg/ml even several weeks after the surgery.

Conclusion The standard treatment of fibroadenomatosis is based on progesterone antagonists administration, for high estrogens concentration was the cause of this disease.

Effectiveness of treatment of remaining glands with an anti-estrogens drug confirmed this thesis. This may be the first such case described in veterinary medicine.

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Analysis of the hematologic values of calves before the 3rd month of life

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Values of hematologic parameters in cattle change during the development of the organism. During the period of fast growing and thereafter, during intensive production they can significantly fluctuate. This is influenced by the fact that many fetal processes remold into processes that allow to survive outside the maternal body, there is a change in the amount of blood cells, hemoglobin concentration and hematocrit. Also, leucocytes variables may be different. All this lead to a situation where reference value ranges that are defined for cattle might not necessarily be good reference points when interpreting new-born calves' test results. The goal of our research was to verify if there are any significant differences in results between calves younger than 3 months of age and adult cattle. For this purpose, blood samples were taken for hematology from 33 calves at a cattle farm. Blood was taken from jugular vein into 2 ml test tubes with anticoagulant (EDTA-K2) and tested for the following 13 parameters: RBC, MCV, MCH, MCHC, MPV, WBC, PLT, HGB, HCT, lymphocyte, monocyte and granulocyte numbers. The results were analyzed and compared with other tests and physiological ranges of adult dairy cattle. There were significant ($p \leq 0.001$) differences for 10 parameters (HGB, HCT, MCV, MCH, MCHC, RDV, PLT, MPV, WBC, lymphocyte number). For more than 90% of the tested red blood cell parameters of calves were different as compared to reference values. We found that the number of leucocytes could be variable. The research shows how important it is to determine ranges of hematological variables for calves.

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The effect of GnRH on the endometrial expression of progesterone receptor mRNA in the dairy cows

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Gonadotropin-releasing hormone (GnRH) consist of mostly direct regulation of pituitary gonadotropin secretion and indirect regulation of ovarian steroid hormone synthesis such as oestrogens and progesterone. Progestin affect through receptors from the nuclear hormone receptor subfamily, which are classified in uterine mucosa as endometrial progesterone receptors-PR, isoforms A and B. Progestin binding in target tissues increases the rate of transcription in the response gene. This study determined changes in the abundance of progesterone receptor isoform A (PRa) in bovine endometrium under the influence of exogenous GnRH. Uterine biopsy samples and blood samples were collected during the anoestrus, before intramuscular GnRH agonist administration (buserelin acetate, 0.0042 mg/ml, 5 ml/animal) and after 3 days from 10 HF no pregnant lactating cows. The biopsy samples were fixed, immunofluorescent stained (IF) using primary (mouse anti-human progesterone receptor 1A6) and secondary (donkey anti-mouse AF 568) antibodies and quantified by scanning cytometry using SCAN[^]R screening station. From corresponding samples full RNA was isolated, underwent reverse transcription reaction onto cDNA and multiplied using specific paired starters (PGR-a-GAPDH) and QuantStudio Real-Time PCR system. The blood samples were centrifuged and progesterone levels were measured in plasma. We demonstrated no differences ($p > 0.05$) in PR mRNA expression (0.56 ± 0.30 ; 0.37 ± 0.23), PRa expression in tissue (9.18 ± 2.80 ; 10.32 ± 3.09) and plasma P level (1.03 ± 0.44 ; 1.58 ± 0.52) in comparison to before: after treatment. Single administration of GnRH analog does not affect progesterone endometrial receptors expression on both mRNA and tissue level.

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Cryopreservation of canine epididymal spermatozoa – comparison of three different extenders

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The aim of the study was to compare three different extenders for cryopreservation of canine epididymal spermatozoa. Testicles of ten dogs that were routinely presented for castration were used in this study. The epididymis was dissected from the testicles of each dog and the cauda epididymidis was minced and placed into 0.9% saline solution at 38°C. The filtered fluid was examined with a computer assisted sperm analysis program (Androvision[®], Minitüb, Tiefenbach) for sperm motility parameters. Further, a hypoosmotic swelling test, a live/dead and a

pathomorphological examination using an eosin stained slide was done. Canine epididymal spermatozoa were cryopreserved with a self-made Tris-Fructose-Citrat extender, a self-made Uppsala extender or the commercial Optixcell[®] extender (imv-technologies, France). The above mentioned examinations were done after cryopreservation as well. Motility was higher for the Uppsala (total motility: $17.2 \pm 12.2\%$, $p < 0.001$; progressive motility: $11.8 \pm 9.5\%$, $p < 0.001$) and the Optixcell[®] (total motility: $11.7 \pm 6.5\%$, $p < 0.001$; progressive motility: $8.9 \pm 4.9\%$, $p = 0.008$) extended epididymal spermatozoa compared to the Tris extender (total motility: $4.7 \pm 4.8\%$, progressive motility: $2.8 \pm 3.7\%$). The differences of motility parameters between Optixcell[®] and UPS extended epididymal spermatozoa were not significant. The average percentage of pathologically altered epididymal spermatozoa was $48.8 \pm 9.8\%$ for Uppsala diluted samples, $44.9 \pm 8.1\%$ for Tris- and $37.5 \pm 10.1\%$ for Optixcell[®] diluted samples, whereas the difference was significant between Uppsala and Optixcell[®] ($p = 0.005$). Uppsala and Optixcell[®] were clearly superior to the Tris-extender in suitability of cryopreservation.

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Recovery of canine epididymal spermatozoa – comparison of two different times of incubation

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There are different methods to collect spermatozoa from the cauda epididymidis such as mincing, flushing, squeezing and percutaneous sperm aspiration. The aim of this study was to evaluate two different times of incubation for canine epididymal spermatozoa to migrate out of the caudal part of the epididymis after mincing. Testicles of ten dogs that were routinely presented for castration were used. The epididymis was dissected from the testicles of each dog, and the caudal part of the epididymis was minced with a scalpel and placed into 0.9% saline solution at 38°C. One cauda epididymidis was incubated for 10 minutes, the other one for 30 minutes. After the previously described time had passed, the fluid was filtered and examined with a computer assisted sperm analysis program (Androvision[®], Minitüb, Tiefenbach) for motility parameters and concentration. Additionally a hypoosmotic swelling test and after eosin staining a live/dead and a pathomorphological examination was done. The remaining tissue of the cauda epididymidis was fixed in formalin and 3 µm thick histopathologic slides were produced and colored with hematoxylin-eosin for evaluation of remaining spermatozoa. Motility and vitality parameters were not significantly influenced by the flotation protocol. Total cell count was $292 \times 10^6 \pm 175 \times 10^6$ for 10 minutes of incubation and $233 \times 10^6 \pm 162 \times 10^6$ for 30 minutes, the difference

was not statistical significant. There was no significant difference between the incubation protocols ($p = 0.56$) regarding intraluminal remaining spermatozoa. Concluding 10 minutes of incubation is sufficient for recovery of canine epididymal spermatozoa from the cauda epididymidis.

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Oral low-dose estradiol-17 β induces intergenerationally altered DNA methylation of CDKN2D and PSAT1

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Estrogenic endocrine disrupting chemicals (EDC) interfere with the natural hormone balance. Epigenetics is an underlying mechanism through which EDC lead to heritable intergenerational changes. In this study, the direct effects of estradiol-17 β (E2) on reproductive and non-reproductive tissues in sows (F0) were assessed. In addition, intergenerational effects were investigated both on post-conception day 10 embryos (blastocysts) and on 1-year old offspring (both F₁). During gestation, sows were orally exposed to two low doses and a high dose of E2 (0.05, 10, and 1000 $\mu\text{g/kg}$ body weight/day, and a control ethanol carrier), respectively. In F₀, perturbed tissue specific mRNA expression of cell cycle regulation and tumour suppressor genes was revealed at low and high dose exposure, being most pronounced in the endometrium and corpus luteum. In contrast, the liver showed the highest significant DNA hypomethylation in three target genes, namely *CDKN2D* putative promoter region, *PSAT1* coding region and *RASSF1* first exon 5'UTR. *CDKN2D* and *PSAT1* were likewise differentially methylated in female offspring as early as the blastocyst stage, highlighting the periconceptual period as a sensitive time window for the action of EDC. Strikingly, while F₁ blastocysts showed significant hypomethylation in selected regions, the liver of 1-year old F₁ offspring showed subtle, but significant corresponding hypermethylation. Our results highlight the importance of assessing the relevance of subtle methylation changes in the development of disease later in life and point towards the potential of epigenetics as additional sensitive parameter for risk assessment of estrogenic EDC.

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The endometrial luminal epithelium cells are most sensitive in the embryo-maternal communication during embryo elongation in roe deer (*Capreolus capreolus*)

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Roe deer display a four-month period of diapause, a period during which the embryonic growth velocity is reduced. A substantial number of earlier studies explored diapause in the roe deer, yet the key molecular signals underlying the reactivation of embryo development and embryo-maternal communication (ECM) have not been identified. Studies investigating the role of specific endometrial cell types in EMC in sheep, mice, and pigs revealed specific gene expression patterns in luminal (LE) and glandular epithelium (GE). In this study, we aimed at identifying differential gene expression in isolated endometrial cell types during diapause (blastocysts) versus preimplantation (elongated embryos) based on a set of 38 target genes with known functions in EMC in cattle, pig, sheep, mink, and mice. Endometrial samples corresponding to blastocysts of 2 mm ($n = 3$) and elongated embryos ($n = 3$) were collected during the course of regular huntings from September to December 2016. They were used for harvesting whole tissue (WT) and specific cell types, namely LE, GE, and stroma (STR), of both intercaruncular (IC) and caruncular (C) endometrium using laser capture microdissection. A clear developmental stage-specific gene expression pattern was found in the LE for both the IC and C endometrium. In addition, the endometrium corresponding to diapausing blastocysts and elongated embryos, showed 16 and 24 differentially expressed genes between all analysed cell types, respectively. Our data underline the importance of cell-specific EMC and further assign candidate genes which play a role in the regulation of embryo reactivation.

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Comparative study of placental characteristics of German Landrace and Hungarian Mangalica Sows and their relationships to fertility parameters

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Specific data of placental characteristics of modern pig lines with high fertility and current data for indigenous fatty pig breeds like the Hungarian Mangalica are not available.

Therefore, this study focusses on a comparative description of placental size of Landrace and Mangalica pigs and their relations to important fertility parameters. As basis for the data collection 55 litters from German Landrace sows (GL) with 15.1 ± 3.5 piglets/litter were used and their placentas were weighted, the length measured and the placental efficiency per litter calculated. Parallel to this 18 Hungarian Mangalica (HM) sows with 7.9 ± 1.8 piglets/litter were investigated and their placentas measured. Mangalica piglets were slightly heavier in average than Landrace piglets (1.5 ± 0.23 Kg vs 1.4 ± 0.23 Kg) however their placentas were lighter (234 ± 37 g vs 277 ± 54 g), which led to a significantly higher placental efficiency in Mangalica compared to Landrace (6.8 ± 1.5 vs 5.1 ± 0.7 ; $p < 0.01$). In GL sows increasing piglet numbers led to significantly decreasing piglet weights and placental lengths ($r = -0.3$ and $r = -0.4$), this might be due to limited uterine space (uterine capacity). However, in both breeds piglet number was still positive correlated with the total litter placental weight (GL $r = 0.7$; HM $r = 0.5$), so that high means of placental weights (GL $r = 0.7$; HM $r = 0.6$) or lengths (GL $r = 0.7$) improved piglet birth weight significantly. Our findings suggest that different biologic strategies for the realization of high fertility could exist. Breeding selection for placental efficiency or for uterine capacity/space might improve piglet weight and therefore their health. (Supported by RCE-1476-4/2016/FEKUT,HU).

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Use of quantitative greyscale analysis for examination of the Alpaca testes

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The quantitative greyscale analysis of the testes is a useful supplement of the B-mode ultrasound. Information about the application of this method is not given for alpacas until now. The aim of this study was to create reference values of this investigation procedure for the alpaca. The investigation was carried out to 46 alpaca stallions with and without pathological testicle findings. The animals were between 9 months and 8 years of age. To verify the ultrasound findings of the testes, 30 animals were castrated and the testicles were examined histologically. The greyscale analysis was carried out with the help of the Honda HS-1500 ultrasound scanner. The testicles were examined in the longitudinal and in the cross section. The middle grey values of the longitudinal section showed not significant differences to the middle grey values in the cross section. However, the comparison of the middle grey values in the longitudinal and in the cross section showed a significant

difference of $p = 0.03$ and $p = 0.09$ between healthy and pathologically changed testicles, respectively. In conclusion, the greyscale analysis could be used as an objective method to detect differences between pathological and physiological testicles of alpaca.

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Using a slow-release GnRH implant to suppress testis development in small ruminants

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In the present study the GnRH-Analogue Deslorelinacetat was tested in the form of a subcutaneous implant (Suprelorin® 4.7 mg, Fa. Virbac) to testis development in juvenile small ruminants. The implantation occurred in the test group to seven sheep and three goat lambs at the beginning of the sexual maturity, at the side from the bellybutton. The control group contained an equal number of animals without treatment. Every two weeks over 5 months, all animals were checked by a general and an andrological investigation including drawing blood samples for testosterone determination and ultrasound of the testicles. At the end of the investigation period, the animals were castrated for histological evaluation of the testicles. No significant difference was found between the test and control group in sheep and goats in all parameters. Nevertheless, for individual animals (one sheep and one goat) the testis development was suppressed. Concluding, testicle development cannot be suppressed with the help of a slow release deslorelin implant.

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Simultaneous profiling of the equine cumulus proteome and metabolome after maturation *in vivo* and *in vitro*

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Maturation of oocytes under *in vitro* conditions (IVM) results in impaired developmental competence compared to oocytes matured *in vivo*. Oocytes are closely coupled to their cumulus complex with a bidirectional exchange of metabolites. Therefore, elucidation of aberrations in cumulus metabolism *in vitro* is crucial for a better mimicking of physiological maturation conditions. The aim of this study was a novel combination of proteomic and metabolomic profiling of single cumulus complexes of metaphase II oocytes

matured either *in vivo* or *in vitro*. Cumulus oocyte complexes (COCs) of the *in vivo* group ($n = 8$) were collected from oestrous mares slaughtered 30h after hCG injection. COCs of the IVM group ($n = 7$) were matured for 30h in DMEM based maturation medium. Methanol based metabolite extraction preceded an adapted filter-aided sample preparation protocol for protein digestion. Proteome analysis was performed by nano-HPLC MS/MS and metabolome analysis by UPLC-nanoESI-MS in negative mode. Progenesis QI for Proteomics and Metabolomics software (Nonlinear Dynamics) were used for data analysis. A total of 1811 quantifiable proteins and 905 metabolites were identified. The proteome contained 216 differentially expressed proteins ($p \leq 0.05$; $FC \geq 2$; 95 increased *in vivo*; 121 increased *in vitro*) and the metabolome 48 differently abundant metabolites (BGA Score $> 66\%$; 32 increased *in vivo*; 16 increased *in vitro*). These results unravel for the first time simultaneously an impact of the maturation condition on the cumulus proteome and metabolome of individual COCs.

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Comparative study of automated and manual thermogram analysis of bovine udders with induced *E. coli*-mastitis

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Infrared thermography is a noninvasive tool to detect clinical mastitis early; however, manual evaluation of the thermograms is very time-consuming. This study concerns the question if evaluation of udder thermograms by an automatic image recognition software provides results comparable to those of manual evaluation in detecting clinical *E. coli* mastitis. Five healthy Holstein-Friesian dairy cows were challenged with *E. coli* into the right hindquarter and a placebo into the left hindquarter. All cows developed signs of clinical mastitis. In a period of 24 hours before and after challenge, thermograms of the hind udder surface were taken in intervals of two hours with the help of an infrared camera. The thermograms were evaluated by an automatic image recognition software ('Aut') that detects the silhouette of the left (HL) and right hindquarter (HR). For comparison, they were also evaluated manually with a polygon-tool ('Man'). Automatic evaluation had a low rate of falsely detected udder borders (2–3%). In both methods, peaks of average ('Avg') and maximum surface temperature ('Max') of both hindquarters occurred 13–15 hours after challenge. Average temperature peaked less in the challenged quarter. The results of both methods are highly correlated: $r = 0.98$ ('Avg HL'), respectively, $r = 0.99$ ('Avg HR'). In ROC-analysis, both methods provide good

results for sensitivity and specificity at different threshold values. Automatic evaluation of thermograms of bovine udders challenged intramammary with *E. coli* provides good results in clinical mastitis detection and is comparably valid as the current gold standard of manual evaluation.

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Dynamic profiles of extracellular vesicles (EVs) in porcine oviductal fluid throughout the estrous cycle

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Recently, extracellular vesicles (EVs) have been considered to be important mediators in the early embryo-maternal dialogue. As oviduct physiology periodically varies throughout the estrous cycle, we hypothesize that content and amount of EV populations released into the porcine oviductal fluid (OF) change accordingly. Thus, we collected oviductal fluid from sows in 3 cycle phases (diestrus $n_D = 3$, estrus $n_E = 4$, postestrus $n_P = 4$), and isolated EVs using ultracentrifugation in combination with polyethylene glycol. The EV population was characterized via transmission electron microscopy (TEM), Western Blot, protein content and nanoparticle tracking (ZetaView). TEM confirmed the presence of EVs with typical spherical, cup-shaped morphology and a majority population resembling the size of exosomes (40–150 nm). EV markers Hsp70, Hsp90, Tsg101, as well as oviduct-specific glycoprotein OVGPI were detected in the EV fraction. Protein concentration of the EV suspension dynamically changed during the cycle (D: 54.51 $\mu\text{g/ml}$, E: 286.95 $\mu\text{g/ml}$, P: 947.10 $\mu\text{g/ml}$), which correlated with the EV concentration (D: 2.23×10^{10} particles/ml, E: 20.38×10^{10} particles/ml, P: 71.75×10^{10} particles/ml). Interestingly, the mean (116.13 nm) as well as median (108.18 nm) particle size in estrus was significantly larger than in both postestrus (mean: 102.35 nm; median: 94.88 nm) and diestrus (mean: 101.83 nm; median: 92.88 nm, $p < 0.05$). In conclusion, we uncovered dynamic EV profiles over different cycle stages in the porcine oviduct and therefore build the basis for further analysis regarding differences in EV cargo and its functional relevance. (Inga Weiss is supported by the H. Wilhelm Schaumann Stiftung, Hamburg, Germany.)

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Detection of *Lactobacillales* spp. in the bovine uterus and the seminal plasma and their influence on endometrial epithelial cells *in vitro*

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A variety of bacteria were found in the bovine uterus as well as in the seminal plasma. In the past, the focus was on pathogenic bacterial strains. The aim of this study was to evaluate the presence of commensal *Lactobacillales* spp. in the bovine uterus as well as in the seminal plasma and to investigate their influence on endometrial epithelial cells *in vitro*. Samples for bacterial analysis were collected from healthy cows as well as from healthy bulls. Obtained colonies on selective agar plates were characterized by sequencing. Endometrial epithelial cells were incubated with bacteria *in vitro* to evaluate the cell viability by trypan blue staining and to analyze the mRNA expression. The presence of several species of *Lactobacillus*, *Aerococcus*, *Weissella* and *Pediococcus* in the bovine uterus was noted. Five *Lactobacillales* strains, which were obtained from three different bulls, were identified as *L. mucosae* and *Leuconostoc mesenteroides*. Viability of the co-cultured epithelial cells was not affected up to 72 hours in presence of most *Lactobacillales* spp.. Real-time PCR revealed a significant increase of the mRNA expression of *IL1A*, *IL1B*, *IL8* and *CXCL1/2* in presence of most *Lactobacillales* spp.. However, some strains did not obviously influence the mRNA expression of the selected genes. In conclusion, the immunological response of endometrial epithelial cells to these *Lactobacillales* isolates varies in a strain specific manner. (This study was supported by the Förderverein Bioökonomieforschung e.V. [FBF].)

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Influence of lipopolysaccharide on uterine contractility in cows *in vitro*

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Up to know there is only limited information about the effect of uterine inflammatory diseases on myometrial contractility during the early puerperal period in cows. Therefore, the aim of this study was to examine the influence of lipopolysaccharide (LPS) on uterine contractility in cows *in vitro*. Myometrial tissue samples (longitudinal strips) were collected from 17 healthy Holstein-Friesian cows during caesarean section on Day 278.2 ± 4.3 after AI. Strips (n = 8) of each cow were incubated in an organ bath with different concentrations of LPS (0 [= control], 0.1, 1.0 and 10 µg/mL LPS) and contractility (isometric force transducer) was recorded for 270 minutes in 9 intervals (T1–T9). The mean amplitude (MA) and area under the curve (AUC) were calculated for each time interval. The LPS concentration affected MA and AUC (p < 0.05). At T1 and T2 the AUC and

MA tended to be higher (p < 0.10) in stripes incubated with 0.1 and 1.0 µg/mL LPS than in stripes incubated with LPS 0 and 10 µg/mL LPS. Furthermore, both contractility parameters were in tendency (p < 0.10) higher at T3 for a LPS concentration of 0.1 and at T6 for LPS concentrations of 0.1 and 1 µg/mL compared to a LPS concentration of 10 µg/mL. However, at T9 AUC and MA of stripes incubated without LPS tended (p < 0.10) to be higher than those of stripes incubated with LPS 0.1 and 1.0. The results of this *in vitro* study show that there are different effects of LPS on uterine contractility depending on dose and time of incubation. Moderate concentrations of LPS enhanced myometrial contractility, but only for a short time period.

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Evaluation of pathogenicity of *Staphylococcus aureus* strains isolated from cow's milk from mammary gland by biotype according to Umeka

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Biotyping is one of the phenotypic methods of classifying *S. aureus* strains based on selected phenotypic traits. The biotyping according to Umeka involves classifying strains into 1 of the 4 biotypes based on the disintegration of three sugars. Biotype I decomposes mannitol, Biotype II decomposes mannose, Biotype III decomposes mannitol and Mannose, Biotype IV decomposes mannitol, mannose and ribose. This is a simple method that enables quick and cheap detection of the most pathogenic strains. This was the aim of the study to compare biotype affinity with other characteristics of pathogenicity such as lipase and betalactamase production, antibiotic, particularly methicillin resistance, adhesion capacity and biofilm production. The study material were 225 *S. aureus* strains isolated from cow's milk with mastitis. The mannose, ribose and mannitol decomposition on the basis of Bailey and Scott was examined. Results: 155 (51.1%) of the tested *S. aureus* strains were included into biotype III, in biotype I 19 (8.5%), biotype IV 91 (40.4%), and no strain was included in biotype II. The strains of biotype IV showed the most pathogenic traits: 63.0% produced lipase, 66.7% had lipase production capacity, and 47.8% were resistant to methicillin. The strains of biotype IV also most commonly produced mucus and biofilm. In addition, after genotyping by ADSRRS-fingerprinting, 58.6% of the biotype IV strains belonged to the D genotype, which exhibited the highest pathogenicity and spreadability.

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Changes in the liver structure and function of intrauterine growth retarded piglets as a model for human neonates predisposed to insulin resistance and obesity in later life

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Introduction Intrauterine growth retardation (IUGR) leads to increased predisposition to obesity and diabetes type 2 in adult life but the mechanisms are still poorly understood. Considering number of functional similarities, the IUGR piglets seem to be a good model to study the development of the syndrome in humans. The aim of the present study was to investigate the ultrastructure and proteomic profile of the liver in IUGR pig neonates to find out early markers of predisposition to obesity and insulin resistance.

Materials and Methods Liver tissue samples were investigated in 7 d old IUGR piglets, and their normal body weight (NBW) littermates using light microscopy, mass spectrometry, and in-tissue cytometry.

Results Liver in IUGR neonates was significantly enhanced Kupffer cells to hepatocytes ratio and increased expression of TNF-alpha as a main cytokine producing by macrophages compared to NBW control. It was found many proteomic changes between IUGR and NBW as in protein metabolism (proteasomes, cathepsin D, phermitin, phosphoglucosylase), carbohydrate metabolism (hexokinase 1, phosphoglucokinase, galactokinase, aldolase B, glucose-6-phosphate isomerase), oxidative stress and chromatin organization and DNA uptake (histones, lamin a/c). In the IUGR liver as well as increased expression of insulin, resistin, adiponectin receptors and uncoupling protein 3 was showed.

Conclusion Reduction of hepatocytes number concomitant with significant modifications of expression of key hormones and enzymes for protein and carbohydrate metabolism in IUGR neonates may predispose to insulin resistance and obesity in adult life.

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Comparison of two clinical endometritis treatment methods in Holstein-Friesian cows: preliminary results

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The aim of the study was to compare clinical endometritis antibiotics vs. herbal infusions therapy. The study covered 30 Holstein-Frie-

sian cows from the herd in north-eastern Poland. The diagnosis of clinical endometritis was based on a character of vaginal discharge (purulent or mucopurulent). Cows were between 30 and 60 days postpartum. They were free from metabolic, infectious and reproductive disorders (except endometritis). All groups were injected with PGF_{2α} after finding corpus luteum (CL), and intrauterine infusions were done 48 hours later. In group A (n = 9) cephalirin was administrated once and in group B (n = 11) aqueous solution of herbs twice 2 days in a row. Cows without CL did not receive any PGF_{2α} injections. The control group C (n = 10) did not receive any intrauterine medicines. All cows were checked 20 days after the intrauterine treatment. The days open (DO) and the number of services per conception (NSC) were also monitored. There were no symptoms of clinical endometritis in groups A, B and C in: 5 cows (55.5%), 5 cows (45.45%), 4 cows (33.33%), respectively. DO and NSC were as follows: 130.44 and 2.33; 131 and 2.27; 143.3 and 2.9, respectively. Although there was no statistical difference between groups ($p > 0.05$), there was a trend for best efficacy after cephalirin therapy. The efficacy of herbal remedies and the control group was similar. Considering DO and NSC, there was a trend towards decreasing DO and NSC in experimental groups, without difference between the antibiotics treatment compared to the herbal one. Results indicate that in long term perspective of reproductive performance antibiotic and herbal treatment are giving similar results, with the later one being cheaper and lacking side effects of antibiotics. Further studies are needed on a larger population.

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Inflammatory cells response in mammary carcinoma with metastases to the uterus in the mare

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A mammary gland squamous-cell carcinoma (SCC) with metastases to uterus was found in an 18-year-old thoroughbred mare. The diagnosis was based on a histological examination and presence of similar tissue in lymph nodes, vulva, vagina and uterus. After euthanasia, tissue samples were collected from primary tumor and metastases, fixed for immunohistochemistry, cut into slices and labeled with primary, secondary antibodies and NuclearGreen or Hoechst. Slides were examined under confocal microscope and scanning cytometer. Immune cells differentiating was based on expression of CD45/CD66 (granulocytes), CD45/CD14 (macrophages), CD45/CD3/CD4 (lymphocytes Th), CD45/CD3/CD8 (lymphocytes Tc). Both in the mammary gland (MG) and uterus (U), the tissue consisted of areas of normal glandular tissue and neoplastic glandular structures with irregularly shaped acini and tubules. Some cells were pyknotic and karyolytic, others have shown polymorphism, anisokariosis and atypia. Endometrial adenocarcinoma diagnosis was based on well circumscribed, non-encapsulated nodular tissue in uterine mucosa which infiltrated the myometrium. Massive inflammatory cells infiltration, haemorrhage and necrosis were present in MG and U where CD45 positive cells accounted 16.2% and 14.9% of all cells, respectively. Similar immune cell infiltration (MG:U) was observed: 62.2%:58.9% granulocytes, 20.1%:24.4% macrophages and 17.7%:16.7% lymphocytes (10.1%:8.9% Th and 7.6%:7.8% Tc). Mares with mammary and endometrial carcinoma have been reported previously, but inflammatory infiltration was not described yet. Immune cell reaction is similar in primary and metastatic tumors and contains a lot of non-activate immune cells like tumor associated macrophages.

Clinical forms of post-service anoestrus in not-pregnant cows in eight dairy herds in north-east Poland

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The cows not-pregnant to the first service should have been observed in oestrus for a second service. However, the percentage of non-pregnant cows showing no oestrus signs until pregnancy examination is high. Anoestrus can be associated with various clinical

conditions. There is no information available about the incidence of anoestrus and their clinical forms after service in dairy cows in Poland. Thus, the objective of this study was to investigate incidence of clinical forms of anoestrus after artificial insemination in not-pregnant dairy cows based on ultrasound examination. The study was carried out on 1543 Polish Holstein-Friesian cows in 8 dairy herds in north-east Poland over a three-year period. Cows were examined for pregnancy on Day 35 after AI using a portable ultrasound scanner Honda 1500 equipped with a 5 MHz linear-array transducer. Cows diagnosed not-pregnant were re-examined on Day 45. Of the 1543 inseminated cows, 328 (21.3%) returned to oestrus within 35

days, 807 (52.3%) were pregnant to artificial insemination, and 408 (26.4%) showed no oestrus signs and were diagnosed not-pregnant by ultrasonography. The incidence of anoestrus after service in non-pregnant cows varied among herds from 10.3% to 32.9% of cows ($p < 0.05$). Based on ultrasound examination silent heat was diagnosed in 324 (79.4%), corpus luteum pseudogaviditatis in 36 (8.8%), ovarian cysts in 26 (6.4%), and ovarian dystrophy in 22 (5.4%) of 408 anoestrous, not-pregnant cows. The results of this study showed that the incidence of anoestrus after service in dairy herds in North-East Poland was high. The most prominent clinical form of post-service anoestrus was silent heat.

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