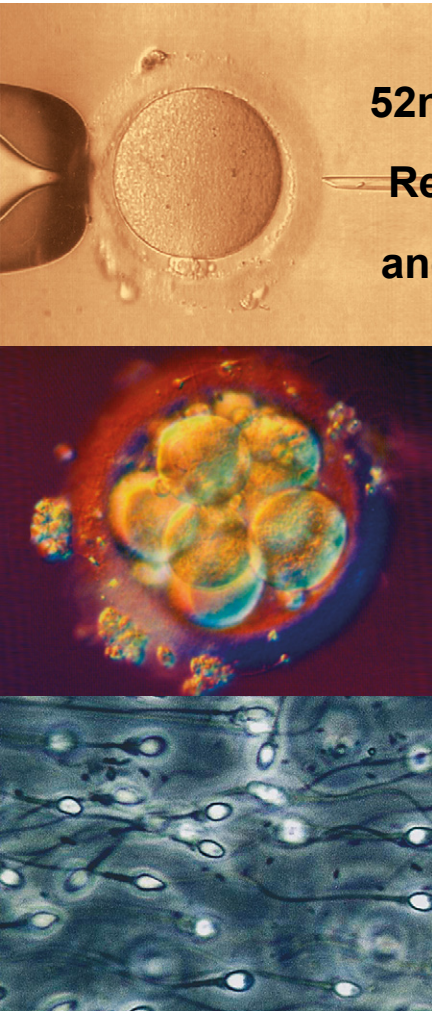


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**52nd Annual Conference of Physiology and Pathology of
Reproduction and 44th Mutual Conference of Veterinary
and Human Reproductive Medicine 20th–22nd February
2019, Göttingen Abstracts**

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52nd Annual Conference of Physiology and Pathology of Reproduction and 44th Mutual Conference of Veterinary and Human Reproductive Medicine 20th–22nd February, 2019, Göttingen

Abstracts*

■ Oral Presentations/ Kurzvorträge

1

Development of a milk-based lateral flow test for pregnancy diagnosis

Entwicklung eines milchbasierten
Lateral-Flow-Tests zur Trächtig-
keitsdiagnose beim Rind

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Pregnancy-associated glycoproteins (PAGs) are commonly used molecular markers for early pregnancy in cattle. PAG concentrations in serum and milk rise steadily throughout pregnancy and enable pregnancy diagnosis by day 28 of gestation [Wallace, Pohler, Smith, & Green, 2015]. Available PAG detection systems in blood and milk are laboratory based, with the exception of one lateral flow blood test (Fassisi AT GmbH, 2017), which enables pregnancy diagnosis from blood drop samples within minutes directly in the stable. The aim of the present study is to develop a similar lateral flow assay for PAG in milk. To this end, cotyledon tissue samples were collected from an abattoir. Samples clustered into four different pregnancy stages: 1. day 20–90 (n = 46), 2. day 91–180 (n = 59), 3. day 181–240 (n = 18) and 4. day 240-calving (n = 5).

Gestation stages were estimated by measuring the crown-rump-length of the fetuses. PAGs were purified from all gestation stages by FPLC. Identification of PAGs in FPLC fractions was achieved by mass spectro-

metry. Antisera were raised against purified PAG fractions for each gestation stage. Ten different PAGs were identified in the third gestation stage. The ability to detect PAGs in blood and milk will be tested in samples from cows throughout pregnancy that were collected on eleven different farms. The first results (obtained from 44 samples) indicate a good detection capacity of the antisera in a sandwich-ELISA (LOD = 15 pg/ml in serum) and a high correlation (R² = 0.91) of measured results with an established test system.

2

Effect of glyphosate on bovine oocyte development during in vitro maturation

Einfluss des Wirkstoffes Glyphosat auf die Entwicklungskompetenz boviner Oozyten während der In-vitro-Maturation

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Glyphosate is the most widely used agent for non-selective herbicides worldwide [Benbrook, Environ Sci Eur 2016, 28: 3]. The aim of this study is to determine, whether the active ingredient alone or in formulation with Roundup, the global market leader of glyphosate-based herbicides, affects the maturation and further development of bovine oocytes. Ovaries of healthy cows were collected at a slaughterhouse. The selected cumulus-oocyte-complexes matured for 24 hours. Glyphosate (96%) and Roundup were added to the maturation medium in concentrations of 1 µg/ml and 10 µg/ml each. After 24 hours of incubation (39 °C, 5% CO₂), the oocytes were stained with Hoechst 33342 to detect the metaphase II stage. The maturation rates of the three tested groups Glyphosate, Roundup and Control showed no significant differences irrespective of the used concentrations. On day seven and eight post-fertilization the embryos were evaluated morphologically. The development rates also

showed no significant differences between test groups and concentrations. After addition of 1 µg/ml, the average rates of development on day eight were 19.2% (± 10.8%) for pure Glyphosate (n = 301), 22.6% (± 12.3%) for Roundup (n = 315) and 24.1% (± 9.4%) for Control (n = 295). After adding 10 µg/ml to the maturation medium, the developmental averages on day eight were 26.9% (± 14.7%) for Glyphosate (n = 523), 24.1% (± 12.6%) for Roundup (n = 479) and 25.6% (± 11.0%) for Control (n = 389). In this study, negative effects of glyphosate and Roundup on bovine oocyte development could not be detected so far, further analysis will follow.

3

The effect of an oil covered culture system on bovine *in vitro* produced embryos

Die Wirkung eines ölbedeckten Kultursystems auf *in vitro* produzierte Rinderembryonen

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Embryos are usually produced in culture systems with an oil overlay, which conveys protection against the evaporation of water and microbial contamination. The oil can also release toxic substances and absorb essential components, such as hormones, which adversely affect the quality of the oocytes, and the resulting development of embryos *in vitro*. This study compares bovine *in vitro* production (IVP) with and without an oil overlay. Cumulus-oocyte-complexes (COC) were collected from abattoir-derived ovaries. Groups of 20 COCs were matured in Tissue Culture Medium 199 supplemented with BSA, eCG, hCG, fertilized in Fert.-TALP medium and cultured in SOF-culture medium with BSA-FAF employed as the standard protocol. The quantity of medium in both groups (with and without an oil overlay) and throughout all stages of IVP was maintained (100 µl). The second group was covered with 75 µl paraffin oil. The maturation stage of oocytes was assessed using a fluorescence

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staining (Hoechst 33342) after 24h of incubation and developmental stages were evaluated on day 8 post-fertilization. So far, our results indicate that oocytes matured in the absence of an oil overlay had significantly ($p < 0.05$) higher maturation rates ($71.56 \pm 6.82\%$) when compared against medium with an oil overlay ($61.74 \pm 7.84\%$). The developmental rate was significantly ($p < 0.05$) higher in IVP systems without oil ($38.19 \pm 17.81\%$) than in oil overlaid systems ($22.75 \pm 9.83\%$). Based on the higher maturation and embryo development rates by bovine oil-free culture systems we suggest this method as an alternative to oil covered culture medium. However, further work is still needed to confirm these results at the molecular level.

(We gratefully acknowledge our colleagues H. Hellmold and D. Teuteberg.)

4

Pre-incubation of bovine sperm used for IVF accelerates the developmental kinetics of the resulting embryos and possibly their sex ratio

Eine Präinkubation der Spermien, die im Rahmen der IVF eingesetzt werden, beeinflusst die Entwicklungsgeschwindigkeit der resultierenden Embryonen und möglicherweise deren Geschlechtsverhältnis

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In vivo, the sex ratio of bovine embryos is approximately 1:1, whereas for bovine IVP embryos it may differ from this ratio as male IVP embryos reach advanced stages earlier. The aim of the present study was to test whether a pre-incubation of sperm prior to IVF will improve the developmental rates and influence the sex ratio. Cumulus-oocyte-complexes were recovered from abattoir-derived ovaries. After maturation, fertilization was realized using a standard protocol. Prior to IVF, sperm cells from two different bulls were treated as follows: pre-incubation in IVF medium for one hour (group A); omission of this step in group B (control). After 19 hours of IVF, presumptive zygotes were cultured in SOFaa for seven days. Cleavage and developmental rates were recorded. Day 7 blastocysts from both groups were sexed using bovine and Y chromosome specific primers. Data were analyzed by ANOVA. Sperm pre-incubation did not affect the cleavage and developmental rates for the individual bull ($p > 0.05$). On

average, at Day 7 a higher number of blastocysts (calculated on the total number of developed embryos) was determined when embryos have been produced from pre-incubated sperm ($p \leq 0.05$). This held true for both bulls (bull 1/group A: $55.2 \pm 11.7\%$; bull 1/group B: $28.1 \pm 8.8\%$; bull 2/group A: $58.1 \pm 10.3\%$; bull 2/group B: $36.5 \pm 11.4\%$). The shift in favor of male embryos was detectable in all groups of embryos, with a drastic one for bull A after sperm pre-incubation. In conclusion, sperm pre-incubation accelerated embryo development and possibly enhanced the proportion of male embryos which was already shifted towards males.

5

Comparison of survival rates of vitrified biopsied in vitro-produced bovine blastocysts using the VitTrans- or the Cryotop-device

Vergleich der Überlebensraten von vitrifizierten biopsierten *in vitro* produzierten Rinderembryonen unter Verwendung des VitTrans- oder Cryotop-Systems

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The implementation of a vitrification method suitable for direct transfer of biopsied embryos would help to increase the genetic improvement of cattle and reduce the costs of embryo transfer. The aim of our study was to compare the success rates of two vitrification devices for intact (I) and biopsied (B) bovine *in vitro*-produced embryos. The VitTrans system (V) allows a one-step in-straw warming procedure using 0.5 M sucrose in holding medium (Group VI; Group VB), while in the Cryotop system embryos are warmed in three steps with 1, 0.5 and 0 M sucrose in holding medium (Group CI; Group CB). In a first experiment, survival rates of immediately vitrified embryos were recorded 24h post-warming. Best survival rates were reached with CI = 75% and CB = 69%. With the VitTrans system results were VI = 61% and VB = 40%. (SAS, PROC GLM; p -value = 0.02). Due to the attachment inside the straw, 20% of the biopsied embryos warmed using the VitTrans system were lost during recovery. In a second experiment, embryos were vitrified 3h after the biopsy using VitTrans (VB3). In this case, re-expansion rates increased from VB0 = 40% to VB3 = 62%. (SAS, PROC GLM; p -value = 0.14). Overall, the Cryotop system showed better results. Vitrification and warming using the VitTrans device was acceptable with intact embryos; after biopsy,

the re-expansion rates were only good in embryos vitrified after a 3h period of culture. However, only the establishment of pregnancies using the VitTrans direct transfer method will prove its possible application in the field.

6

Addition of low IGFBP4 concentration increased the hatching rate of *in vitro* derived bovine embryos

Die Zugabe von niedrigen IGFBP4-Konzentrationen erhöht die Schlupfrate *in vitro* produzierter boviner Embryonen

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It was previously shown that cows with higher concentrations of IGFBP4 and IGFBP2 in maternal serum displayed an increased risk of late embryo/early fetal mortality. However, it is unclear whether IGFBP4 have direct effects on oocytes and/or embryo development. Therefore, the aim of this preliminary study was to investigate the effect of IGFBP4 on oocyte developmental competence. Cumulus oocyte complexes (COCs) were retrieved from slaughterhouse derived ovaries. COCs were matured, fertilized and cultured *in vitro* until day 9 (IVF = day 0). Culture media used for *in vitro* oocyte maturation was supplemented with recombinant bovine IGFBP4 at 74 nM, 20 nM and 2 nM. For each treatment 6 replicates were performed. No difference in cleavage rate, blastocyst rate and hatching rate on day 9 were determined. However, interestingly a higher hatching rate was observed on day 8 when lower IGFBP4 concentrations were added in comparison to the control (18.5% vs 45.5% for 2 nM IGFBP4 [$p < 0.05$], and 50.0% for 20 nM IGFBP4 [$p < 0.05$]). In contrast, this effect is diminished when a high IGFBP4 concentration 74 nM was added, then the hatching rate was only 13.0%, which was comparable to the control ($p > 0.05$). In conclusion, low concentrations of IGFBP4 may stimulate the viability of hatching. Two mechanisms can be speculated. Firstly, low IGFBP4 concentration may stabilize embryo derived IGF-1 whereas high concentrations of the inhibitory binding protein may diminish it. Secondly, IGFBP4 may also have a direct effect via an IGFBP receptor, which accelerates in low concentration the hatching process and improving hatching rates.

7

Application of computer tomography to study preantral follicle features of equine ovaries

Anwendung der Computertomographie zur Untersuchung präantraler Follikelcharakteristika an Pferdeovarien

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The mare has been suggested as a proper comparative animal model to study antral follicular dynamics in women, in view of similar mono-ovulation, age-related decline in fertility, hemorrhagic anovulatory follicles occurrence or preantral follicles development. We focused on the architecture of tissue of equine ovaries as an ovarian translational study of preantral follicle population, density, and distribution. We applied multislice computed tomography (CT) scanner (750 Revolution CT, GE Healthcare, Waukegan, WI, USA), used clinically for imaging of ovaries of women. We scanned fresh equine ovaries in anestrus ($n = 12$) within 2h after collection with following parameters: amperage: 550 mA, rotation: 0.8/s (He), voltage: GSI-QC (Dual Energy). The mean number of preantral follicles in consecutive groups of follicles' size in largest diameter (mm)/volume (mm^3), recorded per ovary during the anestrus phases was: 6.11 in g1: $< 10 \text{ mm}/462 \text{ mm}^3$; 8.32 in g2: $< 20 \text{ mm}/3892 \text{ mm}^3$ and 2.92 in g3: $< 30 \text{ mm}/30493 \text{ mm}^3$. Minimum/maximum radiodensity of each follicles' group (g1–3) (in HU, Hounsfield Units) were for g1: $13.58 \pm 3.53/30.17 \pm 2.48$, g2: $11.38 \pm 3.18/37.38 \pm 6.02$, g3: $19.96 \pm 0.67/44.01 \pm 4.81$, and differed significantly between groups ($p < 0.01$). No differences ($p > 0.05$) were found in average radiodensity of follicles for g1: 21.58 ± 1.25 , g2: 22.15 ± 1.99 and g3: 22.73 ± 2.21 . The area of ovarian structures was positively correlated with the follicle maximum radiodensity. CT provides useful data from 3D structure of ovaries in order to explore the preantral follicle population, which represents the finite and available reserve of gametes that can be recruited during the reproductive lifespan.

(Travel was funded by KNOW [Leading National Research Centre] Scientific Consortium 'Healthy Animal – Safe Food', decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.)

8

Non-enzymatic methods for the isolation of large luteal cells (LLC) in felids

Nicht-enzymatische Methoden zur Isolierung großer Lutealzellen (LLC) in Feliden

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Corpus luteum (CL) is a transient, endocrine gland, which forms on mammalian ovaries. Alongside nonsteroidogenic cells it is composed of small (SLC) and large (LLC) steroidogenic luteal cells, which can be used for *in vitro* studies on luteal functions. Current isolation methods of luteal cells from different species are usually based on enzymatic digestion of CL with collagenase and DNase. The enzymatic method leads to low recovery of LLC due to their extreme fragility towards enzymes. Therefore, we developed a non-enzymatic method for dissociation of luteal tissue. CL from domestic cat ovaries obtained after routine ovariectomy were ground through a cell dissociation sieve $380 \mu\text{m}$ (Sigma). Afterwards, LLC were separated by filtration through a $35 \mu\text{m}$ cell strainer (BD Falcon) followed by percoll density gradient (0–20%). The average cell diameter was $\pm 35 \mu\text{m}$, clearly indicating for LLC. To characterize luteal cell activity, 3β -hydroxysteroid dehydrogenase (HSD3B) staining was used and the medium was collected for progesterone determination following 1 day *in vitro* culture. Results show that LLC were characterized by high activity of HSD3B, which could be specifically blocked by trilostane (2 mM). Culture medium contained $5.25 \pm 2.47 \text{ ng/ml}$ ($n = 3 \times 9$) progesterone per 10,000 cells. In conclusion, our results show that the non-enzymatic method is suitable for isolation of LLC from domestic cats, which is a good prerequisite for cell culture studies on factors supporting survival of LLC.

(The study was funded by DFG (BR 4021/5-1).)

9

Transmigration of immune cells through the testis-immune barrier *in vitro*

Transmigration von Immunzellen durch die Hoden-Immunschranke *in vitro*

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Although the blood-testis barrier (BTB) is known as a very tight barrier, *in vitro* models revealed only a modest function. Further-

more, Sertoli cell ablation showed that the barrier is still intact as shown by blocking the invasion of immune cells, which we therefore have termed the testis-immune barrier (TIB). We aimed to optimize the TIB in *in vitro* models and to investigate the transmigration of immune cells. We used the pubertal immortalized Sertoli cell lines 93RS2, 93RS2-AR17-SC7 (androgen receptor positive cell line), TN4A5 (FSH receptor-positive cell line), SCIT-C8 and the THP-1 cell line (human monocytic cell line). Measurements of the TIB were performed with transepithelial electrical resistance (TER), FITC dextran diffusion assay as well as transwell assays for the transmigration of labeled THP-1-derived macrophages with LeukoTracker. With retinoic acid as well as hydrogels we obtained an increase of the TIB by about 30%. Similar increases were found with testosterone with the 93RS2-AR17-SC7 Sertoli cells. After stimulation of THP-1 cells with PMA followed by polarization with γ IFN and LPS, we obtained M1 macrophages positive for TNF- α . However, differentiation of the THP-1 cells to M2 macrophages with IL4 and IL13, proved to be difficult. We are currently optimizing the polarization protocol and obtained encouraging results with shorter stimulations. Up to date, we found that Sertoli cells form a tight barrier for the unstimulated THP-1 cells (M0 macrophages) with and without the chemoattractant MCP-1.

(This work was supported by the Deutsche Forschungsgemeinschaft [DFG] as part of the International Research Training Group between Justus-Liebig-University of Giessen [L. K., G. S.-B.] and Monash University, Melbourne [GRK 1871/1], on "Molecular pathogenesis on male reproductive disorders".)

10

The difference in contractility between prostatic ducts and glands, and how oxytocin is involved

Die Kontraktilität von Prostata-drüsen in Unterscheidung zu Prostatagängen und der Oxytocin-Einfluss hierauf

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Benign prostatic hyperplasia (BPH) affects up to 80% of 80 year old men and up to 100% of old dogs. Most recently we analysed for the first time separately the contractile pattern of prostatic glands and ducts, allowing predicting effects and local side effects of drugs used for the treatment of BPH by relaxing the smooth muscle cells in the prostate. Most of the experiments were performed in rodent tissue. In the human prostate data on

(i) structure and functional regulation of excretory ducts as well as (ii) the potential of targeting other receptors instead of adrenergic ones (as usually done) are missing. In the present study the duct system of the human prostate was visualized using corrosion cast models, smooth muscle staining and Micro-CT-imaging. The effects of oxytocin were investigated using video microscopy. We were able to clarify the human prostatic ductal system and also got information about the organisation of the surrounding smooth muscle cells. Oxytocin significantly increased the frequency of spontaneous prostatic contractions. There was a visible difference between oxytocin- and noradrenaline-induced contractions, which also differed between prostatic ducts and glands. Revealing differences between ducts and glands in the prostate extends our basic anatomical and physiological knowledge and might allow an even more targeted line of medications. These insights in combination with the oxytocin data we obtained could open up new strategies in BPH treatment especially by comparing our novel oxytocin antagonists in the near future.

11

Different responsiveness of the testis and epididymis to bacterial invasion

Unterschiede zwischen Hoden und Nebenhoden hinsichtlich der Antwortmuster nach bakterieller Stimulation

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The epididymis can be segregated into three distinct regions (caput, corpus, cauda), that each fulfill distinct tasks crucial for sperm maturation. In urinary tract infections, bacteria ascending via the urethra can induce severe inflammation and subsequent damage to the upstream reproductive organs, i.e. epididymis and testis. In a mouse model of bacterial epididymitis, the ascent of uropathogenic *E. coli*, which is a common bacterial cause of epididymitis in men, was detectable along the entire length of the epididymis, and into the testis as well. It was notable, however, that the pathogenic response of the epididymal regions and the testis varied profoundly at 10 days post-infection. Extensive tissue destruction including fibrotic remodeling, leucocytic infiltration and epithelial damage occurred in the cauda epididymis, as the entry site of bacterial invasion. In comparison, much less severe effects were documented for the corpus epididymis, while the caput showed no obvious signs of pathological alterations or leucocytic infiltration whatsoever. In the testis, spermatogenesis

beyond the earliest spermatocyte stages was severely disrupted and interstitial leucocytic infiltration was apparent. Consistent with the pattern of tissue damage, pro-inflammatory gene expression was increased in the testis and cauda, but not in the caput epididymis. It is hypothesized that immune responses toward the pathogen in the caput epididymis are either absent or actively suppressed, thus conferring protection from infection-induced damage. This may be due to caput-specific immune cells, such as the intraepithelial dendritic cells, or other functional properties that are currently under investigation.

12

The potential role of tumor infiltrating T lymphocytes in human testis cancer

T-Lymphozyten und ihre potentielle Rolle bei humanem Hodenkrebs

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In human testicular germ cell neoplasia, i.e. seminoma and pre-invasive germ cell neoplasia in situ (GCNIS), infiltrating immune cells are frequently found and have previously been identified as macrophages, dendritic cells, and lymphocytes, with T cells representing a major component of the tumor-infiltrating lymphocyte (TIL) population. Recent studies indicate that functional polarization and respective subtypes of TIL including regulatory T cells (Treg) and T follicular helper cells (Tfh), play an important role in cancer development and immune surveillance. Therefore, we aim to characterize subsets of TIL/T cells in seminoma and GCNIS. Human testis samples (seminoma, GCNIS ± lymphocytic infiltrates [ly], impaired spermatogenesis [hyp] + ly, and normal spermatogenesis [nsp]; n = 10, each) were analyzed by immunohistochemistry (IHC) and RT-PCR. For cytokine expression profiles, quantitative RT-PCR was performed. IHC confirmed the presence of CD8⁺ and CD4⁺ cells in immune cell infiltrates in GCNIS and seminoma. In contrast to clustering of B cells, T cells show a scattered distribution in seminoma. Preliminary results revealed that both neoplastic and non-neoplastic testes contain CD4⁺/FOXP3⁺ cells (Treg). Further marker analysis (e.g. CXCR5,

ICOS, PD-1) and cytokine expression profiles (e.g. IL-2, IL-6, TGF-β, IL-21) will allow for identification of other T cell subsets (e.g. Tfh). Detailed functional characterization of TIL in testicular neoplasia will help to elucidate the complex mechanisms of „immune editing“ during testis cancer development.

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13

Association between IGF-1, 25-hydroxyvitamin D3 (25-OHD3) and benign prostatic hyperplasia (BPH) in the dog

Zusammenhang zwischen IGF-1, 25-Hydroxyvitamin D3 (25-OHD3) und der benignen Prostatahyperplasie (BPH) des Hundes

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The impact of IGF-1 on proliferation of many tissues is also considered regarding BPH in men [Khosravi, J Clin Endocrinol Metab 2001; 86: 694–9]. Moreover, the prevalence of vitamin D deficiency, in human BPH patients may represent a causal association between BPH and vitamin D status [Espinosa, Can J Urol 2013; 20: 6820–52]. The aim of the present study was to investigate serum IGF-1 and vitamin D status represented by serum 25-OHD₃ in male dogs with normal and hyperplastic prostate gland. A total of 38 dogs (18 Labrador Retrievers/LR, 20 Rhodesian Ridgebacks/RR), assigned to the age groups 18–24 mo (n = 12), 25–48 mo (n = 13), and 49–72 mo (n = 13), were included in the study, 20 of them showing a normal (group 1) and 18 a hyperplastic prostate gland (group 2). Prostatic volume was 25.9 ± 10.7 and 72.0 ± 37.3 cm³, respectively (p < 0.05). Significant differences were detected between LR and RR in all age groups. Serum IGF-1 concentrations were 470.9 ± 129.8 and 419.8 ± 85.6 ng/ml and serum 25-OHD₃ concentrations were 82.8 ± 25.7 and 86.0 ± 47.6 ng/ml in groups 1 and 2, respectively. The age related increase in prostatic volume was significantly more pronounced in RR than in LR. Serum IGF-1 concentrations showed a consistent level in both breeds and all age groups. In RR, 25-OHD₃ concentrations decreased markedly between age groups 25–48 mo and 49–72 mo (100.8 ± 44.5 and 55.5 ± 44.2 ng/ml, p = 0.08). Overall prostate gland volume was positively correlated with age (r = 0.56, p < 0.001), and negatively with IGF-1 (r = -0.33, p < 0.05) and 25-OHD₃ (r = -0.32, p < 0.05). These results indicate a breed dependent age related association between vitamin D status and BPH.

14

Proteomic markers in seminal plasma as a means to predict successful TESE in azoospermic patients

Proteomische Marker im Seminalplasma als Mittel zur Vorhersage einer erfolgreichen TESE bei Patienten mit Azoospermie

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Azoospermia, i. e. complete absence of spermatozoa in the ejaculate, is an acute form of male infertility. So far, testicular biopsies are still the only reliable diagnostic and therapeutic tool. Proteomic markers in seminal plasma could constitute an excellent, non-invasive alternative for the differential diagnosis of azoospermia and the prediction of the outcome of testicular sperm extraction (TESE). Label free mass spectrometry analysis of seminal plasma samples from infertile men ($n = 24$), diagnosed with mixed atrophy (MA), obstructive azoospermia (OA) and Sertoli cell only syndrome (SCO) and fertile men ($n = 8$) identified over 2000 differentially expressed proteins. Out of 37 proteins significantly down-regulated in OA, but not in MA or SCO, DEFB129 (Fold Change [FC]_{NOA} = 15.24; $p = 0.0009$), ELSPBP1 (FC_{NOA} = 5.77; $p = 0.006$), and ADGRG2 (FC_{NOA} = 6.19; $p = 0.003$) were selected as potential obstruction markers. Gene expression analysis confirmed these proteins to be specific to the epididymis with a significantly lower number of transcripts in the testis. Among the proteins found to be significantly down-regulated in SCOS but exhibiting similar abundances in the MA and control groups, CSE1L (FC_{N/SCO} = 8.63; $p = 0.002$), and PFKP (FC_{N/SCO} = 182.27; $p = 0.018$), as well as DNAH2 and SLC52A3, were chosen for confirmation as potential markers of successful sperm retrieval. PFKP and CSE1L's overall expression levels in MA far surpassed those in SCOS, with high variance within each of the groups, while DNAH2 showed significantly higher expression in MA compared to SCO ($p = 0.0026$).

15

Evaluation of activation and course of apoptosis of canine spermatozoa

Bewertung der Aktivierung und des Verlaufs der Apoptose von Hundespermien

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The aim of this study was to explain the mechanisms of activation of sperm apoptosis using intracellular and extracellular pathway inducers as well as auto-activation. Furthermore the course of apoptosis after activation of both pathways in canine sperm was also studied. Ejaculates from 25 healthy dogs were obtained after masturbation and from 29 animals semen from testis, epididymis and vas deferens was collected after castration. The mitochondrial potential, the expression of phosphatidylserine, caspase activity and DNA fragmentation were evaluated using flow cytometry (FACScalibur, Becton Dickinson). The results indicated a lower percentage of sperm with low mitochondrial potential ($29.8 \pm 10.1\%$), lower percentage of sperm with phosphatidylserine expression ($14.8 \pm 2.6\%$) and lower percentage of sperm with DNA fragmentation ($16.2 \pm 6.7\%$) in spermatozoa from the testis in comparison to those from the epididymis ($p \leq 0.05$) and vas deferens ($p \leq 0.05$). The highest DNA fragmentation ($28.8 \pm 4.2\%$) and the highest level of active caspase ($65.8 \pm 12.1\%$) was observed in ejaculates. The induction of intracellular and extracellular pathway and self-activation of apoptosis caused a decrease of mitochondrial potential, an increase in the expression of phosphatidylserine and increased DNA fragmentation in epididymal spermatozoa. In conclusion, apoptosis of spermatozoa isolated from semen, testis, epididymis, and vas deferens of dogs is a caspase dependent, extrinsically and extracellularly activated process. It is also activated spontaneously without the action of external inducers. Apoptotic changes occur during sperm maturation in the epididymis and the vas deferens.

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16

Does single layer centrifugation Bovicoll improve sperm quality of thawed semen in Fleckvieh bulls?

Verbessert die einschichtige Zentrifugation Bovicoll die Spermienqualität von aufgetautem Sperma bei Fleckvieh-Stieren?

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The aim of the present study was to evaluate the effect of sperm selection by single layer centrifugation (SLC) performed before freezing on sperm quality after thawing of Fleckvieh bull semen. Ejaculates from twenty-two bulls were collected by artificial vagina and divided into two aliquots. One of them (unselected – Control) was diluted with Steridyl® and frozen over nitrogen vapor in a Digitool (IMV) freezer. The second aliquot was selected by the SLC technique using Bovicoll colloid and subsequently frozen as unselected samples. After thawing selected and unselected straws were evaluated by CASA (SCA System) for sperm motility parameters. In addition, integrity of the plasma membrane, viability, high mitochondrial membrane potential (HMMP), and acrosome integrity were assessed by using a Guava® easyCyte flow cytometer. Furthermore, morphological examination of spermatozoa was performed with a Leica DMi8 inverted microscope by Differential Interference Contrast microscopy. Immobilized, live spermatozoa were analyzed at magnifications greater than or equal to 6600X. The mean sperm viability decreased from 51.57% to 40.37% after selection ($p < 0.05$). For HMMP it decreased from 40.37% to 28.96% ($p < 0.05$) whereas the mean of live spermatozoa with damaged acrosome increased from 1.63% before selection to 1.95% after selection ($p < 0.05$). The mean total motility percentage of spermatozoa decreased from 76.79% to 75.99% after SLC selection and the progressive motility before selection dropped from 72.69% to 70.58% after SLC. However these differences were not significant at level $\alpha = 0.05$. Therefore, sperm quality after cryopreservation is not improved in Fleckvieh bull semen when sperm selection by SLC was carried out before freezing.

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Improving quality of cryopreserved bull semen through heterospermic processing

Verbesserung der Qualität von kryokonservierten Bullensperma durch heterosperme Verarbeitung

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This study aimed to test sperm mixing from different bulls as a tool for improving the quality of semen within the same breed. Ejaculates of six bulls were collected in two rounds (three bulls per round) at an AI station and separated in aliquots that were either frozen *per se* or mixed 1:1 with semen from another bull. In total 12 samples of semen

resulted, as follows: semen from bulls A, B, C, D, E and F that were processed without mixing, as well as the combinations A+B, A+C, B+C, D+E, D+F and E+F. All samples followed the same procedures of cryopreservation. After thawing, the samples were analyzed by CASA and flow cytometry using a multicolor assay with five fluorochromes (PI: plasma membrane integrity; Peanut Lectin: acrosome integrity; CellTrace™ Calcein Violet AM: esterase activity; MitoProbe™ DiIC1: mitochondrial membrane potential; Fluo-4 AM: calcium level). Examinations were performed immediately after thawing and after three hours of incubation at 38 °C. Compared to the samples from singular bulls semen mixed from different bulls increased the percentage of progressively motile sperm (+6%; $p < 0.05$), as well as the percentage of sperm with an intact plasma membrane and acrosome, high esterase and mitochondrial activity and low levels of calcium (+3%, $p < 0.05$), particularly immediately after thawing. There was a high variability concerning the effect of ejaculate combinations on the examined sperm parameters. While some combinations showed no effects, other improved sperm quality distinctly (up to +14% progressive sperm motility). In conclusion, sperm quality can be improved by mixing sperm from different bulls within the same breed. In ongoing studies we are testing if this phenomenon is repeatable with different ejaculates from the same bulls. Furthermore we investigate *in vitro* fertility of mixed samples compared to samples from singular bulls.

18

Pregnancy risks by hyperglycaemia: Metabolites like methylglyoxal and glyoxal act as histone modifiers in early embryos

Schwangerschaftsrisiken durch Hyperglykämie: Metabolite wie Methylglyoxal und Glyoxal wirken als Histonmodifikatoren in frühen Embryonen

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In the endocrine-metabolic disorder diabetes mellitus, blood glucose concentrations and its toxic metabolites, methylglyoxal (MGO) and glyoxal (GO), are increased in cells and tissues also during pregnancy. The aim of this study was to determine specific histone modifications and the expression of histone modifying enzymes in early mammalian embryos exposed to a diabetic environment *in vivo* and components of a diabetic environment *in vitro*. Therefore, six-day-old blastocysts were

cultured with MGO and GO. *In vivo* blastocysts were obtained from rabbits with an Alloxan induced diabetes mellitus. Histone modifications of blastocysts were examined by LC-MS/MS. Expression of histone modifying genes was analysed by quantitative Real-Time PCR *in vivo* and *in vitro*. High levels of MGO and GO led to a reduction of H3 acetylation at lysine 18 and lysine 23 in the embryoblast and to an increase of di-methylation on lysine 27 (H3K27) in the trophoblast. EZH2 (*enhancer of zeste homolog 2*), a H3K27 methyl transferase, was transcriptionally downregulated in the embryoblast of MGO/GO exposed blastocysts and in the embryoblast from diabetic rabbits. Our data indicate that blastocysts challenged by diabetic components (MGO and GO), changed their epigenetic code and regulate their modifying enzymes. The embryo can adapt to an altered uterine environment in an epigenetic manner by alterations in histone modifications.

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Influence of a metritis on uterine contractility in dairy cows *in vitro*

Einfluss von einer Metritis auf die uterine Kontraktilität *in vitro*

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Metritis is a common disease during the early puerperal period in dairy cows. Up to now there is only limited information about the effect of metritis on myometrial contractility as a component of uterine involution. Therefore, the aim of this study was to examine the effect of occurrence of metritis on uterine contractility in cows *in vitro*. Myometrial tissue samples were collected from 16 Holstein-Friesian cows undergoing euthanasia 1 to 21 days after calving ($n = 6$ without [M–] and $n = 10$ with metritis [M+]). Strips ($n = 4$ longitudinal and $n = 4$ circular layer) of each cow were incubated in an organ bath. Spontaneous contractility and contractility induced by increasing concentrations of oxytocin (Oxy), prostaglandin $F_{2\alpha}$ (PGF), ionized Ca^{2+} (Ca) or nothing (Con) were recorded in an organ bath during 9 intervals of 30 minutes each (T1 to T9; 5 and 4 intervals for SC and IC, respectively). The mean amplitude (MA) and area under the curve (AUC) were calculated for each time interval. Overall, group had no influence ($p > 0.05$) on MA and AUC. However, the myometrial strips of group M+ reached higher values ($p \leq 0.05$) of MA and AUC than the strips of group M– during time period T2. In the M+ group the MA– and

AUC-values of circular strips were higher ($p \leq 0.05$) than those of longitudinal strips during spontaneous contractility. During stimulation with PGF (T9) the longitudinal layer showed higher values ($p \leq 0.05$) of MA and AUC than the circular layer in group M+. The MA– and AUC-values of longitudinal strips of group M+ were higher ($p \leq 0.1$) than those of group M– with the two highest concentrations of PGF. The results of this *in vitro* study show that cows with a metritis show a higher *in vitro* myometrial contractility compared to those of healthy cows. Interestingly, both myometrial layers, especially in uteri with metritis, react differently during spontaneous contractility and to stimulation with PGF.

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Quantitative evaluation of the fetal and maternal microvillous surface in the epitheliochorial porcine and bovine placenta

Quantitative Evaluierung der fetalen und maternalen Oberflächen der Microvilli in der epitheliochorialen Plazenta von Schwein und Rind

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The “parent-offspring conflict hypothesis” is a recent approach to explain the evolutionary force that led to the high diversity of the mammalian placenta. Following this hypothesis, the fetus has an interest to maximise nutrient absorption, while the mother tends to limit transplacental nutrient transfer. In the epitheliochorial placenta, fetal trophoblast cells are apposed to the maternal uterine epithelium and microvilli of both epithelia interdigitate with each other. This structure increases the surface areas and provides the infrastructure for active and passive transport. According to the “parent-offspring conflict hypothesis” we expected that the fetal microvillous surfaces are larger than the maternal ones. To test this hypothesis, we used stereological tools on transmission electron microscopy images to estimate fetal and maternal microvillous surface enlargement factors in ten species with an epitheliochorial placenta. In addition, to support our data, we produced three-dimensional models of feto-maternal interdigitation in bovine and porcine placentae, based on Focused ion beam-scanning electron microscopy images. In all the experimental animals, the fetal surface was larger than the maternal one. This was due to an increased number of fetal microvilli and to the existence of membrane folds at the base of the fetal, but not of maternal microvilli. These results support the “parent-offspring conflict hypothesis”.

Positive correlation between the pre- and post-oestrous secretory activity of successive bovine *corpora lutea*

Positive Korrelation zwischen der prä- und post-östrischen Sekretionsleistung aufeinanderfolgender boviner *Corpora lutea*

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To test further the hypothesis that the development of bovine *Corpora lutea* (CL) indirectly is influenced by the predecessor's (CLp) progesterone (P4) secretion, the ovarian activity of spontaneously cycling Swiss Brown dairy cows and heifers was monitored during 118 peri-oestrous periods (d-12 till d12; d1 = oestrus). Apart from daily blood sampling for P4 radioimmunoassay, all animals were submitted to ovarian palpation at intervals of 1–2 days, in order to register the CLp's and the future ovulatory follicle's (OF) absolute (left; right) and relative (ipsilateral; contralateral) location as well as the OF's rise (first detection). Basically, the luteal activity of the CLp and the CL proved to be positively correlated, but the correlation's manifestation (ranging from non-significant to $r > 0.7$) varied markedly, depending on how specifically the location of the CLp and the OF, as well as the date of the OF's rise, also were taken into account. In particular, the amount of P4 to which the developing OF was exposed before its first detection and the OF's position relative to the CLp appear to be of relevance as for the subsequent CL's P4 secretion. No direct correlation was found between the OF's manually felt lifespan and the activity of the CLp or CL. The data suggest that a CL's luteal capacity is markedly influenced by its predecessor's P4 output during the selection of the future OF out of a cohort of competitors. The obviously intricate interplay of systemic (asymmetrical ovarian activity) and local (intraovarian) factors which, for their part, modulate the CLp's activity remains to be investigated in detail.

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The pattern of tissue architecture in lactating and nonlactating mammary gland in mares

Das Muster der Gewebearchitektur in laktierenden und nicht laktierenden Milchdrüsen bei Stuten

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The equine mammary gland, similarly to other mammals, undergoes a cycle of growth and tissue differentiation after every mating resulting in a pregnancy. The growth of the mammary gland tissue is associated with changes in the natural flexibility of udder. The tissue elasticity may be assessed by elastography, an advanced technology providing information about the stiffness of tissue or local tissue strain. The purpose of this study was to evaluate real-time ultrasound elastography (RTUE) as an adjunct to conventional sonography for discrimination of tissue architecture of mammary gland during and outside lactation. The preliminary research was carried out on 20 Konik Polski mares (4–12 y) in lactation ($n = 10$) and without ($n = 10$). The RTUE protocol was conducted using ESAOTE MyLab Alpha with 3–11 MHz probe. The Average Percentage of Pixels of Each Color (APPEC) was calculated for right and left half of the udder, considered as two separate functional gland units. The quantitative four-level scale (1 = mostly hard, 2 = intermediate hard, 3 = intermediate soft, 4 = mostly soft) and the pattern of tissue architecture were applied. Mammary gland in lactation shown the scattered pattern with mostly soft areas, which was much more extensive than the linear structure in nonlactating gland. During lactation the contribution of soft tissue (scale 4: $57.6\% \pm 2.04$) significantly ($p < 0.05$) prevailed over nonlactating gland (scale 4: $46.1\% \pm 1.54$) with no differences in soft areas (scale 3: $19.2\% \pm 2.13/26.1\% \pm 1.77$), intermediate (scale 2: $4.7\% \pm 0.75/5.1\% \pm 0.68$) and harder tissue (scale 1: $18.2\% \pm 0.89/23.5\% \pm 1.13$). No significant differences ($p > 0.05$) in APPEC parameters between halves of udder were observed. RTUE provides detailed data about tissue in lactating and nonlactating mammary gland in the mare.

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Effects of abomasal supplementation with essential fatty acids and conjugated linoleic acids on fatty acid pattern in follicles and oocytes in dairy cows

Auswirkungen einer abomasalen Supplementation von essentiellen Fettsäuren und konjugierter Linolsäure auf die Fettsäuremuster in Follikeln und Eizellen bei laktierenden Kühen

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Essential fatty acids (EFAs) like linolenic acid and conjugated linoleic acids (CLAs)

are known for their positive impact on animal's health and fertility. This investigation aims to clarify, how a supplementation with a certain amount of EFAs and CLAs will affect fatty acid profiles in different compartments of the ovary. Four groups, consisting of 10 lactating rumen-fistulated Holstein-Friesian cows received from 9 weeks ante- until 8 weeks postpartum twice daily an abomasal infusion either with coconut oil (CONTR, 76 g/d), linseed and safflower oil (EFA, 78 and 4g/d), Lutalin® (CLA, c9,t11 and t10,c12, each 10g/d) or a combination of EFA+CLA, respectively. Finally, oestrus was induced with PGF_{2α} and the cows were slaughtered 48h later. Blood, follicular fluids of the largest follicles, cumulus cells and oocytes were collected for analysis of fatty acids and IVF. Independent of the supplementation, 47% of the animals had a corpus luteum. Preliminary results show that the different supplementations were able to significantly increase the levels of the supplemented fatty acids in plasma and similarly in follicular fluids of dominant as well as of atretic follicles. The cumulus cells also showed comparable changes in their fatty acid profile, but the oocytes contained mostly saturated fatty acids and no changes in their unsaturated fatty acids composition were detected. The results of IVF revealed no differences between the treatments. In conclusion, a dietary supplementation with unsaturated fatty acids alters their proportions similarly in plasma, follicular fluid, and cumulus cells but hardly in the oocyte likely due to barrier functions of cumulus cells.

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Elevated concentrations of free fatty acids alter the expression of the granulosa and sertoli cell identity markers FOXL2 and SOX9 in cultured bovine granulosa cells

Erhöhte Konzentrationen freier Fettsäuren verändern die Expression der Granulosa- und Sertoli-zell Identitätsmarker FOXL2 und SOX9 in kultivierten bovinen Granulosazellen

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Negative energy balance (NEB), experienced by postpartum dairy cows, leads to elevated free fatty acid concentrations in both, serum, and follicular fluid, which could largely affect the normal ovarian function. In the present study, we investigated effects of physiological concentrations of three major free fatty acids i.e., oleic acid (OA, C18:1), palmitic acid (PA, C16:0) and stearic acid (SA, C18:0), on the expression of the granulosa cell identity marker FOXL2 and the sertoli cell marker SOX9 in a granulosa cell culture model. In order to mimic the *in vivo* conditions, we conjugated these fatty acids to bovine serum albumin (BSA). Granulosa

cells (GC) from 2–6 mm follicles were initially cultured for two days followed by treatment with different concentration of PA, SA and OA over the next six days. The results showed that both PA and SA (saturated fatty acids) up-regulated the expression of the granulosa cell marker *FOXL2* along with its downstream genes, *ESR2* and *FST*. On contrary, OA (a monounsaturated fatty acid) down-regulated the transcript abundance of *FOXL2*, *ESR2* and *FST*, but up-regulated the sertoli cell marker *SOX9*. From the above findings, we conclude that fatty acids differentially affect the GC phenotype, depending on their saturation or stereo metric position of double bonds. However, being the predominant fatty acid in follicular fluid, OA could certainly alter GC identity by instigating cell lineage reprogramming processes, which could possibly lead to reduced reproductive performance in postpartum cows.

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Intrauterine growth retardation results in impaired postnatal glucose metabolism in pigs

Intrauterine Wachstumsverzögerung führt zu einer Beeinträchtigung des postnatalen Glukosestoffwechsels bei Schweinen

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Intrauterine growth retardation is a response to reduced nutrient supply via placenta and results in creation of thrifty phenotype. Organs, which are key for survival like a brain, develop normally, but organs as liver, intestine and pancreas are less developed. The aim of our study was to give more insight into carbohydrate metabolism in IUGR piglets in postnatal life. The criteria for IUGR were as follows: piglets born on term with birth body weight below 1.1 kg and head manifesting characteristic shortening of the facial part. Blood samples from 6 pairs of IUGR piglets and their normal body weight (NBW) littermates were collected 2 weeks after weaning. Blood was withdrawn pre- and post-ally for hematology and biochemistry analyses followed by oral glucose tolerance test (1.75 g/kg b.wt. of glucose in 100 ml water given orally). Birth body weight of IUGR and NBW neonates was respectively, 0.99 ± 0.07 and 1.55 ± 0.07 kg ($p < 0.001$). Concerning IUGR fasting hematology and biochemical parameters: thrombocyte number was lower (545 ± 43 vs $660 \pm 105 \times 10^9/l$; $p < 0.05$). Basal plasma glucose did not differ between the groups: 64.4 ± 10.6 vs 72.3 ± 2.7 mg/dl, after 1 h it showed significantly higher concentra-

tion in IUGR compared to NBW (247 ± 58 vs 142 ± 32 mg/dl; $p < 0.05$). After two hours, plasma glucose in IUGR piglets remained elevated (208 ± 77 mg/dl), whereas in NBW it started to return to a basal level (99 ± 17 mg/dl; $p < 0.05$). In conclusion, results of the oral glucose tolerance test in weaned IUGR piglets revealed functional alteration of carbohydrate metabolism.

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Studies on the hormone profile in pigs in the peripartur period in different housing conditions

Untersuchungen über das Hormonprofil bei Schweinen im peripartalen Zeitraum unter Berücksichtigung unterschiedlicher Haltungssysteme

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The creation of a birth-specific hormone profile in pigs (modern crossbreed line Viktoria) was used to investigate whether any influences of different husbandry forms (farrowing crate = K, free farrowing pen = fA) have an effect on the hormone profile. In each group there were 15 female pigs ≥ 2 parturition. On 112th day of pregnancy, a central venous catheter was implanted in the Vena auricularis lat./med., without (45%) or with slight sedation (55%). The sampling took place intrapartum (= i. p.) at intervals of 30'. For the present study, the curves of prostaglandin $F_{2\alpha}$ (= PGFM), oxytocin (= O) and adrenaline (= A) were selected. At birth stage II (expulsion) there was a significant ($p = 0.0001$) increase in concentration (= c) of [PGFM] in both housing systems. Between complication-free and delayed (= obstetric intervention after 60') parturitions, there was no difference. At the same time, at stage II, there was a continuous increase in c [O]. These were tended to be higher in fA than in K. It is striking that in both groups sows (20.8%) showed c [O], which were only slightly above the basal value, which is also correlated with distinctive nest building behavior ($p = 0.013$). Furthermore, under K conditions, the c [A] of the prolonged births were significantly higher ($p = 0.011$). In addition, there was a positive correlation between the protraction frequency and the c [A] ($r = 0.728/p = 0.0002$). With

consideration of the endocrinological situation, it should be noted that some serious differences i.p. are depended on the type of housing.

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Establishment of a robust protocol for generation of clinically relevant number of porcine bone marrow-derived mesenchymal stem cells (BM-MSC)

Etablierung eines Protokolls zur Generierung einer klinisch relevanten Anzahl von Knochenmark-abgeleiteten mesenchymalen Stromazellen beim Schwein

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We hypothesize mesenchymal stem cells implantation into dysfunctional cervix may improve its function. We have established a protocol for isolation and rapid expansion of clinically-relevant numbers of BM-MSC. Bone marrow was aspirated from the head of the humerus. Following mechanical dispersion, extensive washing and density gradient centrifugation the crude fraction comprising stem cells and hematopoietic cells was collected. These cells were cultured in MSC expansion medium. Purity and multipotency of the obtained population was assessed by both flow cytometry and trilineage *in vitro* differentiation. A set of positive (CD73/90/105) and negative (CD19/34/45) surface markers were analyzed, indicating high purity of cultured MSC. The cells were successfully differentiated into adipogenic, osteogenic and chondrogenic lineages, as demonstrated by histochemical staining with Oil Red O, Alizarin S and Alcian Blue, respectively. The undifferentiated cells were cultured under normoxic or hypoxic conditions and a range of FGF concentrations as culture medium additive was tested. Addition of FGF increased growth of the cells over five-fold, with EC50 of 188 and 197 ng/ml and EC90 of 976 and 1009 ng/ml for normoxic and hypoxic culture, respectively. Independently, hypoxic culture further increased yield by 50%. Data presented here demonstrate an establishment of a robust protocol allowing production of high numbers of porcine BM-MSC. Establishment of the protocol allows proceeding to measurements of the impact of MSC implantation on myoelectric functions as measured by electromyography.

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Cortisol regulates oviduct epithelium marker gene expression *in vitro*

Kortisol reguliert *in vitro* die Expression von Markergenen im Eileiterepithel

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Maternal stress during the periconceptional period has detrimental effects on fertilization and early embryonic development. To explore how maternal stress influences oviduct physiology, we tested the effect of cortisol on oviduct epithelial cells *in vitro*. Primary cells were differentiated in a compartmentalized culture system at the air-liquid interface (ALI-POEC) and basolaterally exposed to cortisol (0, 50, 100 and 250nM) for 3 days. Cell height, portion of ciliated and non-ciliated cells and trans-epithelial electrical resistance (TEER) were measured to evaluate morphology and barrier function of the epithelium. RT-qPCR was performed to quantify the expression of marker genes for cortisol bioactivity, oviduct function, glucocorticoid (GC) signaling and inflammation. Cortisol administration neither changed ALI-POEC morphology nor TEER. However, it showed significant effects on marker gene expression. Expression of GC-inducible genes was upregulated proving bioavailability and -activity of cortisol during treatment. Oviduct-specific glycoprotein (OVGP1), estrogen receptor 1 (ESR1) and progesterone receptor (PGR) mRNA levels were downregulated by cortisol. Expression of prostaglandin-endoperoxide synthase 2 (PTGS2) was increased while IL6 mRNA amount was decreased. These results suggest a direct influence of cortisol (and therefore maternal stress) on oviduct epithelium physiology, as its functionality is precisely regulated by steroid hormones during the estrous cycle, and inflammation pathways are involved in early embryo-maternal communication.

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Spatiotemporal gene expression in porcine endometrium at the time of recognition of pregnancy and the onset of conceptus attachment

Räumliche und zeitliche Genexpression im Endometrium beim Schwein während der Phase der Erkennung der Trächtigkeit und des Beginns der Anheftung des Konzeptus

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In the pig, maternal recognition of pregnancy takes place in response to estrogen secretion

by the elongating conceptus around Day 12 of pregnancy. Two days later, the conceptus is already starting to attach to the uterine wall in preparation for implantation. In two recent studies, we analyzed transcriptome changes in different endometrial compartments on Day 12 [Zeng et al. 2018, BMC Genomics] and Day 14 of pregnancy [Zeng et al. 2018, unpublished] by the use of laser capture microdissection and RNA-sequencing. Here, we compared gene expression between pregnant and cyclic endometrium on Days 12 and 14 in luminal epithelium (LE), endometrial glands (GE) and stroma based on these data sets. The comparison of detectable genes between Days 12 and 14 revealed the greatest difference for stroma, followed by GE and LE. The overlap of differentially expressed genes (DEGs) between Day 12 and 14 was low, particularly for GE and stroma with only 3.6% and 6.2%, respectively (LE 18%). This confirms results of a previous RNA-seq study [Samborski et al. Biol Reprod, 2013] of complete endometrial tissue samples collected at the same time points. While on Day 12 the most overrepresented functional categories were related to signaling, estrogen and prostaglandin metabolism, categories involved in cell adhesion, immune response, and tissue remodeling and development were prevalent on Day 14. In conclusion, the cell type-specific RNA-seq approach assigned specific biological processes to the endometrial compartments and localized molecular changes associated with pregnancy recognition and preparation for implantation.

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A transcriptomic approach toward understanding induction of parturition in the dog

Transkriptom-Ansatz zur Klärung der Vorgänge bei der Geburtseinleitung beim Hund

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Parturition in dogs is associated with a rapid drop in progesterone (P4), concomitant with increasing PGF_{2α} levels in the maternal blood. PGF_{2α} originates in trophoblast and can also be induced by blocking P4 receptors (PGR) localized only in placenta materna. To investigate molecular events occurring in canine placenta during parturition and/or abortion, we applied the RNA-seq approach. RNA was isolated from canine placentae (n = 13; 3–5/group) collected at mid-gestation (control, days 35–40), prepartum luteolysis and aglepristone-induced abortion (mid-gestation dogs treated with Alizine®). Datasets were analyzed using SUSHI, a framework developed by FGCZ, and IPA® software. Analysis showed 1937 and 135 differentially regulated

genes (DEG, p < 0.01, FDR 0.1) at prepartum or abortion, respectively, compared with mid-gestation. Both for normal and induced luteolysis, more highly represented transcripts were linked to apoptosis, impairment of vascular function (negative regulation of endothelial cell function and adhesion) and activation of signaling of several cytokines (e.g., IL-8, IL-3, TGFβ). Moreover, both conditions shared the same upstream regulators, which besides P4 and PGR, included, i.a., TGFβ and glucocorticoid. Among P4-regulated genes, we identified PTGS2/COX2 (prostaglandin synthesis), ICAM1 (immune cell migration) and HSD11B2 (cortisol metabolism). In summary, natural and induced P4 withdrawal disrupts the fetomaternal interface, which is reflected by disturbed vascular maintenance, apoptosis and controlled, local immune response.

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Conceptus-mediated peri-implantation modulation of uterine immune responses in the dog

Konzeptus-induzierte Modulation der uterinen Immunantwort im periimplantären Zeitraum beim Hund

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The relationships between pro- and anti-inflammatory processes during the peri-implantation period are critical for pregnancy maintenance, but are little known for the dog. Here, expression of selected immune cell markers and cytokines was investigated by immunohistochemistry and qPCR in the canine uterus during the pre-implantation stage (days 10–12; E+) and in corresponding non-pregnant controls (E–), at the implantation (day 17; Imp), and post-implantation periods (days 18–25; P-Imp). Transcript levels encoding for CD4, CD25, MHCII, NCR, IDO1 and AIF1 were increased in E+ compared with E– uteri, whereas FoxP3, CCL3 and IL12 were suppressed. Later, during Imp, FoxP3, IL12 and NCR increased, with concomitant suppression of CD4, CCR7, IL8 and IL10. The early P-Imp period was characterized by suppressed mRNA levels of FoxP3, MHCII, CD206, NCR, IL12, and the lowest CCL3 abundance. At the cellular

level, CD206, CD163, MHCII, TNF α , and AIF-1 were detected in macrophages, CD4 in lymphocytes and NCR in NK cells localized mainly within endometrium. In summary, our study for the first time shows the possible interplay between pro- and anti-inflammatory factors in the canine uterus during the peri-implantation period. Thus, whereas some of the pro-inflammatory factors, like CD4, MHCII, CCR7 or IL6, are more strongly present prior to implantation, anti-inflammatory events appear to prevail during implantation and early trophoblast invasion, as indicated by upregulated levels of IL12 and FoxP3 and suppressed expression of MHCII, CD4, CCR7, IL6, IL8, IL10 and CCL3.

F-1

Quality audits in AI centers: a ten-year retrospective study in 29 European boar studs

Qualitätsaudits in Besamungsstationen: Eine 10-jährige retrospektive Studie in 29 europäischen Eberstationen

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The aim of this study was to investigate the influence of semen production management in 29 different European boar studs on thermo-resistance (TRT) of boar spermatozoa. TRT is an important semen characteristic, easy to determine and is used to explain variations in pig fertility. During a ten-year study period from 2009 to 2018, 1,523 ejaculates (one ejaculate/boar) were analyzed for TRT on day 7 of semen storage. Statistical models showed that around 40% of the total variability in TRT could be explained by production management factors. Of these factors AI center, extender type, dilution steps, first dilution temperature, bacterial contamination, arrival temperature of ejaculates in the analysis laboratory, electrical conductivity of pure water for extender preparation and total sperm number per dose significantly influenced TRT. Furthermore, year and month of production, boar age, ejaculate volume, semen concentration, semen output, doses/ejaculate, mitochondrial activity and membrane integrity of spermatozoa correlated with TRT and were useful predictors for semen quality. Based on an improved production management during the study period, TRT increased and bacterial contamination decreased in AI doses. Breed, dilution rate and refractive index of extender had no influence on TRT. A holistic view of the requirements in the field of boar semen production is of great importance for future developments of individualized Hazard Analysis and Critical Control Point (HACCP) concepts, prognosis models for boar semen quality and could help to improve the efficiency of AI organizations.

(Supported by FBF).

F-2

Multicolor flow cytometry is a sensitive tool to visualize storage effects in plasma membrane function of liquid preserved boar spermatozoa

Mehrfarben-Durchflusszytometrie ist eine sensitive Methode zur Darstellung von Lagerungseffekten auf die Funktion der Plasmamembran bei flüssigkonservierten Eberspermatozoen

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Multicolor flow cytometry now offers the possibility for simultaneous single cell assessment of changes in sperm membrane architecture and function. The aim was to study storage effects on functional changes in plasma membrane physiology of boar spermatozoa using multicolor flow cytometry. Semen doses (n = 66) from 11 boar studs were stored at 17 °C. At 72 h and 96 h, aliquots were incubated in Tyrode's capacitating medium under CO₂ at +38 °C or in a non-capacitating control medium (Tyrode w/o bicarbonate). Sperm were loaded with flourescences H33342, Yo-Pro-1, Merocyanine (M) 540 and Calbryte (Cbry) 630 and analyzed by flow cytometry (CytoFlex Beckman Coulter) after 3 and 60 min incubation time. Under non-capacitating conditions, storage did not influence the percentage of viable (Yo-Pro neg.) sperm with high membrane fluidity (M540 pos.) and high intracellular calcium (Cbry pos.). After 60 min in capacitation medium, semen stored for 96 h showed a lower percentage of M540 pos. and of Cbry pos.-viable spermatozoa compared to 72 h (p < 0.05). The specific response to the capacitation stimulator bicarbonate based on the decrease of viable, M 540 neg. and Ca neg. sperm revealed a lower responsiveness at 96 h (47.8 ± 22.6%) compared to 72 h (54.8 ± 18.6%; p < 0.01). In conclusion, multicolor flow cytometry is a sensitive tool to visualize storage-induced changes in sperm membrane physiology. The observed small storage effect might be irrelevant for the outcome of field inseminations but ultimately such tools are essential to improve semen preservation techniques.

F-3

Species-specific antibacterial defense in boar and bull ejaculates

Artspezifische antimikrobielle Schutzstrategien in Eber- und Bullenejakulaten

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Natural antibacterial substances in the seminal fluid (SF) can reduce bacterial growth in semen. In the current study the overall bacterial killing activity (BKA) against *Escherichia (E.) coli* and *Staphylococcus (S.) aureus* and the concentration of an important antibacterial enzyme, the lysozyme (LC), of boar (n = 119) and bull (n = 61) SF was compared. In addition, colony-forming units (CFU) in raw semen as well as sperm morphology, sperm motility and thermo-resistance of liquid stored (boar) or frozen-thawed semen (bull) were determined and their association with the antibacterial defenses was tested. In boars, the SF from 32% of samples had BKA against both bacterial strains, 11% against *E. coli* only, 39% against *S. aureus* only and 18% had no BKA. In bulls, 20% had BKA against both bacteria, 77% against *S. aureus* only and 3% had no BKA. Contrary to boars, there were no bulls with BKA against only *E. coli*, implying that 80% of bulls had no BKA against *E. coli*. In both species, individuals with BKA against *E. coli* were significantly older (boar: p < 0.001, bull: p = 0.045). LC was higher in boars compared to the bulls (2.4 ± 1.2 µg/ml and 0.8 ± 0.4 µg/ml, respectively). CFU correlated with LC (r_s = -0.281, p = 0.034) only in bulls. In boars, individuals with BKA against *E. coli* had significantly lower normospermic ejaculates (p < 0.001), but in bulls normospermic ejaculates were highest in individuals with BKA against both bacteria (p = 0.029). Future research is needed to discover the specific antibacterial molecules responsible for BKA of boar and bull SF.

F-4

Effect of cooling velocity on sperm quality and bacterial growth in boar semen preserved at 5 °C in absence of antibiotics

Einfluss der Abkühlgeschwindigkeit auf die Spermienqualität und das Keimwachstum im Ebersamen bei antibiotikafreier 5 °C-Konservierung

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Storage of boar spermatozoa at 5 °C is a novel concept to reduce the use of antibiotics in semen extenders. Determination of optimized cooling regimes is essential to ensure high sperm quality and inhibit bacterial growth. The aim was to test the effect of different cooling rates on sperm quality and bacterial load of boar semen stored at 5 °C with an antibiotic-free extender (Androstar Premium®, Minitube). Diluted semen (20 × 10⁶ sperm/mL) was cooled from 30 to 5 °C using four different cooling rates, 4.8, 3.3, 2.1 and 0.6 °C/h in the temperature

zone from 30 to 15 °C. Sperm quality ($n = 8$ boars) and colony forming units (CFU/mL; $n = 4$ boars) were assessed at 24, 72 and 144 h. At 144 h, progressive motility did not differ between the cooling rates ($77 \pm 6\%$ to $79 \pm 4\%$). Membrane integrity was highest ($85 \pm 3\%$; 144 h) with a cooling rate of 2.1 °C/h ($p < 0.05$). The amount of viable sperm with high membrane mitochondrial potential (Propidium iodide neg/JC-1 pos) were on high levels with all cooling rates ($93\text{--}94\%$, 144 h). The response to capacitation stimulus measured as bicarbonate-induced Ca^{2+} influx (Fluo3 AM) at 72 h was highest ($20 \pm 9\%$) with a cooling rate of 0.6 °C/h ($p < 0.05$). Initial bacterial load in diluted semen (0 h) ranged between 2 and 7.5×10^3 CFU/ml and remained in this range with cooling rates between 4.8 and 2.1 °C/h until 72 h. Interestingly, at 144 h there was a tendency towards lower bacterial load (range 0.1 to 4.5×10^3 CFU/mL) in all groups. In conclusion, moderate cooling rates between 3 and 0.6 °C/h until 15 °C are suitable for hypothermic boar semen preservation.

F-5

***Staphylococcus aureus* (*S. aureus*) mastitis modulates expression of antimicrobial peptides dependent on Holstein genotype**

Staphylococcus-aureus-
(*S. aureus*-) Mastitis moduliert die Expression antimikrobieller Peptide abhängig vom Holstein-Genotyp

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Antimicrobial peptides (AMP) are key molecules for the resolution of intramammary infection (IMI). Genetic selection offers a sustainable tool to breed animals with differing susceptibility towards mastitis. Aim of the study was establishing an *ex vivo* model to study the impact of genetic selection on AMP gene expression in mammary explants from animals with *S. aureus* mastitis. Holstein Friesian heifers were selected for favourable (Q, $n = 12$) and unfavourable (q, $n = 11$) parental chromosome 18 haplotypes regarding somatic cell count, used as surrogate trait for mastitis susceptibility. On day 36 ± 3 post partum animals received an intramammary

S. aureus challenge in both hindquarters. The front quarters remained untouched. Necropsy was performed 96 h after challenge and tissue explants were prepared from the teat cistern and the udder parenchyma of hind right (hr) and front right (fr) quarters. Subsequently tissue explants were cultured for 18 h *in vitro* with either 106 colony forming units of heat killed *S. aureus* or no stimulus. *S. aureus* restimulation of explants originating from hr quarters induced higher ($p < 0.05$) LAP (lingual antimicrobial peptide) expression in the teat cistern compared to explants solely stimulated *in vivo*. *S. aureus* IMI *in vivo* caused elevated levels ($p < 0.01$) of LAP in the teat cistern (hr) of Q-cows but not of q-cows. Furthermore, Q-cows showed significantly ($p < 0.05$) higher mRNA expression for the AMP S100A9 in udder parenchyma (fr) compared to q-cows. In conclusion, genetic selection for mastitis susceptibility has an impact on the pathogen induced expression of innate defense molecules.

D-1

The expression of microRNA processing enzymes Drosha and Dicer is altered in preimplantation embryos and placenta of diabetic rabbits

Veränderte Expression microRNA-prozessierender Gene in Präimplantationsembryonen und Plazenta diabetischer Kaninchen

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MicroRNAs (miRNAs) are regulators and potential markers of embryonic and placental development and their aberrant expression is associated with pregnancy complications. The Drosha-DGCR8 complex processes pri-miRNAs, are subsequently exported to the cytoplasm by Exportin 5 (EXP5) and cleaved by Dicer. Mature miRNAs are loaded onto Argonautes (AGO) to form the RNA-induced silencing complex and regulate the gene expression at the post-transcriptional level. In previous studies we could show that a maternal diabetes type 1 is associated with a distinct downregulation of specific miRNAs in the embryonic trophoblast. Therefore, we have analysed the expression of genes involved in miRNA biogenesis in the embryoblast (EB) and trophoblast (TB) of rabbit blastocysts at day 6 *p.c.* and in placenta at day 12 *p.c.* using qRT-PCR, Western Blot and immunohistochemistry for AGO2, DGCR8, Dicer, Drosha and EXP5. Maternal diabetes led to a marked downregulation of Dicer and nuclear Drosha in EB and TB, respectively, and an increase of AGO2, DGCR8 and EXP5 mRNA levels in blastocysts from

diabetic rabbits. In addition, an altered expression of microRNA processing genes was consolidated and observed in placenta at day 12 *p.c.* from diabetic mothers. We conclude that the perturbation of miRNA processing may contribute to embryonic developmental delay and placental complications in a diabetic pregnancy.

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D-2

Characterization of mitochondrial function during *in vitro* capacitation of boar spermatozoa by multicolor flow cytometry

Charakterisierung der Mitochondrienfunktion während der In-vitro-Kapazitation bei Eberspermatozoen mittels Mehrfarben-Durchflusszytometrie

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Multicolor flow cytometry offers new possibilities to identify functionally relevant changes by more in-depth functional characterization of distinct sperm subpopulations. The aim was to evaluate the interplay of changes in free intracellular calcium content and mitochondrial function during capacitation of viable boar spermatozoa. To this end, aliquots of semen ($n = 9$ boars) were incubated in a capacitating Tyrode's medium (15 mM bicarbonate) or a non-capacitating control (w/o bicarbonate) and analyzed after 3 min and 60 min incubation. Sperm were loaded with SiR700-DNA to distinguish sperm and debris. At 60 min, capacitation led to a significant increase in viable (H33258 negative) sperm with high intracellular calcium levels (Calbryte™ positive; $52.6 \pm 0.16\%$, $p < 0.05$), when compared to the control condition ($23.1 \pm 0.12\%$; $p < 0.05$). A time-dependent increase in intracellular calcium levels during capacitation was mainly observed in sperm with a high mitochondrial membrane potential (JC-1 positive; $35.9 \pm 0.12\%$ of total sperm). Fluorescence intensity of JC-aggregates revealed that viable spermatozoa with low intracellular calcium levels maintained a higher mitochondrial membrane potential under capacitating conditions than sperm in the control medium, although sperm from both media were JC-1 positive. The results support the view that a high mitochondrial activity is closely linked with a successful progression of capacitation, e.g. an increase in intracellular calcium levels. In conclusion, multicolor flow cytometry is a helpful tool to study sperm bioenergetics during capacitation.

D-3

Epithelial cell cultures originating from different regions of the porcine oviduct show divergent phenotypes

Epithelzellkulturen aus verschiedenen Regionen des porzinen Eileiters zeigen unterschiedliche Phänotypen

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The oviduct is composed of different anatomical regions (isthmus, isthmico-ampullary junction, ampulla and infundibulum), each of which is responsible for hosting distinct reproductive events. Our group established differentiated long-term cultures of porcine oviduct epithelial cells using an air-liquid interface (ALI) approach. However, so far we use a pool of cells from the whole oviductal tube. In the present study, we aimed to prove whether primary porcine oviduct epithelial cells isolated from ampulla (A) and isthmus (I) show a divergent phenotype after culture under ALI conditions. Primary porcine oviduct epithelial cells from $n = 5$ animals were grown in hanging inserts for 3 weeks and then evaluated for cell morphology, cell composition, epithelial barrier function, sperm binding index and expression of marker genes. All cultures reached complete differentiation, demonstrating comparable cellular height (A: $17.95 \pm 3.05 \mu\text{m}$; I: $18.02 \pm 2 \mu\text{m}$) and sperm binding capacity (A: $3.77 \pm 0.68 \times 10^6$ sperm/24well insert; I: $3.90 \pm 0.52 \times 10^6$ sperm/24well insert). The ampulla cultures possessed a significantly lower ratio of ciliated cells (A: $69.98 \pm 5.08\%$, I: $80.64 \pm 5.86\%$; $p < 0.05$), and higher trans-epithelial electrical resistance (A: $562 \pm 30 \Omega\text{cm}^2$; I: $521 \pm 6 \Omega\text{cm}^2$, $p < 0.01$). The isthmus cultures showed higher expression of estrogen receptors (ESR1 and GPER), but lower abundance of stem cell marker Nanog, as well as of Follistatin ($p < 0.05$). In conclusion, the anatomical origin of the primary cells has a profound influence on the phenotype of oviductal epithelial cells even after long-term ALI culture.

**■ Poster presentations/
Posterpräsentationen**

1

Loop-mediated isothermal amplification for bovine mastitis: A method to distinguish five relevant pathogens

Loop-mediierte isothermale Amplifikation für die Diagnose der bovinen Mastitis: Eine Methode, fünf relevante Pathogene zu unterscheiden

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Bovine mastitis is the most common infectious disease in dairy cows. While bacterial culture is the gold standard for pathogen identification, it is time consuming and labor-intensive. Molecular techniques such as PCR are faster but require expensive laboratory equipment. As a cost-effective and time-saving alternative we designed loop-mediated isothermal amplification (LAMP) assays to discriminate infection with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Mycoplasma bovis*. LAMP rapidly amplifies DNA under isothermal conditions with a PCR-like sensitivity and specificity. LAMP primers were designed for each of the five pathogens and proof of concept was achieved using DNA extracted from cultivated bacteria. Subsequently, analytic detection limits were assessed using a standard dilution series for each of the pathogens ranging from 10^6 to 10^1 copies of the template DNA. LAMP reactions were run on a Step OnePlus Real-Time PCR System. All five LAMP assays were highly specific for the target DNA. Amplification was rapid and ranged from 5 to 15 min incubation time. Analytic detection limits were between 10^4 and 10^3 copies of the template DNA. Since fast and easy pathogen-detection is beneficial for the diagnosis and early control of bovine mastitis, the newly established LAMP assays show great potential for further application in dairy-cow herd management.

2

Occurrence of mycotoxins in buffaloes ration and their effects on some reproductive hormones and physicochemical composition of the milk

Vorkommen von Mykotoxinen in der Büffelration und deren Auswirkungen auf einige Fortpflanzungshormone und die physikochemische Zusammensetzung der Milch

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The study aimed to explore the effects aflatoxicosis on the reproductive hormones and physicochemical composition of milk in buffaloes. Forty lactating buffaloes were fed total mixed rations (TMR), contaminated by fungi secreting aflatoxin. Animals divided into: The first group (BG, 20 buffaloes), was fed ration rich in fungi and contain aflatoxin above the permissible limit (21.2 ppb). The second control group (CG, 20 buffaloes) fed on ration contain aflatoxin below permissible limit which is (5 ppb). Samples taken from feed, blood and milk to detect the presence of aflatoxin. The amount of aflatoxin in feed and milk determined by HPLC. Hormonal analysis of serum was for FSH, LH, Progesterone, Estradiol, Cortisol and Prolactin. From the results, aflatoxins detected in dietary ration and milk of the buffaloes, were AFB1 and AFM1 respectively. The mean concentration of AFM1 in raw buffalo milk was (0.04 ± 0.002 ppb) in control group (CG), while in that of the second group (BG) was (0.42 ± 0.043 ppb) and the difference was significant ($p < 0.05$). Animals in BG revealed highly significant ($p < 0.001$) decrease in FSH, LH and Progesterone concentration compared with control one. In contrary estradiol and Cortisol increased significantly ($p < 0.01$) in BG compared with CG. Prolactin concentration showed slight elevation. The Lacto scan measured physicochemical indices of milk samples including fat%, solid not fat (SNF)%, Slats%, protein%, total solids%, pH showed no recordable changes. In conclusion, aflatoxicosis leads to hormonal imbalance, which could influence the normal function of reproductive organs and elsewhere the productivity of lactating buffaloes.

3

Small animal repro vets in an ethical dilemma – a survey**Kleintierreproduktionsmediziner im ethischen Dilemma – eine Umfrage**S. P. Arlt¹, H. Ovregaard²¹Clinic for Animal Reproduction, Freie Universität Berlin, Germany; ²AniCura Askoy Dyreklinikk, Kleppestø, Norway

Veterinarians may face ethical dilemmas when dealing with dog or cat breeders. To learn more about potential issues, an online survey was conducted using Google forms in spring 2018. In total, 83 participants (62 EU, 12 USA, 9 other countries) completed the survey. Thereof, 78 stated to provide special services for breeders. Altogether these participants stated to perform 5000 fresh semen transfers, 2300 chilled semen transfers, 1200 transcervical inseminations, and 690 surgical inseminations per year in total. In addition, they carry out 1800 elective C-sections per year. Most vets (n = 72) consider overtaking and heritable diseases compromising health and welfare in specific breeds to be fatal or very fatal. Most participants stated that it is ethical to perform semen transfer in dogs who did not reproduce naturally before (n = 65). However, 52 participants consider surgical insemination not ethical, and 46 participants (vs 37) are of the opinion that insemination is not ethical if natural whelping is not likely to occur. Elective C-sections are ethical for 37 and not ethical for 46 participants. Fourteen vets feel “never”, 61 “sometimes” and 6 “often” compromised by ethical conflicts when performing assisted canine reproduction interventions. This survey suffers from a selection bias and some questions did not fully reflect the complex situations in real life practice. However, the results show that most specialists see considerable ethical issues but the opinions are heterogeneous. This topic should be discussed and evaluated in more detail and strategies to solve ethical conflicts and to support action in diminishing heritable diseases should be developed.

4

Effects of the addition of different concentrations of catalase to TRIS-egg yolk extender before freezing on quality of frozen-thawed bull sperm**Auswirkungen der Zugabe unterschiedlicher Catalase-Konzentrationen zu TRIS-Eidotterverdünner auf die Qualität kryokonservierter Bullenspermien**H. O. Arslan, M. Siuda, E. Malama, H. Bollwein
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The aim of this study was to assess the effects of different doses of catalase (CAT) on

frozen-thawed bull sperm. Two ejaculates of each of 7 bulls were collected. The sperm samples were divided into 5 aliquots and diluted with Trilady¹ extender containing 0 (control), 5, 10, 15 and 20 IU CAT/mL to a concentration of 60×10^6 sperm/mL. All aliquots were equilibrated for 24 h and frozen in liquid nitrogen. Immediately (= 0), 3, 6, 12, and 24 h after thawing and incubation at 38 °C, the percentages of rapid motile sperm (RMS), plasma membrane and acrosome intact sperm (PMAI) and of sperm with a high degree of DNA fragmentation (%DFI) were investigated by using CASA and flow cytometry, respectively. Considering the whole time interval, all doses of CAT increased RMS and PMAI and lowered %DFI compared to control ($p < 0.001$). Sperm frozen with 15 IU and 20 IU CAT showed the highest PMAI and the lowest %DFI at 24 h and did not differ ($p \geq 0.05$) from each other. In conclusion, the addition of 15 IU/mL CAT to Trilady¹ extender is recommended to enhance motility, plasma membrane and acrosome integrity as well as DNA integrity of frozen-thawed sperm.

5

First insights into diversity and health status specific fluctuations of intrauterine anaerobic microbiota in postpartum dairy cows by culturomics**Erste Einblicke in Diversität und Fluktuation der intrauterin anaeroben Mikroflora von postpartalen Milchkühen mittels Culturomics**P. Ballas¹, K. Wagener², M. Drillich², M. Ehling-Schulz¹¹Functional Microbiology, Institute of Microbiology;²Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria

Postpartum uterine environment is rapidly changing after calf delivery. Thus, the identification and understanding of the bacterial population dynamics is extremely important to elucidate the pathogenesis of uterine diseases. We previously showed that the aerobic microbial community in the postpartum uterus is highly diverse and fluctuations are linked to the uterine health status [Wagener et al. 2015]. By contrast, the composition and dynamic within the anaerobic part of the microbial community is largely unexplored. Therefore, we employed MALDI-TOF to gain insights into the anaerobic bacterial population in the postpartum uterus and shed light in the dynamics of the postpartum uterine anaerobic flora. For that purpose, strains that have been collected from dairy farms in Austria were analyzed by MALDI-TOF. A broad variety of species were found, with the major representatives of the anaerobic flora being bacteria that are considered facultative anaerobic, such as *Trueperella pyogenes* (19%), *Escherichia coli* (9.5%) as well as *Streptococcus pluranimalium* (18%). In ad-

dition, strictly anaerobic species that have been linked to endometritis, such as *Fusobacterium necrophorum* (0.05%), were found as well. In conclusion, the changing uterine environment in the postpartum period, maybe in combination with different conditions in different parts of the uterus, provide a suitable niche for the growth of facultative anaerobic bacteria. However, hitherto the contribution to the overall uterine health of the majority of these bacteria in the uterine environment and whether they have a beneficial or detrimental effect still needs to be elucidated.

6

Elevated oleic acid concentration inhibits ovulation in preovulatory bovine follicles**Eine erhöhte Ölsäurekonzentration in präovulatorischen Follikeln des Rindes inhibiert deren Ovulation**

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Excessive lipid mobilization after calving to meet energy requirements is inevitable in high-yielding dairy cows. Therefore, the concentrations of free fatty acids (FA), among them Oleic acid (OA; C18:1), increase in the blood and in the follicular fluid. We investigated the effects of elevated concentrations of OA in dominant follicles on gene expression and ovulation in 14 heifers (HF). In total, 45 ultrasound guided injections were done into dominant follicles during induced heat of the animals. Twenty follicles were treated with OA and 25 follicles were used to check the impact of the transport medium BSA. According to the diameter of the follicles (10 to 19 mm) an adapted amount of BSA without/with 400 µM OA was injected. Follicular fluid and cells were aspirated 48 h later to estimate focused transcript levels. Finally, four follicles were aspirated without any previous injection; however 14 injected follicles were not aspirated with the aim to check their ovulatory competence. In total, 77% of these follicles injected with BSA ovulated within 96 hours while only 20% of the OA injected follicles ovulated. Results from the aspirated follicles demonstrate, that OA increased the transcript levels of the FA translocase CD36 ($p < 0.05$). It plays an important role in the homeostasis of FA within the follicle. However, OA downregulated the transcript abundance of steroidogenic genes aromatase CYP19A1 ($p < 0.05$) and StAR ($p < 0.01$). No significant effects were found regarding the cell cycle regulator CCND2. Our results suggest that increased OA concentrations specifically alter mechanisms of folliculogenesis and ovulation in cattle.

Interferon- α and pregnancy associated interferon- τ induce different gene expression in peripheral leucocytes

Interferon- α und trchtigkeitsassoziiertes Interferon- τ induzieren unterschiedliche Genexpressionen in peripheren Leukozyten

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Interferon- τ (IFN- τ) is crucial for pregnancy establishment in bovine whereas IFN- α is a regulator of antiviral immunity. Both share about 50% structural similarity and bind to the type I IFN receptor, activating the transcription of > 2000 Interferon stimulated genes (ISG) with a multitude of biological functions. To examine the biological effect of IFN- τ compared to IFN- α in respect to activation of (non-)classical IFN associated pathways and the induction of ISG, bovine leucocytes were stimulated *in vitro* with recombinant IFN- τ and IFN- α (6.0 and 0.6 ng/ml). Western blots were used to analyse protein level of IFN activated cell signalling pathways (Janus-kinase/Stat-pathway [JAK-STAT], mitogen activated protein kinases [MAPK] 42/44 and Akt-kinase/protein kinase B [pAKT]). Furthermore, qRT-PCR was performed to determine mRNA-expression of MX1, MX2, ISG15, OAS and USP18. All *in vitro* experiments were carried out in triplicates. IFN- τ increased the protein level of pSTAT1 ($p < 0.05$) whereas IFN- α increased protein level of MAPK and pAKT ($p < 0.05$). IFN- τ raised MX1, MX2, ISG15, OAS and USP18 mRNA expression significantly after 120 min. stimulation compared to IFN- α . Expression levels of MX2 were ~50 fold higher, MX1, ISG15 and USP18 were ~14 fold higher and expression levels OAS were ~4 fold higher in leucocytes after 120 min. stimulation with IFN- τ compared to IFN- α (6 ng/ml). These results show that IFN- τ and IFN- α differ in the activation of IFN associated pathways and in the expression of ISG in leucocytes, leading to the assumption that this causes different biological effects.

8

Studies of the injury pattern of the soft birth canal in Holstein Friesian heifers and cows after spontaneous parturition

Untersuchungen zum Verletzungsmuster des weichen Geburtsweges bei primi- und pluriparen Holstein-Friesian-Khen nach Spontangeburt

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While there is numerous of literature describing the involution of the uterus after unassisted birth, there is little information available about possible alterations in the soft birth canal. Hence, the goal of this study was to describe potential changes of the soft birth canal post partum (p.p.). For this purpose, 50 cows (25 heifers und 25 cows) were assessed vaginally using a cylindrical speculum within eight hours after birth and on day 1, 5, 10, 15 and 21 p.p.. Special attention was given to lacerations, hematomas, hyperemia and suffusions in the area of the cervix, vagina, hymenal ring, vestibulum vaginae, and vulva. Immediately after birth, all animals presented, with varying degrees, lacerations in the dorsal commissure of the vulva, which had healed off completely on day 15 p.p. in approximately 50% of the heifers, and on day 10 p.p. in approximately 70% of the cows. Additionally, all heifers and 41% of the cows presented with lacerations of the hymenal ring, which had healed almost completely by day 15 in 75% and 99%, respectively. Hyperemia and suffusions were present in all heifers in this area. While the hyperemia had disappeared by day 10 p.p., suffusions were still present in approximately 45% of the animals on day 21 p.p.. In summary, there are injuries in the soft birth canal even after unassisted birth and it is possible to describe a unique injury pattern. Particularly heifers have significant injuries in the area between the vulva and the hymenal ring; however, these heal entirely within a short period of time.

9

Structural and contractile peculiarities reflect the unique function of the postnatal epididymal duct

Strukturelle und kontraktile Besonderheiten spiegeln die einzigartige Funktion des postnatalen Nebenhodenganges wider

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In adult epididymis smooth muscle cell (SMC) contractions are essential for the transport of the immotile spermatozoa and the expulsion of stored sperm cells during the ejaculatory process. In agreement, spontaneous contractions are present in the proximal part of the adult epididymal duct but not in the distal sperm-storing part. Our recent study showed spontaneous contractions also in the postnatal epididymal duct and the transport of exfoliated epithelial cells. In this study we compared postnatal and adult epididymal tissue regarding histological and functional differences. The typical histological characteristic of the adult epididymal

duct, increasing thickness of the SMCs layer from proximal to distal, was not found in postnatal rat epididymis. Smooth muscle actin (SMA) immunostaining revealed a thick circular layer of SMCs in all regions of the postnatal epididymal duct in contrast to the adult ones. Time Lapse Imaging showed a consistent frequency of spontaneous contractions in all parts of the duct reflecting the uniform postnatal SMC layer whereas in adult tissue the contractions varied between caput, corpus and cauda. Interestingly spontaneous contractions were also found in the most distal part of the postnatal cauda, which is without any spontaneous contractions in adult epididymis. These structural and contractile peculiarities of the postnatal epididymal duct emphasize the completely underestimated epididymal function of waste disposal during the development to prevent subsequent obstruction-induced infertility.

10

The effects of different treatments of recipient mares on the day of embryo transfer on post-transfer pregnancy rates in a large commercial embryo transfer program

Effekt verschiedener Behandlungsregime bei Empfngerstuten am Tag des Embryotransfers auf die Trchtigkeitsraten in einem groen kommerziellen Embryotransferprogramm

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In large herds of horses environmental stress has been assumed to reduce pregnancy rates after embryo transfer. The objective of this retrospective study was to evaluate the efficacy of established adjunctive therapies in recipient mares in a large commercial embryo transfer program. For insemination of donor mares frozen or cooled semen from stallions with proven fertility was used. Embryos were flushed from healthy donor mares transvaginally at days 7–9 post ovulation. Embryos classified grade 1, grade 2 and grade 3 were transferred and a total of 303 embryos were transferred into 260 recipient mares (~10 years), which were treated once on the day of embryo transfer. Before transfer all recipients were sedated (Detomidine 0.008 mg/kg BW). Group RAA (n = 100) received intestinal relaxant (Butylscopolamine bromide 0,13 mg/kg), broad-spectrum antimicrobial, and anti-inflammatory drugs (flunixin meglumine 0.9 mg/kg, and dexamethasone 0.01 mg/kg). Group AA (n = 100) received gentamicin (5 mg/kg) and flunixin meglumine (0.9 mg/kg). Group C (n = 103) did not receive any treatment. At day 16 (post donor ovulation) pregnancy rates were higher in Group RAA compared to Group AA, and

Group C ($p < 0.05$). Proportions of recipients showing pregnancy loss at D45 were not different between groups. In conclusion, in a large commercial embryo transfer program a single administration of intestinal relaxant, broad-spectrum antimicrobial, and anti-inflammatory drugs at the time of embryo transfer improved post transfer pregnancy rates without affecting the proportion of recipients suffering from pregnancy loss.

11

Parturition progress in the pig under different housing conditions

Zum Geburtsverlauf beim Schwein unter verschiedenen Haltungsbedingungen

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The aim of the study was to examine the influence of three different housing conditions on farrowing process in a modern pig genetic. Surveys were carried out on 69 sows at the age of 2.45 ± 0.98 years, which at least had reached second gestation. Mothers were either housed in farrowing crate (K, $n = 38$), free farrowing pen (fA, $n = 25$) or group housing (G, $n = 8$). Gestation length was 114.3 ± 1.2 for K, 114.9 ± 1.4 for fA and 114.5 ± 1.2 days for G. The overall parturition length (min.) did not differ between housing in K (277 ± 117) and in fA (275 ± 99), but averaged 202 ± 74 min in G ($p > 0.05$). Eutocic partus lasted 207 ± 86 in K, in fA and G 190 ± 57 resp. 145 ± 61 minutes ($p > 0.05$). In G 50%, in K 55.6% and in fA 60% of births were prolonged, but the degree of prolongation was more severe in K than in fA and G ($p > 0.05$). Thus, in 30% of the dystocic farrowings in K four or even more prolongations occurred. Birth in fA and in G had a maximum of two intrapartum prolongations. While between birth interval in dystocic farrowings averaged 25.0 ± 1.7 (K), 26.6 ± 1.5 (fA) and 18.0 ± 2.7 minutes (G), it only lasted 11.4 ± 1.4 , 9.9 ± 1.3 and 8.7 ± 1.6 minutes in eutocic births ($p < 0.01$). The vitality of the offspring correlated positively with the birth weight ($p < 0.01$), as well as negatively with the partial degree of prolongation. The average placental weight per piglet was negatively related to litter size ($p < 0.01$). On the basis of clinical characteristics mentioned above there is evidence that farrowing without confinement is beneficial for parturition success in modern pig breeds. Therefore, further research on a larger population of sows is needed.

12

IGF-1 as farm independent prognostic biomarker of high ketosis risk after parturition in dairy cows

IGF-1 als betriebsübergreifender Prognosebiomarker für das Risiko einer Ketose nach der Geburt

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It was previously shown that cows with postpartum clinical ketosis had lower insulin-like growth hormone 1 (IGF1) concentrations antepartum compared to healthy cows. However, the previous studies involved solely cows from individual farms, and it was not clear if a farm specific threshold for IGF-1 is necessary. Therefore, the aim of the present study was to test if IGF-1 can be used as predictive biomarker for ketosis from cows housed in different commercial dairy farms. Blood samples were collected in 30% of the total stock of four farms. Blood samples were taken from 224 cows on d -28 ± 4 , d -14 ± 4 , d 1 ± 4 , d 7 ± 4 and d 14 ± 4 relative to parturition and measured for IGF-1 with validated immunoassays. The incidences of ketosis given in % and the overall mean IGF-1 concentration on each farm was 23.9% and 131 ± 147.3 g/ml in farm 1, 22.9% and 101.2 ± 116.1 ng/ml in farm 2, 17.0% and 106.6 ± 141.8 ng/ml in farm 3 as well as 24.0% and 115 ± 150.4 ng/ml in farm 4. Neither the incidence nor the mean IGF-1 concentration differed significantly ($p > 0.05$). The mean IGF-1 concentration on d -14 was 164.1 ± 52.8 ng/ml in healthy ($n = 111$) and 148.8 ± 43.0 ng/ml in cows with ketosis ($n = 50$), ($p < 0.05$). In all 4 farms IGF-1 was lower on d -14 in cows developing a ketosis compared to metabolically healthy cows. The results of this study indicate that an overall threshold for IGF-1 as prognostic biomarker for ketosis is possible but depended on days relative to calving. Therefore, in future studies an algorithm should be evaluated to calculate a threshold according to days relative to calving in order to increase the practicability and prophylaxis schedules for the farm for cows at risk.

13

In vitro ageing in boar spermatozoa increases calcium release from intracellular stores by sensitizing receptor-gated channels

In-vitro-Alterung von Eberspermien erhöht die Freisetzung von Kalzium aus intrazellulären Speichern durch Sensibilisierung rezeptorgesteuerter Kanäle

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A finely tuned calcium homeostasis is essential for the fertilizing capacity of spermatozoa. Recently, we showed that a thimerosal-sensitive pathway regulates the release of calcium from intracellular stores in boar spermatozoa. The aim of this study was to test whether semen storage and subsequent thermic stress influence the sperm's response to thimerosal, a sensitizer of inositol 1,4,5-trisphosphate (IP3R) and ryanodine (RyR) receptor-gated calcium channels. Semen ($n = 3$ boars) was diluted in Beltsville Thawing Solution and stored at 17°C . After 24 h, 72 h and 120 h storage, Fluo-4/AM-loaded aliquots were incubated in a bicarbonate- and calcium-free Tyrode's solution at 38°C . Thimerosal ($100\ \mu\text{M}$) was added after 3 min and 120 min incubation and subsequent changes in the free intracellular Ca^{2+} concentration (Fluo-4) were monitored in viable spermatozoa (propidium iodide negative) using continuous flow cytometry measurements. After 3 min incubation, intracellular calcium levels increased during 210 second measurement the more the longer the semen had been stored, i.e. 1.4 fold after 24 h, 1.5 fold after 72 h, and 1.7 fold after 120 h storage, respectively ($p < 0.05$). After 120 min incubation, the intracellular calcium increase after Thimerosal addition was significantly lower in samples stored for 120 h compared to 72 h ($p < 0.05$). In conclusion, semen ageing *in vitro* leads to an initial sensitization of intracellular calcium channels in boar spermatozoa, accompanied by a later refractoriness. Both situations reflect an altered functional state with a potential impact on pre-fertilization events.

14

Blood chemistry in newborn healthy puppies – Establishment of reference values with the Samsung PT10V with the use of small blood volume

Klinische Chemie bei neugeborenen gesunden Welpen – Erstellung von Referenzwerten mit dem Samsung PT10V unter der Verwendung kleiner Blutmengen

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With the introduction of laboratory devices that use only small volumes of blood, it is possible to determine clinical chemistry parameters in newborn puppies. To properly assess the results, it requires reference values of healthy neonates. Aim of the study was the establishing of reference values for newborn puppies. Blood was taken from 46 puppies within the first 24 h of life. Four puppies were delivered naturally; 42 puppies were delivered by caesarean section. The puppies belonged

to 10 different breeds. All puppies were vital. 0.25 ml blood was taken from the V. jugularis and was examined using the Samsung PT10V device according to the instructions. Thirteen blood parameters were evaluated. Following reference values were calculated: Blood Urea Nitrogen (mmol/L): 2.1–10.4; Creatinine ($\mu\text{mol/L}$): 62–270.8; Phosphate (mmol/L): 2–3.5, total Calcium (mmol/L): 2.6–3.4; Total Protein (g/L): 39.1–55.9; Albumin (g/L): 25.1–36; Globulin (g/L): 9.1–24.9; Alanine-Aminotransferase (U/L): 20–259.4, Alkaline Phosphatase (U/L): $256 \geq 1000$, Total Bilirubin ($\mu\text{mol/L}$): 2.1–17.9; Cholesterol (mmol/L): 1.7–5.5; Triglyceride (mmol/L): 0.6–2.5; Amylase (U/L): 220.7–962.6. The established reference values show significant differences to the reference values of adults in some parameter. Alkaline Phosphatase values are known to increase significantly after colostrum intake [Center SA et al. 1991, Am J Vet Res 52: 499–504], which might led to the high discrepancies in the results. Due to this, no real upper reference value was determined. The established reference values can be used as a diagnostic tool in canine neonatology.

15

Deviation of blood chemistry parameters in puppies with diarrhea during the first five days of live

Klinisch chemische Blutparameter bei Welpen mit Diarrhö in den ersten fünf Lebenstagen

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With the possibility to determine blood chemistry parameters in puppies and the establishment of reference values [Conze et al. Blood chemistry in new born healthy puppies – Establishment of reference values with the Samsung PT10V with the use of small blood volumes. Abstract submitted], blood evaluation can be used as a diagnostic tool in sick puppies. The aim of the study was to evaluate blood chemistry parameters and their deviation from reference values in puppies with diarrhea during the first five days of life. Blood was taken from 18 puppies with diarrhea. One puppy was one-day old, eleven puppies were two days old, one puppy was three days old and five puppies were five days old. 0.25 ml blood was taken from the V. jugularis and was examined using the Samsung PT10V device according to the instructions. The range of the results were as followed: Blood Urea Nitrogen (mmol/L): 6–37; Creatinine ($\mu\text{mol/L}$): 35–150, Blood Urea Nitrogen/Creatinine: $21 \geq 100$, Phosphate (mmol/L): 1.74–3.17, Total Calcium (mmol/L): 1.95–2.83, Total Protein (g/L): 31–54; Albumin (g/L): 21–34, Globulin (g/L): 6–22; Albumin/Globulin: 1–4.7; Alanine-Aminotransferase (U/L): 11–992, Alkaline Phosphatase (U/L): $259 \geq 1000$, Total Bilirubin ($\mu\text{mol/L}$): 2.91–28.04; Cholesterol

(mmol/L): 1.76–5.78; Triglyceride (mmol/L): 0.52–4.54; Amylase (U/L): 124–444. Especially the results of Blood Urea Nitrogen, Creatinine, Alanine-Aminotransferase and Total Bilirubin show aberrations from the reference values of newborn puppies (see reference above). Blood Urea Nitrogen as well as Creatinine were lower than values of new born, although a hemoconcentration due to the diarrhea could be suspected. All of the puppies during the first two days of life had ALP values > 427 U/L including six puppies with values > 1000 U/L. Puppies of the age of five days had values between 259 and 368. The increase in the first two days might be due to the intake of colostrum. The reason for the increase of Alanine-Aminotransferase and Total Bilirubin has to be further investigated.

16

Influence of ovarian steroids on the expression of membrane progesterone receptors in the bovine placenta

Einfluss von Ovarialsteroiden auf die Progesteronrezeptoren in der Rinderplazenta

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Progesterone (P4) affects the function of cells by the nuclear P4 receptor and by the membrane P4 receptors. The expression of mRNA and protein for PGRMC1 and 2, PGRMC1 binding partner – SERBP1 (serpine 1 mRNA binding protein) and mPR α , β and γ was found in the bovine uterus and placenta, but the regulatory mechanism of this process is not explained. In this study we hypothesize that ovarian steroids may regulate of membrane P4 receptors mRNA in the bovine placenta. Endometrial and chorion slices obtained from cows in the II trimester of pregnancy were incubated for 6 and 24h ($n = 5$) with: P4 (10^{-7} ; 10^{-6} ; 10^{-5} M), E2 (10^{-10} ; 10^{-9} ; 10^{-8} M) and P4 together with E2 ($10^{-6}/10^{-9}$; $10^{-5}/10^{-8}$; $10^{-7}/10^{-10}$ M). The concentration of P4, E2 and prostaglandin (PG) E2 and F2 α and mRNA expression for studied genes were determined by EIA and Real Time PCR, respectively. In the chorion, P4 alone (each dose) decreased expression of mPR γ mRNA, but P4 jointly with E2 (in each doses) decreased expression of mRNA for PGRMC2, mPR β and mPR γ after 24h. Moreover, P4 with E2 (10^{-5} M/ 10^{-8} M) increased expression of SERBP1 mRNA after 24h. After 6h, only P4 with E2 (10^{-6} M/ 10^{-9} M) decreased PGRMC2 mRNA level in the chorion. In endometrium, P4 with E2 ($10^{-5}/10^{-8}$ M and $10^{-7}/10^{-10}$ M) decreased expression of mPR γ mRNA after 6h and 24 h respectively, but P4 with E2 ($10^{-5}/10^{-8}$ M) increased PGRMC2 mRNA expression after 24h. These results suggest that ovarian steroids can regulate the expression of PGRMC2, mPR β and mPR γ in the bovine placenta and this way they may influence on the placenta function.

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17

The hierarchy of the features of uterine myoelectrical activity during estrus cycle in sows

Die Hierarchie der Merkmale der uterinen myoelektrischen Aktivität während des Östruszyklus bei Sauen

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Certain myoelectrical activity (bursts) pattern prevalence differs at each phase of the estrus cycle in the porcine uterus. In this study, the hierarchy of bursts' features was determined according to information they carry using the 3rd cumulant's tensor and Joint Skewness Band Selection (JSBS). The electromyographic (EMG) signal was recorded in sows ($n = 8$) from uterine corpus and horn. The concentration of LH (ng/ml)/P4 (ng/ml) and estrus induction (d. –4 eCG; d. –1 hCG) was used to distinguish diestrus (d. –6/–5; LH < 1.0 ; P4 > 1.0), proestrus (d. –4/–1; LH < 1.0 ; P4 < 1.0) and estrus (d. 0/1; LH > 2.0 ; P4 < 1.0). The bursts' features for time (D: duration; P: pause; A: amplitude; RMS) and frequency (MaxP: max power; MinP: min power; DF: dominant frequency) domains were extracted. The cumulant's tensor calculated from all features was a $7 \times 7 \times 7$ array of 3rd order correlations between features. The Higher Order Singular Value Decomposition (HOSVD) of this tensor was performed in order to determine information significance and hierarchy of features. In corpus uteri and the middle part of horn hierarchy of features demonstrated the same characteristics in estrus (P $>$ Min P $>$ Max P) and slightly different in diestrus (corpus: MinP $>$ MaxP $>$ P; horn: P $>$ MinP $>$ MaxP). Only in the tip of the horn, which is suspected to act as tentative anatomical region with the strong pacemaker activity, amplitude and RMS were significant only in estrus (MinP $>$ P = A $>$ MaxP = RMS). In proestrus, the features exhibited daily changes from diestral to estral pattern. The combination of the time and frequency dependent features was meaningful in multivariate EMG signal analysis. With JSBS method we demonstrated the synchronization of uterine corpus and horn and the taking over the tip of the regulatory function in estrus.

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Characterization of the F1 generation of transgenic common marmoset monkeys (*Callithrix jacchus*)

Charakterisierung der F1-Generation transgener Weißbüschelaffen (*Callithrix jacchus*)

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Common marmosets are a valuable animal model in biomedical research, and their genetic modification (GM) may be crucial for gaining insights into complex human diseases. We previously generated our transgenic common marmosets by injection of a lentiviral construct into the subzonal space of preimplantation embryos. Mating of the founders to wildtype marmosets demonstrated their fertility and provided the F1 generation mainly born as twins and triplets. Embryos from multiple pregnancies develop placental anastomoses resulting in blood cell-chimerism. Weight of the offspring was documented weekly. At the age of 6 months, blood, skin, and skin fibroblasts were collected and checked for the presence of the transgene by PCR. Buccal swaps and hair samples were also collected from selected animals for the development of non-invasive DNA sampling. Eighteen out of 39 F1 animals were constitutively EGFP-transgenic at the genomic level, but only two of them exhibited visible fluorescence. Ten animals were chimeric and presented the transgene only in blood and skin samples but not in immune cell-depleted fibroblasts due to a blood cell-chimerism from shared placental anastomoses of twins during pregnancy. Eleven F1 animals were non-transgenic. Females were overrepresented among the transgenic offspring (8/12) and also the two animals with visible fluorescence were female. One founder generated 100% EGFP-positive animals (5/5) indicating a very efficient germline transmission. In summary, the transgene was efficiently transmitted to the F1 generation, but silenced in most cases. However, the fluorescent monkeys will be bred in order to establish a larger group of these monkeys, which may be a valuable tool for transplantation studies. Blood cell-chimerism resulted in chimeric animals, which need to be further characterized.

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Applicability of bovine hepatocytes in co-cultivation with macrophages to mimic systemic effects of embryo-maternal interaction *in vitro*

Verwendung boviner primärer Hepatozyten in Ko-Kultivierung mit Makrophagen zur Abbildung systemischer Effekte embryo-maternalen Interaktion *in vitro*

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It was previously shown that Interferon- τ (IFN τ) induces interferon stimulated gene expression (ISG) in the liver already on day 18 in pregnant dairy cows. Experiments in sheep indicated that IFN τ enters the blood circulation; however, it is not clear, whether ISGs play a biological role in embryo-maternal interaction on the systemic level. Therefore, the objective of this study was to test the applicability of a well-established hepatocyte *in vitro* model to mimic systemic effects of IFN τ . Liver of Holstein Friesian cows was used to gather primary hepatocytes and macrophages for defined mono- and indirect co-cultivation in a sandwich-culture system. Recombinant IFN τ and IFN α was added (0.6 ng/ml resp. per well with 500.000 hepatocytes, 0.06 ng/ml resp. per well with 500.000 hepatocytes) on day three and four for 6 h. qRT-PCR was performed to measure the relative expression of ISGs (ISG-15, MX-1, MX-2 and OAS-1) in the hepatocytes after removal of macrophages situated in the second collagen layer of the co-culture system. The expression of all measured ISGs was higher after the stimulation with IFN τ ($p < 0.05$) in mono- and co-cultured hepatocytes. In the co-culture system, the expression of ISGs was lower compared to hepatocyte cultured in the monoculture system before day three of cultivation. These results indicate that the described model can be used to study type-I IFN signaling on the hepatocyte-macrophage compartment in future studies in detail with regard to influenced metabolic and immunologic adaptations of the maternal liver due to the embryo signal.

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Comparison between a calving predictive system and a routine prepartal examination in Holstein-Friesian heifers and cows

Vergleich zwischen einem abkalbungsprädiktiven System und einer routinemäßigen präpartalen Untersuchung bei Holstein-Friesian-Färsen und -Kühen

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The aim of this work was to examine the efficiency of the calving predictive system Moocall compared with a routine clinical examination at a cattle farm in Lower Saxony, Germany. Moocall is a device based on an accelerometer system detecting tail raising and overactivity. Placed at the tail of cattle 4 days prior to the predicted calving date (pcd), the Moocall sensor produces alarms in form of short message services (SMS) in case of overactivity. Thirty-eight animals were enrolled (Moocall group [n = 24]: 16 heifers and 8 cows; control group [n = 14]: 9 heifers and 5 cows). Clinical examinations were carried out every 6h during 7 days before pcd. The examined traits were changes in pelvic ligament relaxation (PLR), teat filling (TF), vaginal secretion (VS), and flexibility of the tail tip (FTT). Moocall sent 6.1 ± 3.9 SMS (max: 14; min: 2) in heifers and 6.5 ± 4.3 SMS (max: 13; min: 2) in cows. There were no differences in Moocall activity related to SMS between heifers and cows ($p > 0.05$). The time lag between the first SMS of Moocall and the clinical recognition of labor was 36.9 ± 32.1 h (max: 95h; min: 1h) for heifers and 61.1 ± 66.5 h (max: 177h; min: 0h) for cows ($p > 0.05$). The positive predictive value (PPV) of Moocall 24h prior to calving was 0.4 in heifers and 0.5 in cows. The traits PLR, TF, VS and FTT changed 24h prior to calving and their PPV was 0.6 (heifers: 0.5; cows: 0.8), 0.2 (heifers: 0.2; cows: 0.4), 0.3 (heifers: 0.4; cows: 0.3) and 0.2 (heifers: 0.2; cows: 0.1), respectively. Further calculations are necessary to determine the efficiency of Moocall.

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Characterization of macrophages in the bovine placenta at term

Charakterisierung von Makrophagen der Rinderplazenta zum Geburtstermin

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At the fetomaternal interface, macrophages are involved in various functions during all stages of pregnancy. The functions of these cells are highly dependent on the local tissue microenvironment and properly and timely regulated macrophage polarization has been considered a crucial factor of a successful pregnancy. The aim of this study is to evaluate the expression of three cytokines, which are either anti-inflammatory (interleukin (IL) 10) or pro-inflammatory (IL12A, IL12B), and the pro-inflammatory transcription factor Interferon regulatory factor 5 (IRF5) in bovine term placenta. Placentomes were obtained at caesarian sections (n = 3) and manually separated into fetal (cotyledons) and maternal (caruncle) parts. Expression levels were studied by semi-quantitative Real Time RT-PCR. Using identical calibration values for all the four transcripts a relatively high expression of IL10 was detected in the cotyledons, while IL12A, IL12B, and IRF5 were significantly higher expressed in the caruncles (p = 0.03, t-test). These results suggest that in the maternal caruncle macrophages are more numerous and are predominantly of M1 type, while the fewer fetal cotyledonary macrophages appear to be M2-polarized. The predominance in M1-polarized macrophages over the M2 subset has been considered as an inflammatory event. Furthermore, the relatively high expression of the inflammatory interleukins in the maternal part might also point towards pro-inflammatory events during parturition. Accordingly, such an inflammatory event at term has been previously proposed to be involved in promoting the contraction of the uterus, expulsion of the calf and the fetal membranes, and uterine involution.

Effects of different concentrations of lecithin on maturation of camel oocytes vitrified in various vitrification solutions at the germinal vesicle (GV) stage

Auswirkungen verschiedener Konzentrationen von Lecithin auf die Reifung von Kameloozyten, die in verschiedenen Vitrifikationslösungen im Germinalvesikelstadium vitrifiziert wurden

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The work aimed to elucidate the effects of different concentrations of lecithin on maturation of vitrified camel oocytes. Dromedary ovaries were collected from an abattoir in Cairo within 15–30 minutes post slaughter and were transported in sterile 0.95% saline solution within 1h to the IVF lab. All follicles (2–8 mm) were punctured and under a stereomicroscope cumulus oocyte complexes (COCs) were recovered, evaluated and then distributed into 4 groups. All groups (three replicates) were exposed to holding media (HM) for 1 min, and then transferred to an equilibration solution for 4 min, and subsequently COCs were transferred to a vitrification solution. The vitrification solution in group I was HM+ EG 40% (v/v) + 0.01, 0.05 or 0.1 mg/ml lecithin for 30 sec in a 0.25 ml straw. The vitrification solution in group II was HM+ DMSO 40% (v/v) + lecithin as in group I. The vitrification solution in group III was HM+ EG 40% (v/v) + DMSO 40% (v/v) + lecithin as in group I and group IV was left in the medium without lecithin as control. To warm up vitrified oocytes, straws were thawed at 37 °C (water bath) for 1 min, then transferred to 1 ml warming solution WS1 (20 ml BM+ 2 ml EG+ 7.56 g trehalose), WS2 (20 ml BM+ 3.79 g trehalose) and WS3 (20 ml BM+ 1.89 g trehalose) at room temperature for 3 min each. The maturation medium was TCM-199 with 10% FCS, 10 µg LH, 5 µg FSH, 1 µg estradiol and 50 µg sulfate per ml. The pH of the media was adjusted to 7.4 and osmolarity 295–310 mosm. After 42hr of *in vitro* culture post thaw, COCs were washed and examined for the maturation rate. The classification was: fully expanded, partially expanded and not expanded. After evaluation cumulus cells were mechanically denuded by gentle pipetting. Data were analyzed by ANOVA. P < 0.05 was considered to be significant. The results showed that vitrification of camel COC with 0.05 mg/ml

lecithin with EG 40% + DMSO 40% and EG 40% resulted in significantly higher maturation rates (72.5% and 58.9%, respectively) than with 0.05 mg/ml lecithin DMSO 40% and 46.7%. In conclusion, vitrification of immature camel oocytes by the addition of 0.05 mg/ml lecithin to the solution is a suitable method to limit drawbacks of vitrification.

Serological and molecular detection of Brucella infection in aborted ewes in Upper Egypt

Serologischer und molekularer Nachweis der Brucella-Infektion bei abortierenden Mutterschafen in Oberägypten

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Generally, abortion cause enormous economic losses in livestock animals. The most important pathogens involved in the abortion of ewes are *Brucella melitensis*, *Campylobacter fetus*, *Salmonella abortusovis*, and *Chlamydia abortus*. Five flocks of mixed breed ewes showed unexplained high percentage of abortions (33.3%) and the etiology of abortions was not well understood. Therefore, the objective of the current study was to estimate the cause of late abortion in that flocks in Assiut governorate (Upper Egypt). A total number of 94 recently aborted ewes and 47 aborted fetuses with related placenta were examined and correlate its possible association with *Brucella melitensis* the most important abortive diseases in sheep. Serum samples were tested by Rose Bengal and ELISA for brucellosis. The infected tissues and serum were used in polymerase chain reactions (PCR) for detection of DNA of *Brucella* spp. The results revealed that, serological tests were positive in (21.28%) of examined cases. *Brucella* spp. DNA was detected in (34.04%) of serum samples, while was reported in (25.5%) of tissue samples. In conclusion, there was an association between *Brucella* infection and abortion in sheep in Assiut governorate. PCR could be an accurate method for diagnosis of Brucellosis, thereby could control the infectious diseases in sheep and minimize reproductive losses.

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Comparison of three methods for the quantification of progesterone blood concentrations in bitches

Vergleich von drei Methoden zur Bestimmung von Progesteron im Blut von Hündinnen

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The aim of this study was to compare a new commercial system (Speed ProgesteroneTM, Virbac) for the measurement of progesterone concentration in the serum of bitches with an already established commercial method (Mini-Vidas, Biomerieux) and a well validated inhouse radioimmunoassay (RIA) as a reference method. A total number of 45 client-owned clinically healthy bitches of different breeds were included. All dogs were presented for reproductive examinations at the Clinic. Blood samples collected for estrous cycles studies and the prediction of the due time for mating. Fifty-two blood samples collected from the Vena *Vena cephalica antebrachii* or Vena *saphena lateralis*, centrifuged at 3000 rpm for 10 min and the harvested serum divided into three aliquots. One measured immediately using Speed ProgesteroneTM. The other two aliquots were stored at -20 °C till analysis using RIA and enzyme-linked fluorescence assay (Mini-Vidas). The results revealed high positive correlations between the two non-radioactive methods (and the RIA, respectively (Mini-Vidas vs. RIA: $r = 0.99$; Speed ProgesteroneTM vs. RIA: $r = 0.94$). Moreover, the results also showed a strong linear correlation between Mini-Vidas[®] and Speed ProgesteroneTM, Virbac[®] ($r = 0.91$). In conclusion, when applied for the determination in peripheral blood of bitches the Speed ProgesteroneTM enables rapid and reliable measurements of progesterone highly consistent with results from Mini-Vidas and RIA.

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Ensuring high quality of feline ovarian follicle RNA during isolation

Sicherung hoher RNA-Qualität während der Isolierung von Ovarfollikeln der Hauskatze

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In vitro folliculogenesis is an invaluable tool for studying follicular growth and oocyte development in mammals. Gene expression profiles of early follicles are planned to be investigated to identify developmental bio-

markers. To achieve this, high quality RNA must be obtained for downstream analysis. In this study, we first established a protocol to isolate primordial < 45 µm (PrF), primary 55–70 µm (PF), and secondary 85–110 µm (SF) ovarian follicles from domestic cat based on size in µm and presence/absence of a zona pellucida. Secondly, based on PrFs different storage solutions were tested, phosphate-buffered saline supplemented with bovine serum albumin (PBS-BSA, MP-Bio and Sigma Life Science), RiboLockTM RNase Inhibitor (RiboLock, Fermentas Life Sciences), or Lysis Buffer (LB, Macherey-Nagel). Follicles were collected in respective solutions, snap-frozen in liquid nitrogen and stored at -80 °C until RNA extractions were prepared. To determine high sample quality, isolated RNA was measured utilizing a TapeStation with a High Sensitivity RNA Kit. Quality was tested and determined as “good” when RNA integrity number equivalent (RINe) values and 28S:18S rRNA ratios were ≥ 6 and ≥ 2.0 , respectively. Storage in PBS-BSA led to low results (3.2 ± 2.8 and 0.2 ± 0.4) whereas RiboLock (4.8 ± 1.7 and 0.7 ± 1.3) and LB (6.7 ± 0.9 and 2 ± 0.4) values were improved. Overall, LB demonstrated the highest and most consistent values in maintaining “good” PrF RNA quality. To conclude, the protocol for ovarian follicle isolation from domestic cat has been optimized to storage in LB for further downstream gene expression analysis.

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Characterization of dogs with ovarian cysts who fail conservative therapy

Charakterisierung von Hunden mit Ovarialzysten, bei denen die konservative Therapie versagt hat

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Ovarian cysts (OCs) have a high clinical relevance in bitches. They can cause a variety of gynecological disorders, e.g. prolonged estrus and uteropathies. While therapy of first choice is ovariohysterectomy (OHE), there are a number of negative side effects associated with the removal of the ovaries. Therefore, a conservative therapy could provide an acceptable alternative. In this study, we investigated five bitches with OCs, where multiple conservative treatment with human chorionic gonadotropin and/or buserelin failed consistently. We characterized these dogs regarding endocrine status in peripheral blood, histopathological classification of OCs, and additional macroscopic findings during OHE. We predicted that conservative therapy non-

responding individuals have an increased E2- and P4-concentration in the peripheral blood. Results showed, that E2 was above basal-level (>15 pg/ml) in three bitches, P4 in all cases (>1.5 ng/ml). Histopathological and partly endocrinological classification revealed Follicular cysts (FCs) in all cases, and Lutein cysts (LCs) in three animals. Here, clinical symptoms of FCs and LCs overlap. Bitches showed an estrogen-characterized clinical picture. Physiological *Corpora lutea* were macroscopically detected in two cases. The endometrium showed a glandular cystic hyperplasia in three patients. Our study demonstrates that failure of conservative therapy in bitches with OCs, may result from simultaneous occurrence of hormonal active LCs besides FCs. Subsequent studies should enroll more patients to confirm our initial findings.

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Expression of intestinal Granulocyte-Colony Stimulating Factor Receptor (GCSF-R) is induced by colostrum intake in neonate calves

Expression des intestinalen Granulozyten-Kolonie-stimulierenden Faktorrezeptors (GCSF-R) wird durch die Aufnahme von Kolostrum in neugeborenen Kälbern induziert

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Worldwide, high mortality rates of calves due to infectious diseases during their first weeks of life is a major economic and welfare concern in dairy industry. Adequate colostrum supply as well as an intact immune system including existence and functionality of polymorphonuclear neutrophils (PMNs) are essential for survival of the affected calves. Production and activation of PMNs is regulated via cytokines such as Granulocyte-Colony Stimulating Factor (GCSF), which in turn is mediated via the GCSF-Receptor (GCSF-R). The objective of this study was to test, whether colostrum intake regulates the expression of intestinal GCSF-R in the mucosa of abomasum, jejunum and caecum. Calves (total $n = 11$) enrolled in this study received either no colostrum or milk (“no-Col”, $n = 3$), only colostrum (“Col”, $n = 2$) or colostrum and milk or milk replacer (“Col+”, $n = 6$). Mucosa of abomasum, jejunum and caecum of each calf was dissected within 1 hour post mortem and stored in RNAlater at -80°C until analysis of GCSF-R gene expression via RT-qPCR. Within each compartment expression of GCSF-R was higher in Col and Col+ compared to noCol ($p < 0.01$). In animals without colostrum intake, gene ex-

pression was comparable between the three compartments, whereas GCSF-R expression in groups Col and Col+ was significantly lower in abomasum compared to jejunum and caecum ($p < 0.05$). In conclusion, expression of GCSF-R is induced by colostrum intake in neonate calves. The potential impact of GCSF-R expression on immune function deserves further attention.

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Cellular mechanisms of canine species-specific decidualization: *In vitro* effects on remodelling processes, and roles of PGE2 and progesterone

Dezidualisierung beim Hund im Zellkulturmodell: Einfluss auf Umbauprozesse und die Rollen von PGE2 und Progesteron

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Recently, we established an *in vitro* model with immortalized dog uterine stromal (DUS) cells for investigations of canine-specific decidualization. Their capability to decidualize was assessed with our cAMP-mediated protocol. We also showed the basic decidualization capability of PGE2, revealing increased levels of PRLR, PGR, IGF1, PTGES and EP4 receptor. Here, in addition to our previous findings, we show that these effects are mediated through both cAMP-mediating PGE2 receptors (EP2/EP4). Their functional inhibition significantly suppressed both PRLR and PGR mRNA in DUS cells. We also assessed the effects of cAMP and PGE2 on selected extracellular matrix components and Cx43 in DUS cells, and were able to show that cAMP, but not PGE2, increases COL4, ECM1 and Cx43 protein levels. By addressing the role of progesterone (P4) during *in vitro* decidualization, we show that P4 upregulates PRLR and PGR in DUS cells, but these effects are not potentiated by PGE2; both P4 and PGE2 appear to act independently. Interestingly, P4 does not affect IGF1, but suppresses IGF2 mRNA, also in the presence of PGE2. Similarly, P4 does not affect PTGES, but suppresses the expression of EP4, also in the presence of PGE2. Consequently, based on our results, a mutually regulatory loop between PGE2 and P4 is suggested during canine *in vitro* decidualization: whereas P4 may be involved in regulating PGE2-mediated decidualization, PGE2 ameliorates PGR levels.

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Influence of the administration of meloxicam on the development of clinical parameters in neonatal calves after dystocia

Einfluss der Gabe von Meloxicam auf die Entwicklung klinischer Parameter bei Kälbern *post natum* nach Schweregeburten

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The time around the birth is a disturbance-sensitive phase in the life of a dairy cow and her calf. Especially after dystocia, calves show an increased incidence of diseases and a consequently reduction in performance. One cause is pain during delivery, which reduces colostrum intake and weakens the immune system. In the present study, the following question should be answered: Is there an influence of administration of the non-steroidal anti-inflammatory meloxicam in newborn calves from dystocia on clinical parameters and frequency of diseases during the first ten days of life? The study was carried out on 50 Holstein-Friesian calves from four dairy cattle farms. The allocation of the animals into two groups was randomized. The following criteria lead to the inclusion in the data collection: Manual extraction or operative development of a calf due to a dystocia. Exclusion criteria of the calves were malformations, rectal body temperature ≥ 39.5 °C and recumbency. The animals in group I were given a subcutaneous injection of 0.5 mg meloxicam (Metacam® 20 mg/ml, Boehringer Ingelheim)/kg body weight 2–8 hours after birth. The animals of group II received a control substance (Aminin®, Merial) with the same volume. The clinical examination of the newborn calves occurs on the first and the tenth day of life. Information about the days in between was provided by questioning the farmer. As a result, in the present study no influence of meloxicam on the calves was observed. It has to be determined in further studies, whether and which effects can be achieved for calves *post natum* with an increase in the frequency of meloxicam administration.

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Inhibition of ovulation and formation of cystic ovarian follicles in cattle by using an intrafollicular injection of non-steroidal anti-inflammatory drugs

Inhibierung der Ovulation und Bildung ovarieller Zysten bei Rindern durch den Gebrauch einer intrafollikulären Injektion von nicht-steroidalen Antiphlogistika

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The aim of this study was to establish a model to inhibit the ovulation and to form cystic ovarian follicles (COF) in German Holstein cattle. Due to the knowledge that the upregulation of cyclooxygenase-2 (COX-2) is the key step to ovulate, specific COX-2 (meloxicam and NS-398) and unspecific COX-1/-2-inhibitors (indomethacin and flunixin-meglumine) were used to interrupt the follicular function. Ovulation was synchronized with PGF_{2α} followed by GnRH administration 54 h later. Ultrasound-guided transvaginal intrafollicular injections with 0.2 ml of the different inhibitors in decreasing concentrations as well as vehicle-controls were performed 16h after GnRH administration. Further ultrasonographic examinations documented ovulation or development of a COF. Indomethacin (INDO) and flunixin inhibited the ovulation already in low concentrations (35 µM, 169 µM, respectively) and the follicles formed COFs with a diameter of 36.9 ± 4.5 mm (mean $\pm$ SE) on day 11. Whereas the specific inhibitor meloxicam blocked the ovulation in a high concentration of 1725 µM while lower concentrations, NS-398 and controls failed. Additional follicles, treated with 35 µM INDO or 60 µM NS-398 were aspirated 5 h after injection, as well as control follicles 21 h after GnRH treatment. Interestingly, INDO and NS-398 showed comparable bioactivity as indicated by a significant decrease of PGE2 compared to controls (428 ± 115 pg/ml, 337 ± 204 pg/ml, 4290 ± 833 pg/ml, respectively). In conclusion, these findings give the possibility for further investigations and characterizations of COFs in a bovine system.

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Endometrial gene expression of pro-inflammatory cytokines is reduced 7 days after induction of persistent endometritis in susceptible mares

Die endometriale Genexpression pro-inflammatorischer Zytokine ist 7 Tage nach der Induktion einer persistierenden Endometritis bei empfänglichen Stuten reduziert

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The aim of the study was to evaluate the gene expression of inflammatory cytokines (IL1 β , IL6, IL8, IL10, TNF α), CASP3, and COX2 in endometrial tissue of mares classified as resistant or susceptible to persistent endometritis based on their uterine inflammatory response to infusion of killed semen. Ten mares were examined by transrectal ultrasonography daily during estrus and ovulation (Day0) was induced by application (iv) of 1500IU hCG at the first time when a follicular diameter of at least 35mm was observed. 24h after application of hCG, 4mL of killed semen or physiologic saline was instilled into the uterine corpus. Endometrial biopsies were obtained 24h before, and 2 and 7d after infusion, and gene expression was determined by qRT-PCR. All mares were short cycled on Day8 and one estrous cycle without treatment was awaited before the trial was repeated using the opposite treatment (semen vs saline). Intrauterine fluid retention (diameter > 20 mm for at least 3d) was observed after infusion of killed semen in five mares (susceptible). There was no treatment effect (semen vs saline; $p > 0.05$) on gene expression; therefore, averages of semen and saline cycles were used. In resistant mares, expression of TNF α increased ($p = 0.03$) between 24h before and 48h after treatment, whereas expression of interleukins, CASP3, and COX2 remained unchanged ($p > 0.05$). In susceptible mares, gene expression did not differ ($p > 0.05$) between 24h before and 48h after treatment but expression of IL1 β , IL6, IL8, and TNF α decreased ($p < 0.05$) between 2 and 7d after treatment, probably declining the release of PGF_{2 α} and uterine contractility.

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The thermographic imaging of pregnant mares during mid-gestation

Die thermografische Darstellung trächtiger Stuten während der mittleren Trächtigkeit

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Thermography is highly sensitive in assessing superficial temperature changes that may indicate physiological responses during development of pregnancy. In recent studies, it has been reported that the flank thermography is a good utility for confirming mid-to-late gestation pregnancy in mares and the external temperature of the peri-vulvar area may be used alone for pregnancy determination. The major point of this study was to compare the flank and the vulva thermography during mid-gestation pregnancy (3rd to 9th mo) in mares. Thermographic images of the flank (T_f) and the vulva (T_v) were obtained from the group of 40 Konik Polski mares (4–12 years) housed under the same environmental conditions. The pregnancy was confirmed by transrectal ultrasonography at day 24 after mating and one week before imaging. Thermographic images were taken indoor, with ambient temperature 19.5 °C, using an infrared radiation camera with an emissivity (e) between 0.99 and 1.0 (FLIR Thermo CAM E25, FLIR Systems Brasil, Brazil). The mean TV increased significantly ($p < 0.01$) from the 3rd mo (30.3 ± 0.2 °C) to constant values between the 4th and the 8th mo (33.0 ± 0.4 °C) and afterward decreased back to the baseline (30.8 ± 0.3 °C) in the 9th mo. The mean TF remained stable until the 7th mo (26.0 ± 0.5 °C), after which it significantly increased ($p < 0.05$) in the 8th and 9th mo (29.0 ± 0.5 °C). During mid-gestation pregnancy an increase of blood efflux in the peri-vulvar area may depend upon the level of estrogens secreted by the fetoplacental unit. The external temperature of the vulva may be useful for the presumption of pregnancy in early mid-gestation, whereas the flank imaging was more significant in late mid-gestation.

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Role of adhesion, slime and bio-film formation by *Staphylococcus aureus* isolated from milk of the cows from Nord-East Region of Poland

Die Rolle von Adhäsion, Schleim und Biofilmbildung bei *Staphylococcus aureus*, isoliert aus Milch von Kühen aus der Nord-Ost-Region Polens

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Staphylococcus aureus is an important etiological factor of mastitis in cows. The ability of *S. aureus* to adhere to the mammary gland epithelium is important in the pathogenicity of staphylococci, enables tissue colonization and development of infection. Biofilm formation helps *S. aureus* to survive environmental within the host and is considered to lowering susceptibility to antibiotics. The adhesion capacity, formation of slime and biofilm of *S. aureus* strains isolated from cow's milk with clinical and subclinical mastitis from the East-Nord Poland region and sensitivity to selected antibiotics were tested. Slime formation was investigated with the Freeman method on Congo Red Agar (CRA). Biofilms were detected on the Microtitre Plate. The ability to adhesion was tested using Freeman's method with small modification. Among 92 tested strains, 31 (33.7%) grew on Congo Red Agar (CRA) in the form of black smooth colonies indicating slime production. Fifty-two (56.5%) strains were capable to produce biofilm. The adherence of the *S. aureus* strains to mammary epithelial cells was significant and the average number of bacteria adhering to a single epithelial cell was $7,839 \pm 0,751$. Biofilm-production lowered sensitivity to antibiotics. Fifty-five-point-two percentage of slime production and 69% biofilm formation were formed by *S. aureus* strains belonging to the D genotype (after genotyping by ADSRRS-fingerprinting) which were predominant in this region. Greater adhesion capability was shown by strains of this genotype and were equal $10.46 (\pm 0.5)$. *S. aureus* strains with adherence, slime and biofilm production capability are the problem in that region which can be a reason of increased isolation in that region causing troubles during therapy.

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An application of semiautomatic quantification of histological biopsy in assessment of the progesterone effect on cows endometrium

Anwendung der halbautomatischen Quantifizierung histologischer Biopsien für die Beurteilung der Progesteron-Wirkung auf das Endometrium von Kühen

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Endometrial biopsy specimens have been used widely for evaluating the bovine uterus. During estrus cycle, histological parameters include the height of the surface epithelium (HSE), the height of the gland epithelium (HGE), glands' features including surface (GS) and diameter (GD) and the properties of the stroma. This study determined an application of semiautomatic histometry to evaluate changes in the bovine endometrium under the influence of exogenous progesterone. Uterine biopsies were collected over the anoestrus before (b1) intravaginal progesterone administration (progesterone insert, 1.55 g, 7 days) and after 7 days (b2) from HF non-pregnant lactating cows (n = 20). Samples were stained according to HE staining protocol and examined histometrically with semiautomatic quantitative analysis (Olimpus BX53 with advanced cellSens Standard image analysis software). Glands were divided into: low-developed (LG), high-developed with a lumen (HLG) and high-developed with secretion in the gland lumen (HSG). The number of each glands, HGE, GS and GD and HGE were counted. The surface epithelium was significantly higher ($p < 0.05$) after treatment (b1: $18.96 \mu\text{m} \pm 2.72$; b2: $21.65 \mu\text{m} \pm 3.49$). The morphology of LG changed ($p < 0.01$) (b1: HGE $20.84 \mu\text{m} \pm 3.08$; GS $4315.40 \mu\text{m}^2 \pm 115.64$; GD: $75.65 \mu\text{m} \pm 29.82$; b2: HGE $18.77 \mu\text{m} \pm 2.58$; GS $3814.94 \mu\text{m}^2 \pm 858.99$; GD: $82.52 \mu\text{m} \pm 20.98$) with no differences in number, whereas the number of HLG decreased ($p < 0.05$) (b1: 7.57 ± 3.21 ; b2: 4.01 ± 3.02) with no differences in HLG morphology. The features of HSG did not differ ($p > 0.05$) after the treatment. Basing on our findings we may conclude, that the semiautomatic quantification of histological biopsy is a useful tool and may be applied in fast and specific cows biopsy evaluation.

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The comparison of peripheral concentration of reproductive hormones after induction of estrus cycle using eCG with hCG or GnRH

Der Vergleich der peripheren Konzentration von Fortpflanzungshormonen nach Induktion des Östruszyklus unter Verwendung von eCG mit hCG oder GnRH

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Induction of ovulation for controlled breeding is available for use around the world, and conditions for practical application appear promising. Many commercially available hormones are competent to synchronize ovulation after induction of follicular growth with FSH or their natural agonist eCG. The most effective ones are porcine LH or their natural agonist hCG and natural GnRH or their synthetic analogs, such as Buserelin and Goserelin. The aim of the study was to evidence the dynamic of blood hormone concentrations (LH, P_4) according to the onset of standing heat and eCG/hCG/GnRH administration in 12 pigs. The blood was sampling every 4 h from 44 h before and 44 h after the first signs of standing estrus/hCG/GnRH administration and the concentration of LH and P_4 was assayed with RIA method. The peripheral P_4 concentration was at the same level during spontaneous (mean 0.15 ± 0.04 ng/ml) and both hCG (mean 0.55 ± 0.17 ng/ml) and GnRH- (mean 0.58 ± 0.48 ng/ml-) dependent induced estrus. The LH (> 2.0 ng/ml) surge occurred earlier (4 h) and lasted longer (20 h) after GnRH-dependent induction and occurred earlier (4 h) and lasted the longest (28 h) after hCG-dependent induction in comparison to spontaneous estrus (16 h). Moreover, the LH concentration during LH surge was significantly higher ($p < 0.05$) after GnRH stimulation (7.01 ± 1.94 ng/ml) and the highest after hCG (14.11 ± 9.96 ng/ml) as opposite to spontaneous LH surge (4.97 ± 2.21 ng/ml). Recent studies testing use of hCG or GnRH in gilts and sows have reported that when given at the onset of estrus, LH was increased as was ovulation rate. Therefore, the use of natural LH agonist, acting at the ovaries level, seems to be the most favorable of commercial protocols of estrus induction.

Genome-wide transcriptional effects in the early corpus luteum (CL) of the dog after *in vivo* inhibition of prostaglandin synthesis

Genomweite Transkriptionseffekte im frühen Corpus luteum des Hundes nach der In-vivo-Hemmung der Prostaglandinsynthese

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Prostaglandins (PGs), mainly PGE₂, are considered to be among the main regulators of the early, gonadotropin-independent, canine CL. This was further substantiated in experiments *in vivo* in which inhibition of PTGS2/COX2 affected luteal development, decreased intra-luteal PGE₂ and lowered circulating levels of progesterone. Here, we investigated the effects of COX2 inhibition on the early canine CL by using a transcriptomic approach by RNAseq. RNA was isolated from CL of bitches treated either with firocoxib (COX2 specific inhibitor) or with a placebo, for 5, 10, 20 and 30 days (n = 3–4 each) after ovulation. Analysis of sequencing results was done with SUSHI (framework developed by FGCZ) and IPA®. Time-dependent effects were assessed in control animals allowing for additional validation of results. Then, treatment-dependent effects were assessed. As expected, when compared with early CL (days 5, 10), in fully developed CL (days 20, 30) upregulated differently expressed genes (DEG, $p < 0.01$, FDR < 0.1) and functional terms were associated with steroidogenesis, while down-regulated transcripts related to proliferation and inflammation. Interestingly, the effects of treatment were luteal developmental stage dependent: higher numbers of DEG (close to 2000) were affected on days 20 and 30. The overrepresented functional terms and upregulated genes indicated decreased activation of cellular functions, e.g., cell adhesion and signalling, and negative regulation of steroidogenesis in fully developed CLs in response to firocoxib during their transition towards gonadotropic dependence.

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Influence of high maturation temperatures on the developmental competence and the abundance of Sirtuins in bovine oocytes of two different breeds *in vitro*

Einfluss hoher Reifungstemperaturen auf die Entwicklungskompetenz und die Konzentration von Sirtuinen in Rindereizellen zweier verschiedener Rassen *in vitro*

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Increased environmental temperatures represent an increasing problem worldwide, also in livestock husbandry in terms of animal health and performance. It can be assumed that different breeds and subspecies bred under different climatic conditions have developed different adaptation strategies to the stress caused by high temperatures. In this context, the regulation of the intracellular energy metabolism should be of importance. Sirtuins are central switching points in energy metabolism. Therefore we investigated the influence of different temperatures (37 °C, 38.5 °C, 40 °C) in IVM on the developmental competence (IVF/IVC) as well as the abundance (western blot & confocal laser scanning microscopy) of sirtuins in two breeds differently adapted to high environmental temperatures (Nelore [*bos taurus indicus*] & Simmental [*bos taurus taurus*]). The oocytes were obtained from slaughterhouse material and pre-selected by brilliant cresyl blue staining (BCB+ & BCB-). The different maturation temperatures had no influence on the proportion of oocytes with excluded polar bodies after IVM. At 38.5 °C matured oocytes presented the highest blastocyst rate after IVF/IVC with an average of 37% (Simmental) and 23% (Nelore). Oocytes matured at elevated temperature showed the lowest blastocyst rate (Simmental: 22%, Nelore: 7%). In general, the blastocyst rates were higher for BCB+ oocytes and the differences between the groups were much larger (37 °C: 38%; 38.5 °C: 52%; 40 °C: 24%). The Sirt1 and Sirt2 protein was increased at elevated and reduced (Sirt1 only) maturation temperature in cumulus cells and oocytes as well. These differences were more distinct in Nelore, which may indicate a stronger response to temperature increases.

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Expression of mRNA, protein and localisation of coactivators and corepressor of progesterone receptor and histone acetyltransferase and histone deacetylase activities in the reproductive system of cows

Expression von mRNA, Protein und Lokalisation von Koaktivatoren und Korepressoren von Progesteronrezeptor-, Histon-Acetyltransferase- und Histon-Deacetylase-Aktivitäten im Reproduktionssystem von Kühen

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The last step in progesterone receptor (PGR) activation is attachment of coregulators containing coactivators and corepressors, which modulate receptor function. Coactivators demonstrate an internal histone acetyltransferase (HAT) activity, whereas corepressors show histone deacetylase (HDAC) activity. Changes in mRNA and protein levels of coregulators as well as HAT and HDAC activity can affect on the PGR function of corpus luteum (CL) and uterus thereby regulate the action of progesterone (P4) on target cells. As a research material luteal and endometrial tissues from days 2–5, 6–10, 11–16 and 17–20 of the estrous cycle were used (n = 5). The mRNA expression of P300, CREB, SRC-1 and NCOR-2 was determined by means of Real Time PCR, the proteins level by using western blot whereas cellular localisation by means of immunohistochemistry. Activities of HAT and HDAC were determined using commercial kits. The highest expression of mRNA for all of the studied coregulators were found on 6–16 days in CL, while on 2–16 days in the uterus. Their protein products were the highest on day 2–5 and decreased in the following days in both tissues. Activity of HAT and HDAC were the highest on days 6–10 in CL and 2–10 in endometrium as compared to other days, respectively. Localization of all tested coregulatory proteins has been demonstrated in nuclei of luteal and endometrial cells. It is suggested that P4 can regulate CL function via the expression of coregulators, modulation of HAT and HDAC activity and coregulators can compete for a binding site located in the PGR receptor.

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Effect of feeding different amounts of milk replacer on the granulosa cell count in small follicles in German Holstein calves

Einfluss der Fütterung von unterschiedlichen Mengen eines Milchaustauschers während der Aufzucht auf die Anzahl von Granulosazellen kleiner Follikel bei deutschen Holstein-Kälbern

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The amount of fed milk or milk replacer during rearing is known to influence the onset of puberty and the growth of reproductive organs in cattle. The aim of this study was to investigate the effect of different amounts of milk replacer on the number of small follicles (< 6 mm) and the amount of recovered granulosa cells (GC). Two groups of each 11 calves received either 10% (Group A) of bodyweight milk replacer per day or 20% (Group B) during their first 12 weeks. During the last 2 weeks (A) and the last 4 weeks (B) the amounts were gradually decreased. After weaning both groups were fed the same mixed ration *ad libitum*. The calves were slaughtered at 8 month of age and the ovaries were collected. All follicles (> 6 mm) were aspirated and the number of live GC was counted with the trypan blue exclusion method for each calf. Although the animals of group B were significantly heavier (A: 279.8 kg; B: 312.5 kg; p = 0.01) the proportion of animals having a corpus luteum was not different between groups (A: 3/11; B: 6/11; p = 0.39). The results showed that the mean number ($\pm$ sd) of antral follicles (A: 37.91 ± 25.12 , B: 43.91 ± 34.09 , p = 0.64) and the number of recovered GC (A: $5.34 \times 10^5 \pm 2.17 \times 10^5$, B: $14.8 \times 10^5 \pm 14.57 \times 10^5$, p = 0.24) were statistically not different between the two groups. Considering the number of follicles compared to the number of GC, no significant correlation for group A (r = 0.23; p = 0.49) was observed, whereas for group B (r = 0.71; p = 0.01) a correlation between the number of GC and the number of follicles exists. The results indicate that the plane of milk replacer feeding in early life may have an effect on later GC proliferation.

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The differentiation ability of mesenchymal stem cells (MSC) after transplantation into the uterine cervix in swine

Die Differenzierungsfähigkeit von mesenchymalen Stammzellen (MSZ) nach Implantation in den Gebärmutterhals beim Schwein

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MSC seem to be a promising source for cell-based treatment in regenerative medicine. We hypothesize MSC implantation into cervix may improve its function, so we conducted our study on pigs as a referential experimental model. The aim was to evaluate the MSC viability at the places of transplantation and their capability to differentiate into smooth muscle cells. In a first step, bone marrow was gathered from the head of the humerus from six gilts. MSC were isolated and cultivated *in vitro* during five weeks. Thereafter, laparotomy was performed under general anesthesia to expose the uterine cervix. Approximately 30 million mesenchymal stem cells were transplanted into the muscle layer of the cervix in six injections. Directly before implantation the cells were harvested and labeled with DID deep red fluorescent dye. The uterus was positioned back in the abdominal cavity and the laparotomy was closed routinely. Five weeks later the gilts was neutered using similar surgical procedure to obtain the material for further examinations. Cervices were sectioned into 0.5 cm thick sections for imaging in Bruker infrared imager in order to identify areas containing implanted cells. Positive thick sections were cryosectioned into 5 µm sections and stained for smooth muscle actin. High magnification imaging revealed red-labeled cells retaining normal morphology. The cells were also smooth muscle actin negative. Five weeks after the implantation the live MSC were found in all transplantation places. The cells were not successfully differentiated into myogenic lineages, as demonstrated by immunohistochemical staining. The result of the study is promising for the future research on the reproductive system in swine.

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The administration of meloxicam to cows after dystocia does not lead to an increase of retained placenta

Die Gabe von Meloxicam an Kühe mit Dystokie führt nicht zu einer Erhöhung der Nachgeburtsverhaltungen

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Dystocia causes pain. Non-steroidal anti-inflammatory drugs should be applied in animal welfare and performance to reduce birth pains in cows. However, it is known from the literature that the administration of some nonsteroidal anti-inflammatory drugs leads to an increased rate of retained placenta. Therefore, the aim of this study was to answer the question, whether a single administration of meloxicam to cows after dystocia leads to an increased rate of this disease. The study was conducted on 63 Holstein-Friesian cows in four farms, randomized into two groups: group I (n: 27): 0.5 mg meloxicam/kg body weight subcutaneously (Metacam® 20 mg/ml, Boehringer Ingelheim), group II (n: 36, control), the same amount of Aymnin® (infusion solution for cattle, Merial). The injections occur within 2–8 hours after calving. The clinical examination of the cows takes place at the day of birth and at the 21st day *post partum*. Information about the days in between was obtained by questioning the farmer. The following relevant results can be summarized: The control animals developed more udder-leg dermatitis (p = 0.03) in comparison to the ones of the treatment group. There was no increase in the development of retained placenta amongst the treated cows. It can be stated that a single administration of meloxicam to cows after dystocia had no negative clinical effects as described for other nonsteroidal anti-inflammatory drugs.

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Anesthesia concerns for mesenchymal stem cells implantation into reproductive tract and MRI evaluation in pigs as an animal model

Anästhesie beeinflusst die Implantation von mesenchymalen Stammzellen in den Reproduktionstrakt und die MRT-Untersuchung bei Schweinen als Tiermodell

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Pig (*Sus scrofa f. domestica*) is an established model for research about reproductive tract physiology. Normal reproduction performance requires a close cooperation between uterus and cervix. The aim of the study was the bone marrow harvest, mesenchymal stem cells isolation and culture and its implantation into the cervix during surgery then follow up examinations with MRI at certain intervals. Twelve landrace pigs (5–6 months old, 80–90 kg) were enrolled into the study. The elective, surgical, procedures and multiply MRI diagnostics under balanced general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) were performed in all animals following intramuscular premedication (Medetomidine 0.1 mg/kg; Cepetor 1 mg/ml CP-Pharma Germany, Butorphanol 0.1 mg/kg; Butomidol 10 mg/ml, Richter Pharma AG, Ketamine 1 mg/kg; Bioketan 50 ml Vetoquinol Biowet PL), Preemptive Analgesia (Meloxicam 0.4 mg/kg; Metacam 5 mg/ml; Boehringer Ingelheim Vetmedica) and intravenous induction (Etomidate 4–8 mg/kg; Etomidate-Lipuro 2 mg/ml, B. Braun Melsungen AG, Germany) was performed and polyionic balanced crystalloid (Solutio Ringeri Lactate, Fresenius Kabi Poland) at dosage 10 ml/kg/h with synthetic colloid at dosage 10 ml/kg/h (Gelofusine, Aesculap Chifa Poland) was given to maintain both normotension and normovolemia. No general side effects were observed during all procedures. The recovery process after endotracheal tube removal was fast (5 min-standing position). The anesthesia procedure allows for optimal safe, painless management of all multiply procedures on reproductive tract. The goals on anesthetic management should focus on using a balanced, multimodal approach.

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Anesthesia concerns for multiple MRI follow up evaluations after mesenchymal stem cells implantation into swine cervix

Anästhesie-Bedenken für mehrere MRT-Follow-up-Bewertungen nach der Implantation mesenchymaler Stammzellen in den Gebärmutterhals beim Schwein

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Swine cervix is a reliable model for research about reproductive performance physiology. High reproduction efficiency relies on essential interdependence between cervix and uterus. The aim of the study was a multiple MRI tracking and follow up evaluation after mesenchymal stem cells implantation into the cervix to improve its function at certain intervals. Twelve landrace pigs (5–6 month old, 80–90 kg) were enrolled into the study. MRI diagnostics under balanced general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) were performed in all animals following intramuscular premedication (Midanium 500 mcg/kg; Midanium 5 mg/ml Polfa Warszawa S.A.), preemptive analgesia (Meloxicam 0.4 mg/kg; Metacam 5 mg/ml; Boehringer Ingelheim Vetmedica) and intravenous induction (Etomidate 4–8 mg/kg; Etomidate-Lipuro 2 mg/ml, B. Braun Melsungen AG, Germany) and polyionic balanced crystalloid (Solutio Ringeri Lactate, Fresenius Kabi Poland) at dosage 10 ml/kg/h was given to maintain both normotension and normovolemia. No general side effects were observed during all MRI diagnostics procedures. The recovery process after endotracheal tube removal was smooth and immediate (5 min-standing position). The anesthesia protocol allows for optimal and safe management of all multiply, noninvasive, painless MRI procedures of reproductive tract and maximizes patient management success for future surgeries. The goals on anesthetic management should focus on using a balanced, multimodal approach because MRI is the technique of choice for multi dimension, detailed imaging of cervix but usually takes 60–90 min and therefore generally require a safe general anesthetic plan carefully tailored to the future needs.

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Hysteroscopic catheterisation of the equine oviduct

Hysteroskopische Katheterisierung des equinen Eileiters

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Equine reproductive pathologies are well understood in most parts, diagnostic and therapeutic methods are well established. Still, the number of healthy mares incapable of conceiving in spite of professional veterinary management and no pathologic findings is conspicuous. In women infertility is mainly associated with fallopian tube pathologies. Findings of intraepithelial cysts and collagenous masses, clogging the lumen of the salpinx in mares have been described as well [Medenbach et al. Pferdeheilkunde 1999; 15:

560–7]. Diagnostic evaluation, imagery and therapy of the oviduct are significantly limited in horses due to its anatomical structure and poor accessibility. Mostly laparoscopic techniques for evaluation and treatment are described. A catheter with inner tube for hysteroscopic access to the oviductal papilla and hydrotubation of the fallopian tube [Inoue, Equine Vet J 2013; 45: 761–5] enabled oviductal catheterisation in seven euthanized mares using ink to visualize oviducts consistently and accessibility. The procedure was performed in 14 standing, sedated mares of different sizes and breeds. In five mares access to any utero-tubal junction was not possible, as a consequence of excessive swelling of the papilla due to manipulations. In three mares catheterization of only one salpinx could be conducted. Subsequently, catheter's diameter was reduced, an angiography guide wire was added, and cap-assisted endoscopy was performed. These modifications allowed for successful hysteroscopic oviductal catheterization in standing sedated mares. Irritation of the papilla should be reduced to a minimum to ensure successful hysteroscopic catheterisation of the mare's oviduct.

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Elastography imaging of bovine uteri: a preliminary study

Elastographie-Bildgebung von Rinderuteri-Modellen: eine Vorstudie

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Since the beginning of bovine gynecology, palpation has been used to detect the presence of abnormalities in tissue consistency according to mechanical properties of diseased and normal tissue. Quantitative estimation of the mechanical properties of tissues may be conducted using elastography, an ultrasonographic technique providing information about tissue strain, where strain with an external force is applied. We introduced, at the first time, the elastography imaging of bovine uterus in diestrus. The conventional and elastographic ultrasound examinations were carried out on HE cows (n = 10) at 5, 10, 15 days of the estrus cycle. The size of CL was determined by measurement of the Maximal Cross-Sectional area of the Luteinized tissue (MCSL) and the, whereas the Average Percentage of Pixels of Each Color (APPEC) was calculated using real-time ultrasound elastography protocol (probe 3–11 MHz, ESAOTE MyLab Alpha). No significant differences (p > 0.05) in MCSL and APPEC (scale 1–4) for uterine horns were demonstrated between day 5 (MCSL: 21.2 ± 0.16 mm; scale

1: 35.4% ± 1.12, scale 2: 5.9% ± 0.61, scale 3: 26.9% ± 1.02, scale 4: 33.1% ± 2.02), 10 (MCSL: 24.8 ± 0.31 mm; scale 1: 34.0% ± 0.92, scale 2: 6.4% ± 0.55, scale 3: 27.1% ± 0.98, scale 4: 32.9% ± 1.41) and 15 (MCSL: 22.5 ± 0.09 mm; scale 1: 32.9% ± 1.56, scale 2: 6.0% ± 0.84, scale 3: 28.1% ± 1.33, scale 4: 33.9% ± 1.31). However, the cervix was mostly hard at day 10 (scale 1: 55.0% ± 1.37, scale 2: 6.8% ± 0.77, scale 3: 14.3% ± 1.32, scale 4: 20.7% ± 1.15), stiffer than at day 5 and 15 (scale 1: 48.1% ± 1.06, scale 2: 7.9% ± 0.52, scale 3: 16.6% ± 1.11, scale 4: 27.5% ± 2.24). Although the biggest challenge was related to technical limitations, we concluded that elastography can be utilized in the gynecological examination of cattle.

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The involvement of hypoxia-inducible factor (HIF1α) in maturation of cumulus oocyte complexes (COCs) in cattle

Beteiligung des Hypoxie-induzierbaren Faktors (HIF1α) an der Reifung von Kumulus-Oozyten-Komplexen (COCs) beim Rind

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The role of reduced oxygen tension in maturation of cumulus-oocyte complexes (COCs) and resumption of meiosis is unclear, in bovine and in other species. HIF1α is the best-known master regulator of cellular response to hypoxia. Several metabolic and hormonal factors expressed in cumulus cells are positively associated with in vitro maturation (IVM) competence of oocytes. The involvement of HIF1α in regulating their expression was not yet studied in detail. Therefore, in our experiments (n = 7) we investigated the role of HIF1α during IVM of bovine COCs and on blastocyst rates following IVF. COCs from slaughterhouse ovaries were divided into groups: immature, IVM/control (24h, standard protocol), and IVM/E (IVM with Echinomycin, a specific HIF1α blocker). Morphologically, cumulus cell expansion was inhibited in the IVM/E group. Expression of 13 selected factors was assessed in cumulus cells by qPCR. Between IVM/control and IVM/E groups we found decreased (p < 0.05) expression of TMSB4, TMSB10, STAR, COX2, PTGES, HAS2, GATM, TNFAIP6, NPR2, Cx43 and GLUT1. Unaffected were 3βHSD and 20αHSD. Concomitantly, progesterone output decreased in the IVM/E group. Importantly, apparent functional alterations in cumulus cell expansion and corresponding gene expression in the IVM/E group lowered

oocyte maturation rates by 44% ($p < 0.05$). Additionally, blastocyst rates from COCs matured with E decreased by 40% ($p < 0.05$). Our results indicate the importance of HIF1 α for IVM of bovine oocytes and its association with decreased fertilization competence.

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Investigation of the relationship between some maternal factors and teat canal length in dairy cows

Untersuchung der Beziehung zwischen einigen maternalen Faktoren und der Zitzenkanallänge bei Milchkühen

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The objectives of the current study to elucidate the relationship between the teat canal length (TCL) and some maternal factors as race, age, lactation period as well as difficulty of milking. A total fifty clinically healthy cows of different breeds (holstein, simmental, swiss brown) and ages were included. The data of age, race, parity, lactation period, udder health and milking-convenience collected and recorded in previously designed sketches for further statistical analysis. The ease of milking at all teats tested by manual milking, which always conducted by the same person. After the general examination and cleaning of the udder, the teat canals examined by ultrasonography (5–7.5 MHz linear probe, Honda HS-1500) and TCL recorded. The results revealed that, mean TCL were 9.80 mm (right) and 9.89 mm (left) anterior teats and 10.22 mm (right) and 10.28 mm (left) in the rear teats. The measurements of TCL were longer in the rear than the anterior teats ($p < 0.05$). There were no correlations between the TCL and race, age, lactation number, lactation period on TC length as well as easy or difficulty of milking.

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Impact of β -mercaptoethanol and/or Epidermal Growth Factor supplementations on in vitro maturation of buffalo oocytes (*Bubalis bubalis*)

Einfluss der Zugabe von β -Mercaptoethanol und/oder epidermalen Wachstumsfaktoren auf die In-vitro-Reifung von Büffeloozyten (*Bubalis bubalis*)

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The study aimed to assess the effects of supplementation of β -mercaptoethanol (β -ME) and/or epidermal growth factors (EGF; Sigma E5036) to *in vitro* maturation medium (IVM) on the development of buffalo oocytes and subsequent fertilization. The ovaries were collected at a slaughterhouse and were transferred directly to the laboratory. Cumulus oocytes complexes (COCs) were aspirated from follicles of 3–8 mm $\varnothing$ with evenly cytoplasm and multilayered (> 3) COCs. In experiment 1, COCs matured in IVM medium (TCM-199) supplemented with 0 (control), 10, 20, 50 or 100 ng/ml EGF for 24 hours under humidified environment condition (38.5 °C, 5% CO₂ in air and 95% relative humidity). In the second experiment, COCs were matured in medium supplemented with 0 (control), 25, 50, 100 and 200 μ M β -ME for 24 hours at the same conditions. In the third experiment, COCs were matured in medium supplemented with 0 (control) group and a combination of 20 ng/ml EGF + 50 μ M β -ME for 24 hours at the same conditions. After 24 hours of incubation, maturation rates of the oocytes were recorded. The maturation rates (cumulus expansion and MII), fertilization rate and the proportion of embryos reaching the blastocyst stage increased when cultured with 20 ng/ml EGF (76.96 $\pm$ 9.04, 67.96 $\pm$ 2.76 and 58.90 $\pm$ 4.85, respectively) compared to the other concentrations. The maturation rates were significantly ($p < 0.05$) higher after IVM with β -ME 50 μ M β -ME compared to the control (76.96 $\pm$ 9.04 vs 51.33 $\pm$ 4.6%, respectively). In conclusion, the cleavage and blastocyst rates were significantly improved using the combination of growth factors with antioxidants (EGF + β -ME).

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Efficacy of GnRH and hCG at the time of artificial insemination in repeat breeder Holstein-Friesian cows: preliminary results

Wirksamkeit von GnRH und hCG zum Zeitpunkt der künstlichen Besamung bei Umbullern der Rasse Holstein-Friesian: Vorläufige Ergebnisse

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Administration of LH surge stimulants like GnRH analogues or hCG at the time of artificial insemination (AI) is the most common treatment in case of early repeats of estrus i.e. < 25 days after AI. The aim of it is the

prevention of delayed ovulation, which is one of the major causes of early repeats. The objective of the study was to compare efficacy of GnRH vs. hCG with the later being more expensive and occasionally causing anaphylactic reaction. The study included 53 cows from one herd in northeastern Poland. They were between 2nd and 5th lactation, 150 $\pm$ 10 days after calving, cycling normally, without clinical abnormalities and infectious diseases, have failed to conceive after two consecutive inseminations. Cows were also negative for subclinical endometritis and metabolic disorders. Group A ($n = 19$) were injected with GnRH analogue at the time of AI, group B ($n = 18$) received hCG and control group C ($n = 16$) got saline solution injections. Cows that showed estrus after AI were inseminated once again (groups A and B with hormonal injections), and those which did not express signs of estrus were checked for pregnancy 40 $\pm$ 10 days post AI. In groups A, B, C number of pregnant cows was 15 (78.9%), 13 (72.2%), 9 (56.2%) respectively and those who conceived after first experimental AI was: 8 (53.3%), 8 (61.5%), 2 (22.2%) respectively ($p < 0.05$). Results show that both hormonal injections are effective; however, there is a trend for equal efficacy of them. Cows that did not conceive may have had other than delayed ovulation causes of early repeats, which require further investigation.

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Comparison of inflammation severity in mare's endometrium and myometrium in acute and chronic state

Vergleich von Entzündungsgraden im Endometrium und Myometrium der Stute im akuten und chronischen Stadium

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Endometritis is considered as the main cause of infertility in mares, altering microenvironment inside the uterus and is being divided into be acute (AE) and chronic (CE) based on histological evaluation. We have proposed an immunofluorescent assessment of uterus' sections on TissueFAXs Plus as a method for inflammation diagnosis. The aim of this study is to compare the severity of inflammation in endometrium and myometrium during endometritis based on infiltration of immune cells: granulocytes (PMN) for AE and lymphocytes (Lym) for CE. Immunofluorescent staining has been performed on 36 sections of uteri

collected from a slaughterhouse with endometritis being confirmed via HE staining and compared with sections without endometritis (WE). The percentage (mean% $\pm$ SD) of all cells, single positive (Leukocytes: CD45) and double positive (PMN: CD45/CD66; Lym: CD45/CD3) were calculated with TissueQuest Plus. Percentage of immune cells was higher ($p < 0.05$) in AE and CE in endometrium ($11.30\% \pm 17.01$ in AE compared to $0.77\% \pm 0.70$ in WE and 13.75%

± 20.36 in CE compared to $3.16\% \pm 3.06$ in WE), while in the myometrium it was higher ($p < 0.01$) only at the chronic state ($1.74\% \pm 1.57$ in AE: $1.64\% \pm 2.21$ in WE and $9.65\% \pm 6.29$ in CE: $0.83\% \pm 1.16$ in WE). In AE immune cells represented $11.30\% \pm 17.01$ with $67.13\% \pm 16.82$ of all PMN in the endometrium and $1.74\% \pm 1.57$ with $41.18\% \pm 28.36$ of PMN in the myometrium. In CE on the other hand, $13.75\% \pm 20.36$ with $61.26\% \pm 17.96$ of Lym in endometrium and 9.65%

± 6.29 with $68.25\% \pm 24.04$ of Lym were in the myometrium. We have found that CE is affecting myometrium as well, which may be influencing its contractility. Type of infiltration in myometrium and endometrium does not vary in both AE and CE.

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