

ABSTRACTS**POSTER PRESENTATIONS****P 1 | Stearoyl-CoA-desaturase activity in cumulus cells protects the oocyte against lipotoxicity**H Aardema¹; HTA Van Tol¹; JF Brouwers²; BM Gadella^{1,2}; BAJ Roelen¹¹Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; ²Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

A rise in free fatty acid levels is a characteristic of energy rich and poor metabolic conditions, both in human and animals. Saturated free fatty acids can be toxic for oocytes and may be the cause of reduced fertility after metabolic stress. Cumulus cells that surround the oocyte appear to protect the oocyte against free fatty acids (Aardema et al. 2013, *Biol Reprod* 88:164). The current study investigates the function of stearoyl-CoA-desaturase (SCD-1), converting stearic (C18:0) and palmitic acid (C16:0) into respectively oleic (C18:1) and palmitoleic acid (C16:1), in cumulus cells. Cumulus-oocyte-complexes (COCs) were retrieved from bovine slaughterhouse ovaries, matured for 23 h, fertilized and presumed zygotes were cultured until day 8 according to our standard protocol. Cumulus cells expressed high levels of SCD-1 gene and protein as detected by qRT-PCR and immunoblotting, while expression in oocytes was low. Inhibition of SCD-1 activity (1 µM, Biovision) in the presence of C18:0 (100 µM) and C16:0 (150 µM) during maturation of COCs resulted in a reduction in the blastocyst rate when compared to the control group where SCD-1 was active (12.5 ± 7.2% and 29 ± 8.7%, respectively; $p < 0.001$, $n \approx 280$ in 3 runs per group). In addition the C18:1/C18:0 ratio, determined by HPLC mass spectrometry, was reduced when SCD-1 was inhibited in the presence of C18:0 (0.5 ± 0.03 vs. 1.3 ± 0.46; $p < 0.01$). Lipid droplet storage in cumulus cells was decreased when SCD-1 was inhibited during exposure to C18:0 ($p < 0.001$). Combined, the data indicate that SCD-1 in cumulus cells converts the potentially toxic saturated into less harmful mono-unsaturated fatty acid and is subsequently stored in lipid droplets. This function of cumulus cells protects the oocyte against lipotoxicity.

P 2 | Infectiousness of equine semen in prepatent phase of DourineY Ahmed¹; A Hagos²; B Merga²; T Alemu²; A Van Soom³; L Duchateau³; B Goddeeris³; J Govaere³¹Wollo University, Dessie, Ethiopia; ²Addis Ababa University, Bishoftu, Ethiopia; ³Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Dourine, caused by *T. equiperdum* (*T.eq.*), is a notifiable disease (OIE 2013). Since knowledge about the prepatent infectiousness of semen is lacking, introduction of the disease is a relevant threat. Aims of the study were: (1) To determine the infectiousness of stallion semen in the prepatent phase of Dourine; (2) to assess infectiousness of artificially inseminated (AI) *T.eq.*-spiked semen. Stallions (N = 4) were infected by transfusion of *T.eq.* (Dodola 943). Semen collected in the prepatent period (14 days post infection, N = 1) or epididymal semen (N = 1) or 4 weeks post Cymelarsan treatment (N = 2), was inoculated in mice (resp. in 7, 5 and 10 mice) hereafter *T.eq.*-isolation in the mice was performed by the Wet blood smear-test (Wbs-t). Mares (N = 4) were AI using *T.eq.* spiked semen (38.000T.eq./AI) using conventional techniques. Mice inoculated by collected or epididymal semen from infected, non-treated stallions were positive for *T.eq.* (7/7 and 5/5). Mice inoculated with epididymal semen of treated animals were parasitaemic in one stallion (5/5) but not in the second one (0/5). Mares inseminated with spiked semen got Dourine (4/4), were positive on Wbs-, Woo test and CATT serology. Although the presence of *T.eq.* is shown in seminal fluid and genital tissues (Lelli et al. 2012, *J Equine Vet Sci* 32:S70–71), disease spread after AI is not reported and no knowledge about infectiousness in prepatent period is reported so far. This study shows (1) the infectiousness of *T.eq.* during prepatent phase and (2) when used for AI.

P 3 | Does the adding of seminal plasma after thawing influence stallion sperm quality?E Al-Essawe¹; A Johannisson¹; M Wulf²; C Aurich³; JM Morrell¹¹Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; ²Graf Lehnndorff-Institute for Equine Sciences, Neustadt (Dosse), Germany; ³University of Veterinary Medicine, Vienna, Austria

Most of the seminal plasma (SP) is removed when processing semen for cryopreservation; adding SP after thawing might have a beneficial effect on sperm quality. Objective: to determine if adding SP from "good" (GF) or "bad" (BF) freezer stallions to semen after thawing improves sperm quality. Semen from 8 stallions, (three ejaculates per stallion) was processed by Single Layer Centrifugation (SLC) to remove SP prior to freezing. Straws were thawed at 37°C for 30s; the contents were divided into three aliquots; i) control (C); ii) pooled BF-SP (5%) was added; iii) pooled GF-SP (5%) was added. Membrane integrity (MI), mitochondrial membrane potential (MMP) and chromatin integrity (DNA fragmentation index; %DFI) were evaluated using a FACSVerse flow cytometer. Data were analyzed by mixed model

using the (SAS[®] 9.3). Significance was set to $p \leq 0.05$. All values are LSMEAN \pm SE for C, BF and GF, respectively. The proportion of living spermatozoa was higher in C than in groups with SP ($42.7 \pm 3.2\%$, $41.1 \pm 3.2\%$, $41.3 \pm 3.2\%$; C vs. BF $p = 0.02$; C vs. GF $p = 0.05$); there were fewer spermatozoa with low MMP and more with high MMP for C than for SP-treated samples (Low MMP: $70.6 \pm 4.1\%$, $76.1 \pm 4.1\%$, $78.5 \pm 4.1\%$; C vs. BF, $p = 0.06$; C vs. GF $p = 0.006$; high MMP: $28.5 \pm 4.1\%$, $23.1 \pm 4.1\%$, $20.5 \pm 4.1\%$; C vs. BF $p = 0.08$, C vs. GF $p = 0.006$). The % DFI was lower in C than in BF or GF ($8.3 \pm 1.1\%$, $9.9 \pm 1.1\%$, $9.5 \pm 1.1\%$; C vs. BF $p < 0.0001$, C vs. GF $p < 0.0003$). No differences were found between BF and GF for any parameters of sperm quality. In conclusion, adding SP after thawing does not have a beneficial effect on sperm quality, regardless of whether it is from good or bad freezer stallions, and may have a deleterious effect on sperm DNA.

P 4 | Bovine oviductal extracellular vesicles mRNA content changes across the oestrous cycle

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Extracellular vesicles (EVs), exosomes and microvesicles, are important components of the oviductal fluid and have been suggested as modulators of gamete/embryo-maternal interactions by transferring their molecular cargo (proteins, small ncRNA and mRNA). Previously, we identified miRNA and protein profiles of bovine oviductal EVs (boEVs) across the oestrous cycle, which can have a direct impact on gametes/embryos. Here, we focused on boEVs mRNA content that can lead to the generation of functional proteins by them, thus enabling horizontal transfer of genetic information. Oviducts with their attached ovaries were collected from cyclic bovine reproductive tracts at the abattoir and classified in 4 different stages according to Ireland et al. (1980). Oviducts were flushed and boEVs were isolated by ultracentrifugation. BoEVs total RNA was isolated, RNA-Seq libraries were prepared and sequenced on an Illumina HiSeq 2500. The obtained sequence data was processed using a local Galaxy pipeline. Transcripts derived from 12,087 genes were identified in boEVs (Bioconductor R, EdgeR). BoEVs mRNA signature from stage I (1–4 days post-ovulation, period in which the embryo develops up to 16-cells) differed significantly from the other stages analyzed (II–IV). Comparative analysis of stage II, III, and IV (pre-ovulation) mRNA levels to stage I revealed significant differences for 1,027 (597 up and 430 down), 624 (339 up and 285 down) and 1,362 (796 up and 566 down) genes (FDR 1%), respectively. Our study represents the first screening of boEVs mRNA content and revealed changes across the oestrous cycle. Moreover, we identified boEVs transcripts related to early embryo development and gamete interactions. (Supported by EU-AgreenSkills267196, EU-FECUND312097 and COST-EPICONCEPT-FA1201.)

P 5 | Castration effect on echotextural characteristics of the vesicular glands of mature goats

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The aim was to analyze echotexture characteristics of vesicular glands in goats under castration effect. Ten mature goats were castrated by orchietomy (G1, $n = 6$) or Burdizzo techniques (G2, $n = 4$). B-mode ultrasound exams were made immediately before procedure (D0), 30 (D30), 45 (D45), 60 (D60), 75 (D75) and 90 days after castrations (D90), using MyLab 30 Vet equipment (Esaote, Italy) connected to 7.5 MHz transrectal linear transducer. Echotextural analyses were made using Image ProPlus[®] software, and computed the numerical pixel values (NPVs) and pixel heterogeneity (standard deviation of NPVs) within circular regions of interest placed within the vesicular glands parenchyma. Data (means \pm SD) were analyzed under effect of castration technique, days and their interaction (ANOVA for repeated measures with Tukey post hoc test, $p < 0.05$). There was no interaction between both effects. There were no difference between groups for NPVs (137.6 ± 8.9 vs. 135.0 ± 13.5) and pixel heterogeneity (20.9 ± 2.3 vs. 21.6 ± 1.9) for G1 and G2, respectively. Similar values were observed between days for NPVs (D0: 137.9 ± 11.4 , D30: 137.0 ± 14.2 , D45: 139.2 ± 8.0 , D60: 138.3 ± 15.7 , D75: 128.3 ± 7.3 and D90: 135.7 ± 10.8) and for pixel heterogeneity (D0: 21.8 ± 1.8 , D30: 21.7 ± 1.9 , D45: 21.1 ± 1.7 , D60: 21.4 ± 2.2 , D75: 21.3 ± 3.0 and D90: 20.5 ± 2.1). In conclusion, the echotextural characteristics of the vesicular glands parenchyma were not altered after castration, regardless of the technique used. (Financial support: Fapesp (no. 2015/22823–9).)

P 6 | Intraluteal administration of cloprostenol in dairy cows

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The aim of the study was to determine the luteolytic dose of cloprostenol administered directly into the corpus luteum (intraluteal treatment, ILT) in dairy cattle. Intraluteal treatment was performed by modified equipment for ultrasound guided transvaginal ovarian aspiration. Thirty synchronized lactating Holstein-Friesian cows were randomly divided into six equal groups. First control group (C1) was treated by 500 μ g of cloprostenol intramuscularly. Second control group (C2) was treated by ILT with 0.2 ml physiological solution (F1). Four experimental groups were treated by ILT with cloprostenol (Estrumate, MSD) in different doses: group 1 (E1) 5 μ g, group 2 (E2) 25 μ g, group 3 (E3) 50 μ g and group 4 (E4) 100 μ g, respectively. Estrumate in groups E1 and E2 was diluted in F1 to reach a volume of 0.2 ml, group E3 was treated by 0.2 ml of Estrumate and group E4 was treated by 0.4 ml of Estrumate. Size of CL and progesterone concentrations (P4) were

evaluated to control the luteolysis at times 0, 0.5, 1, 2, 4, 8, 24 and 48 h after ILT. Cows in groups C2 and E1 were unaffected by ILT. The cloprostenol dose $\geq 25 \mu\text{g}$ (groups E2 – E4) significantly affected the size of the CL and P4 concentration. However, P4 concentrations $<1 \text{ ng/ml}$ 48 h after ILT were only determined in group E4, as well as in group C1. In conclusion, the cloprostenol dose of $100 \mu\text{g}$ administered intralutally can be established as luteolytic in cows.

P 7 | Estrous response and time to ovulation in nulliparous and multiparous goats treated with progesterone and human chorionic gonadotropin during non-breeding season

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The optimal time between artificial insemination and ovulation is crucial for high fertility rates in goats. The aim of the study was to determine the effect of parity (nulliparous vs. multiparous) on estrous response and time of ovulation in Criollo goats primed with progesterone (P4) plus human chorionic gonadotropin (hCG) during the anestrus season in northern Mexico (25°N). Nulliparous ($n = 9$; 9 to 12 mo. $29 \pm 2.9 \text{ kg}$ and 2.3 ± 0.1 body condition score (BCS)) and multiparous goats ($n = 13$; 2 to 5 yrs. $39.7 \pm 4.3 \text{ kg}$ and 2.3 ± 0.2 BCS) were treated with 20 mg i.m. of P4 and 24 h later, goats were treated with 100 IU of hCG i.m. Twelve hours after hCG administration, the onset of estrus was determined every 6 h with the use of an aproned buck. At the moment of estrus, the ovulation time and the number of ovulations were assessed by transrectal ultrasonographic scanning using a 7.5 MHz linear prostatic probe every 6 h until disappearance of preovulatory follicle(s) presented at the previous scanning. No differences ($p > 0.05$) occurred between nulliparous and multiparous goats neither for estrus nor for ovulation response (100 vs. 92%, interval hCG to estrus: 52 ± 3 and $59 \pm 14 \text{ h}$, interval hCG to ovulation: 85 ± 11 and $90 \pm 13 \text{ h}$ and ovulation rate: 1.5 ± 0.1 and 2.0 ± 0.2 , respectively). To conclude, parity did not affect estrus response, time to ovulation and ovulation rate in goats treated with P4 plus hCG. Results could be important to determine optimal time for artificial insemination as well as to improve the fertility rate during the anestrus season.

P 8 | PRID Delta based treatment approaches with GnRH/eCG for anestrus cows: preliminary results

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The objectives of this study were to investigate duration of PRID Delta[®] (PRID Δ , 1.55 g progesterone/CEVA/France), usage of first (Day0/D0) GnRH and also equine chorionic gonadotropine (eCG) with removal of PRID Δ on pregnancy rates in anestrus cows. For these aims, in group 1 ($n = 8$), anestrus cows received GnRH (100 mcg, i.m., Ovarelín[®]/CEVA/France) and PRID Δ on D0. PRID Δ was removed on D9, following PGF_{2 α} (25 mg, i.m., Enzaprost[®]/CEVA/France) injection on D8. In group 2 ($n = 6$), anestrus cows were treated with GnRH on D0, PRID Δ were simultaneously inserted and removed 9 days later. PGF_{2 α} and eCG (600 IU, i.m., Oviser[®]/HIPRA/Spain) were administered on D8 and D9, respectively. FTAs of group 1 and 2 were carried out 60 h, following removal of PRID Δ . In group 3 ($n = 8$), PRID Δ were inserted and withdrawn on D7. PGF_{2 α} injection was applied on D6 and FTAs were carried out 48 h following removal of PRID Δ and anestrus cows were treated with GnRH at FTAI. All pregnancies were determined with transrectal USG (7.5 Mhz/Draminski[®]/Poland) between 25–30 days following FTAs. Pregnancy rates in group 1, group 2 and 3 were 37% (3/8), 66% (4/6) and 57% (4/7, one lost PRID Δ = 1/8), respectively. In conclusion, 7Ds PRID Δ usage may be effective in treatment of anestrus cows without D0 GnRH; also eCG may be effective in 9Ds PRID Δ . However, new studies must be carried out on usage of GnRH and eCG with PRID Δ with enough number of anestrus cows to determine exact correlations.

P 9 | True vaginal prolapse with sub-vaginal retroflexion of the bladder and uterus in a 45-day pregnant dog

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A true vaginal prolapse in a bitch is a very rare condition. Most frequently it is seen near parturition when progesterone declines and estrogens increase. A 3-year-old female Chou Chou dog was presented with a history of progressively enlarged protruding mass from the vulva. Rectal temperature, heart rate, respiratory rate, blood and serum biochemistry profiles were within normal limits. Clinical examination revealed prolapsed mass with dark-red colored, congested and edematous walls. Bladder and uterus failed to be visualized by abdominal ultrasound examination and ultrasonography of the prolapsed vagina was done. It revealed anechoic, urine containing bladder and next to it hyperechoic skeletons of two dead fetuses without any amniotic fluid. A diagnosis of true vaginal prolapse with retroflexion of the bladder and the uterus was made. Exploratory laparotomy displayed cranial part of stretched uterine horns, without visualization of the uterine body, cervix and bladder. By gentle pressure of the prolapsed vagina outside and gentle pulling of the uterine horns inside, uterus and bladder were repositioned into the abdominal cavity. Ovariohysterectomy was performed and cervicopexy was done to prevent relapse of the vaginal prolapse. The

dog recovered completely and recurrence of the prolapse was not observed. As a complication of vaginal prolapse in the bitch, other organs, such as bladder, uterus and part of the colon, may be retroflexed sub-vaginally.

P 10 | Expression pattern of TLR4 mRNA and protein in the ovine corpus luteum during the early pregnancy and PGF_{2α} induced luteolysis

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Toll-like receptor 4 (TLR4) is an important part of innate immune system and generally produced by different types of immune cells. Nevertheless, its expression is also detected in corpus luteum cells. The aim was to elucidate expression profile and cell specific localization of TLR4 in the ovine corpus luteum during early pregnancy and PGF_{2α} induced luteolysis. CL samples were collected from both pregnant and cyclic ewes on days of 12 (n = 4), 16 (n = 4) and 22 (n = 4). For the induced luteolysis model, ewes were injected PGF_{2α} on the 12th day of the estrous cycle and CL samples were collected at 0 h (no PGF_{2α} injection, n = 4), 1 h (PG1, n = 4), 4 h (PG4, n = 4), and 16 h (PG16, n = 4) after injection. The qPCR was used to evaluate expression profiles, while immunohistochemistry was used to define temporo-spatial localization of TLR4 in CL. Although, there was no change in TLR4 mRNA expression between pregnant and cyclic CL samples, an upregulation of TLR4 mRNA was only detected in PG16, compared to the other groups (p < 0.05). TLR4 protein was particularly localized in endothelial cells of CL in cyclic ewes on day 12, but prominent TLR4 protein signal in CL was clearly apparent in luteal cells in PG16. The result indicates no critical role of TLR4 in ovine CL during early pregnancy. These data also suggest that a possible involvement of TLR4 in late luteolytic mechanism in ovine CL, as indicated by the increased expression level for TLR4 in PG16. Moreover, the present study may suggest that TLR4 signaling mediated pathway in ovine luteal cells may involve regressing of the CL due to the expression of TLR4 in luteal cells. (This study funded by DUBAP.)

P 11 | Expression patterns of epigenetic chromatin modification enzymes in equine endometrium during early pregnancy

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DNA methylation and histone modifications in mammalian cells control the activity of chromatin modification and serve as inheritable

epigenetic marks. Mechanism of DNA packaging and chromatin modification regulates the gene expression depending on time and tissue/organ. Endometrial gene expression is tightly controlled, and also has a critical influence on maternal recognition of pregnancy and embryo implantation. The objective was to investigate mRNA expression patterns of genes (total of 32 genes) from a set of families (Histone Acetyltransferase, Histone Methyltransferases, SET Domain Proteins, Histone Phosphorylation, Histone Ubiquitination, DNA/Histone Demethylases, Histone Deacetylases) of chromatin modification enzymes during the oestrous cycle and early pregnancy. Biopsies were obtained from equine on day of ovulation (d0, n = 4), late dioestrus (LD, n = 4, high progesterone [P4]), and after luteolysis in the beginning of the oestrus phase (AL, n = 4, <1 ng/ml P4) of the cycle. Biopsies were also taken on days 14 (P14, n = 4), 18 (P18, n = 4), and 22 (P22; n = 4) of pregnancy. Relative mRNA expression levels were quantified using real-time quantitative RT-qPCR. Expression of all chromatin modification enzymes were detected in equine endometrium during the oestrous cycle and early pregnancy. Each gene family were observed to be regulated differentially either by cyclic changes during the cycle or by the presence of the embryo inside the uterus. It may be inferred that chromatin modification enzymes are steadily expressed and regulated to some extent to establish well coordinated transcriptional activity with respect to epigenetic regulation in equine endometrium during oestrous cycle and early pregnancy.

P 12 | Effects of season and pregnancy on insulin secretion and glucose clearance in horses

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In pregnant mares, peripheral insulin antagonism channels glucose preferentially to the foetus. On the other hand, horses reduce their metabolic activity in winter. Taking both aspects together, we hypothesized that glucose clearance from blood and the insulin response to glucose do not only change throughout gestation but also with season. To test this hypothesis, the glucose and insulin response to an oral glucose test was analysed in pregnant mares (n = 12) and in geldings (n = 10), as controls. Animals were tested in June, September, December and March (geldings) and on day 320 of gestation (mares). Differences between groups and changes over time were analyzed by GLM-ANOVA with month and time within tests as within subject factors and group as between subject factor. Plasma glucose concentration increased in all tests (p < 0.001). The increase was more pronounced in mares (p < 0.05) and steadily rose from June to December in mares (p < 0.001) but not in geldings (month x group p < 0.05). This indicates a constant glucose clearance in geldings but reduced clearance in pregnant mares. A pregnancy-induced partial insulin resistance is thus independent

from season. Plasma insulin concentration increased after glucose feeding ($p < 0.001$) with no difference between pregnant mares and geldings. The insulin response to glucose increased from June to December ($p < 0.001$) indicating seasonal changes in β -cell sensitivity to glucose in horses.

P 13 | Relationships between maternal serum and fetal fluid glucose, non-esterified fatty acids and beta-hydroxybutyrate levels at the time of Cesarean section in dogs

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The influence of the bitch's metabolic environment in the end of gestation on fetal amniotic fluid (AMF) and allantoic fluid (ALF) composition was studied by measuring metabolic parameters, i.e., glucose, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in the serum of 22 bitches undergoing Cesarean section, and in AMF and ALF from one of their fetuses. Wilcoxon signed-rank test with Bonferroni correction, linear regression, t-test and Mann-Whitney U test were used for data analysis. Glucose was higher ($p < 0.0001$) in maternal serum than in AMF or ALF, and serum and fetal fluid levels showed a strong positive relationship ($p < 0.0001$). NEFA and BHB levels were higher in serum than in the fetal fluids ($p < 0.0001$ and $p = 0.005$, respectively). Bitches of small size breeds (≤ 20 kg pregnant body weight, $n = 12$) had increased serum NEFA and BHB ($p = 0.008$ and $p = 0.003$, respectively), but similar glucose concentrations to larger (> 20 kg, $n = 10$) dogs. However, AMF and ALF metabolite levels from pups of small and large bitches were similar. The total weight of the litter in dogs ≤ 20 kg represented a significantly higher percentage of their pregnant body weight ($11.6 \pm 4.8\%$) than in dams > 20 kg ($5.7 \pm 3.6\%$; $p = 0.004$). In conclusion, fetal AMF and ALF glucose concentrations are strongly dependent on maternal serum glucose levels in dogs. Increased serum NEFA and BHB in small size bitches indicate pronounced lipid mobilization due to the high metabolic demand in the end of pregnancy, but the concentration of these metabolites in fetal fluids remains relatively low compared to serum levels.

P 14 | Factors influencing twin pregnancy/calving in dairy cows

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Frequency of twin calvings increased in the last 3–4 decades in dairy cows. Since twin delivery is undesirable due to the negative effects

on uterine involution and increased calf mortality, recognition of twin pregnancy and possible elimination of one embryo could be desirable in dairy industry. Examinations were carried out in 3 large Hungarian dairy farms (780–1100 cows/farm) between 2012 and 2016. During this period 509 of 8019 calvings were recorded as twin calving (6.4%). At the same time-range fully quantitative ELISA tests were used for early pregnancy detection (PSPB; BioPRYN©) between 28–40 days after Insemination (p.i.), and pregnant cows were re-examined by rectal palpation at 60 days p.i. Pregnancy losses were detected in 1797 cows (22.4%) and 456 of the 6222 pregnant cows delivered twins (7.3%). Effects of season (month of calving/Insemination), age of cows, serum PSPB concentrations were determined. Generalized additive models were used to analyze relationship of twinning frequency and independent variables. We found that month of insemination ($p = 0.037$), the age of the cow ($p < 0.0001$) and the PSPB concentration ($p < 0.0001$) had non-linear effects on the probability of twin calving. The highest probability of twin calving was found if cows were inseminated in October. Probability of twinning is increasing until up to five years of age. Analysis of the PSPB level as a predictor of twin calving by ROC showed that the AUC was 0.71, and the best predictive threshold was 2.958 ng/ml with a sensitivity of 71% and a specificity of 63%. In conclusion, older cows inseminated in October with high PSPB concentration 28–40 Days post AI have higher risk for twin pregnancy.

P 15 | Effect of astaxanthin on extended boar semen stored at 17°C for 48 h

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The aim of the study was to investigate the effect of astaxanthin (ASX) on extended boar semen. ASX is a carotenoid providing a strong antioxidant capacity. Twenty ejaculates (10 boars; 2 ejaculates/boar) were collected, extended to 50×10^6 sperm/ml (pre-trials conducted considering ASX's properties and sperm concentration), and split into 3 groups: Control (1; no treatment), Solvent (2; semen with DMSO, the diluent of ASX) and ASX (3; semen with ASX at $0.5 \mu\text{M}$). Parameters of spermatozoa [total motile, progressive, rapid, viability, mitochondrial potential, morphology (including acrosome integrity), and functional integrity of sperm plasma membrane by HOST test] were assessed 0, 24 and 48 h of storage at 17°C. Data were analysed with a repeated measures mixed model. Percentage of total motile, progressive, rapid and HOST positive sperm did not differ significantly between examination time points in group 3, while they decreased in the other groups ($p < 0.001$). Regarding viability, group 3 performed better than group 1 at 24 and 48 h (91.9 ± 1 vs. 85.7 ± 2.5 and 90.3 ± 1.3 vs. 80.8 ± 2.9 , respectively) and regarding mitochondrial potential better than group 2 at 24 and 48 h (95.7 ± 0.7 vs. 91.3 ± 1.3 and 92.3 ± 1.5 vs. 87 ± 2.1 , respectively). In group 3, the acrosome

integrity decreased after 48 h (85.1 ± 3 vs. 80.3 ± 3 vs. 75.6 ± 3.6 for 0, 24 and 48 h, respectively), while in groups 1 and 2, this decrease was noticed after 24 h ($p < 0.001$). In conclusion, our results suggest that ASX supports extended boar semen preservation.

P 16 | DNA fragmentation assay in defrozen spermatozoa of Holstein bulls and insemination index

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Sperm DNA is an integral element in the success of animal reproduction. The aim of this study was to evaluate the sperm DNA integrity of frozen/thawed bull spermatozoa in order to establish the potential use of sperm DNA fragmentation (SDF) assay for improving the screening of fertility following AI. For evaluation of DNA fragmentation we used the kit «DNA fragmentation sperm chromatin dispersion test» Pvt. Ltd, India. Thawed samples were assessed by the DNA fragmentation assay of 10 Holstein bulls on the dairy farm «Amiran» LLC and 6 Holstein bulls on the dairy farm «Bayserke-Agro». A total number of 100 spermatozoa per sample (smears) were counted and divided in three distinct categories: non-fragmented DNA, fragmented DNA and degraded sperm. Five replicates of the analysis were performed. Average observed percentage of spermatozoa of the three categories in the samples of the 16 bulls with different status of chromatin is as follows: non-fragmented DNA – 65.7%; fragmented DNA – 32.8%; degraded sperm – 1.5%. Insemination index in the group (16 animals) ranged from 1.23 to 1.31. The maximum level of indicators for DNA fragmentation observed was: fragmented DNA – 37.9%, degraded sperm – 3.78%. These indicators correlate with the results of artificial insemination. Insemination index after AI of 125 cows by sperm of this 'maximum' bull was 1.87. It can be concluded that breeding bulls may be screened for their sperm DNA integrity in addition to the regular method for sperm evaluation to have a better conception rate after AI.

P 17 | May stereotypic behaviours affect sperm production of Arabic Stallion?

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This study aimed to study the link between stereotypies, considered as poor welfare indicators, and sperm production of stallions. Fourteen Arabic stallions housed in the National Stud Farm of Sidi Thabet,

situated in the north of Tunisia, were used during the mating season. In a first step, each horse was observed for three months, in its box using focal sampling and instantaneous scan sampling to identify qualitative (different types) and quantitative (respective frequencies) stereotypies that may be present. In a second part, semen of 11 stallions (total ejaculates = 33), was collected by an artificial vagina (AV). The collected fresh semen was evaluated for seminal parameters: volume, sperm concentration, percentage of motile spermatozoa and percentage of dead spermatozoa. Sexual behaviour parameters such as preparation time, collection duration and number of mounts in AV, were also recorded during semen collection. Mann-Whitney U test was used to compare fresh semen and sexual behaviour parameters between stereotypic and non stereotypic stallions. The threshold of significance was fixed at 5%. Our results revealed that 40% ($n = 6$) of the stallions showed at least one stereotypic behaviour. Weaving was the main stereotypy observed. There was no difference between stereotypic and non stereotypic stallions for the collection duration and the number of mounts in AV. However, the preparation time was shorter in the stereotypic animals than in the non-stereotypic ones (respectively 2.27 ± 2.0 s vs. 42.5 ± 14.0 s; $p < 0.05$). Besides, there was no significant difference between both groups regarding semen parameters. The results of this study suggest that stereotypies may not affect stallion's fertility; however, we recommend to extend this study to a larger number of animals.

P 18 | Influences of maternal progesterin concentration on conceptus development in mares

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Periovulatory progesterin concentration has been suggested to influence endometrial function and conceptus development. We investigated influences of postovulatory maternal progesterin concentration on early conceptus development in mares. Genitally healthy oestrous Haflinger mares ($n = 8$; age 4 to 14 years) were inseminated with 500 Mio progressively motile spermatozoa at 48 h-intervals until ovulation (ov). Mares were treated with either altrenogest (0.044 mg/kg once daily orally) on d5 to 10 after ov (ALT), cloprostenol (125 mg once daily intramuscularly) on d0 to 3 after ov (CLO) or were left untreated (control). ALT and CLO treatment aimed at increasing and decreasing total progesterin concentration in blood, respectively. Every mare received each treatment in consecutive cycles at random order. Blood for determination of progesterone was collected once daily. From d10, transrectal ultrasonography was performed daily for detection and measurement of a conceptus. Statistical analysis was performed by ANOVA using a general linear model for repeated measures with treatment and day as between subject factors or with chi-square analysis (pregnancy rates). Pregnancy rates per cycle were 100 (8/8), 72 (8/11) and 62% (8/13) after ALT, CLO and control treatment ($p = 0.056$). Progesterone concentration from d0 to 14 was lower after CLO than ALT and control

treatment ($p < 0.001$). Conceptus size on d14 was 1.7 ± 0.1 , 1.4 ± 0.1 and 1.6 ± 0.1 cm (\pm SEM) after ALT, CLO and control treatment (n.s.). In conclusion, CLO treatment but not ALT treatment during the early luteal phase impaired corpus luteum function in early pregnant mares. Despite an increased per cycle pregnancy rate in ALT treated mares, early conceptus size was not affected.

P 19 | DNA damage induced by addition of hydrogen peroxide is detectable with the COMET assay, but not with the SCSA™

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Oxidative stress in sperm causes DNA damage and adverse pregnancy outcomes. We aimed to investigate the effect of increasing oxidative stress on sperm DNA damage assessed using the SCSA and the COMET assay. For this purpose 44 cryopreserved bovine ejaculates were thawed and incubated for 1 h without H_2O_2 (non-oxidized sperm: NOX S) or with different concentrations of H_2O_2 (50, 100, 1000 μ M, oxidized sperm: OX S). Control sperm (CON S) were analysed immediately after thawing. COMET indices increased continuously upon incubation and H_2O_2 exposure ($p < 0.05$). The SCSA values behaved differently: during incubation time %DFI values increased ($p < 0.05$), but not after H_2O_2 exposure. Catalase, as an enzyme catalysing the decomposition of H_2O_2 to H_2O and O_2 , reversed ($p < 0.05$) the effect of incubation time on %DFI. The addition of DTT, an inducer of DNA decondensation, led to a rise of %DFI values ($p < 0.05$). This effect could be blocked by H_2O_2 . In conclusion we found that only the COMET assay, but not the SCSA™, is able to detect DNA damage after addition of hydrogen peroxide. High concentrations of H_2O_2 possibly induce a sperm DNA over-condensation resulting in reduced sensitivity to acid-induced denaturation measured by the SCSA™.

P 20 | Mammary gland edema as a cause of postpartum dysgalactia in the sow – a case report

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A herd with 1700 sows located in Finland reported an acute problem with postpartum dysgalactia in 30 to 40% of the sows. Sows were moved to the lactation unit five days before parturition where they received 3.8 kg per day of a 10.1 MJ/KG pregnancy diet containing 4.3% crude fiber. Parturient sows showed constipation, reduced water intake, inadequate colostrum quality (estimated with Brix refractometer) and abnormal swollen mammary glands. Ultrasound of the mammary glands (USM) showed thickened dermal and subdermal tissues, hyperechoic lobuloalveolar tissue with enlarged blood vessels and severe shadowing. Blood chemistry showed decreased potassium

and increased sodium-potassium-ratio compared to reference values. The sows were diagnosed with mammary gland edema due to a diet containing too high energy and too low fiber content. As a treatment, a gradual decrease in the amount of feed (3.8 to 2.7 kg/day) and increase in fiber (addition of 200 g/day of sugar beet pulp) during the last week of gestation was suggested. A control visit took place four weeks later. According to the producer, milk production had improved. Parturient sows had improved colostrum quality and constipation was reduced. Ultrasound of the mammary gland showed normal dermal, subdermal and lobuloalveolar tissue with mild shadowing. Blood chemistry showed normal potassium and sodium-potassium-ratio compared to reference values. This case described for the first time mammary gland edema as a herd problem and reason for postpartum dysgalactia and inadequate colostrum quality, seemingly caused by a diet low in fiber and high in energy during late pregnancy and parturition. Blood chemistry and USM proved to be valuable diagnostic tools.

P 21 | Intramammary infection with coagulase-negative staphylococci species in Swiss dairy cows: A longitudinal study

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Bacteriological status, evaluation of udder symmetry, udder hygiene and teat end scores of 92 dairy cows were assessed on 3 Swiss dairy farms in a longitudinal 1-year study to determine risk factors for intramammary infection (IMI) defined as ≥ 100 CFU per ml of milk with a coagulase-negative staphylococci (CNS) species. Farm visits were performed monthly including sterile quarter milk sampling and udder evaluation of all lactating cows. Milk samples were cultured using selective agar plates facilitating for detection of CNS. Species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Overall, 3,151 quarter samples were included in the statistical analysis. *S. chromogenes*, *S. haemolyticus*, *S. xylosum*, and a *S. warneri*-like species were the 4 most prevalent species found. Risk factors for *S. chromogenes* IMI were accorded to herd B, season and presence of udder edema. For *S. haemolyticus* season, co-infection with *S. xylosum* and other CNS species ("Others") were relevant risk factors. For herd B, early lactation, season and co-infection with *S. haemolyticus* and "Others" remained in the final multivariable model for *S. xylosum*. Mid- and late lactation, season and co-infection with *S. xylosum*, were identified as risk factors for *S. warneri*-like IMI. The estimated variance was highest on sample level except for *S. chromogenes* and the *S. warneri*-like species where 26% and 21%, respectively, were attributable to the cow level. Occurrence of different CNS species was herd specific and risk factors differed between species. However, season and co-infections seemed to be important for several species.

P 22 | Effect of super weak static magnetic fields on reactive oxygen species generation in bovine spermatozoa

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The interaction of static magnetic fields (SMF) with biological objects is a rapidly growing field of investigation. Previous research showed that strong SMF influences somatic mammalian cells causing proinflammatory changes and increasing reactive oxygen species (ROS) production. On the other hand, super weak SMF (0.3 μ T) affects proliferation and differentiation of skeletal muscle cells in the primary culture. Investigation of gametes' functioning in a shielded SMF is of great interest due to enhancing human presence in space, and agricultural animals are suitable models for the research. The aim of this study was to evaluate the effect of super weak SMF (0.3 μ T) on ROS generation in bovine sperm. Fresh semen was obtained from Holstein bulls. A special camera (Russian Patent No. 2324989) was constructed for shielding down geomagnetic field. Semen was washed twice in Sp-TALP medium and then incubated for 4 h in a CO₂-incubator at 5% CO₂, 95% humidity and 38°C. To detect ROS production sperm cells (12.5x10⁶ sperm/ml per sample) were supplemented with luminol (25 mM) and horse-radish peroxidase (11.52 U/ml). Chemiluminescence was monitored for 900 s. in a luminometer (Lum-5773) and the results expressed as integrated counts. Seven replicates were done in this study. There was a significant increase in the level of ROS generation in bovine spermatozoa incubated in the super weak SMF (678.9 \pm 47.5 eV \times s in experimental group vs. 490.7 \pm 98.2 eV \times s in control group, $p < 0.05$, Student's *t*-test).

P 23 | Claws dehydroepiandrosterone and dehydroepiandrosterone sulfate concentrations in newborn dogs

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DHEA and DHEA sulfate (DHEAS) in human newborns at birth are considered markers of fetal stress (Tegethoff et al. 2011, *Biol Psychol* 87:414–420). The hair and the claws could be useful, non invasive matrices for long time-frame retrospective hormonal analysis (Veronesi et al. 2015, *Theriogenology* 84:791–796). The aim of this study was to assess DHEA and DHEAS concentrations in the claws of newborn puppies, and to evaluate the possible influence of newborn age (premature, born dead, dead 1–10, 11–20, 21–30 days old), gender, and breed size on DHEA and DHEAS concentrations. The study, performed on 138 spontaneously dead purebred puppies, showed that

the overall mean DHEA and DHEAS concentrations in claws were significantly different ($p < 0.05$) even if highly correlated ($p < 0.0001$) (28.8 \pm 17.00 pg/mg and 36.5 \pm 23.13 pg/mg, respectively). DHEAS levels were significantly higher ($p < 0.05$) in small as compared to large breeds (39.2 \pm 25.45 pg/mg vs. 34.8 \pm 21.51 pg/mg), and in premature vs. born dead and vs. 11–20 days old puppies (48.7 \pm 28.63 pg/mg vs. 34.3 \pm 20.89 and 29.9 \pm 17.19 pg/mg, respectively) ($p < 0.05$). The effect of age on DHEAS levels in claws was previously reported also for DHEA hair concentrations in newborn puppies (Bolis et al. 2015, *Proc EVSAR* 33:283–290). DHEA and DHEAS in claws of newborn puppies can be considered markers of perinatal stress, useful for research investigations and for future clinical application.

P 24 | A case of simultaneous pregnancy, pyometra and fetal mummification in a cat

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Cases of concurrent pregnancy and pyometra in cats are described, but additional presence of a mummified fetus is an extremely rare finding. A free-roaming, around 4-year-old, domestic short hair cat was presented with loss of appetite. Clinical examination revealed mild dyspnea and abnormalities on abdominal palpation. Complete blood count showed leukocytosis, while serum biochemistry was within normal limits. Thoracic and abdominal radiographs in DV and lateral projections were performed. There was one normal in size and shape, mineralized fetus and one with decreased bone density and irregularly located, multiple ossification centers. Abdominal ultrasound was made using a 6.5 MHz microconvex probe (Samsung). It revealed a properly developed, approximately 55-day-old fetus in one uterine horn and enlargement of the other horn, with thickened wall and fluid accumulation. Additionally, in the abdominal cavity there was a 36 \times 24 mm mass of unknown origin, which contained focal mineralizations inside. No other abnormalities were noted in the abdomen. Ovariohysterectomy was elected, during which a free mummified fetus was found in the peritoneal cavity, between the intestinal loops. The fetus was not attached to any neighbouring organs. One of the uterine horns contained living fetus, while focal pyometra was present in the second. Uterine wall specimens were submitted for histopathological examination. The cat was treated with a standard dose of amoxicillin/clavulanic acid, recovered well and was set free 10 days after surgery.

P 25 | Fungal isolation from bovine milk samples in an Italian dairy farm: preliminary results

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Aim of the study was to isolate yeasts from quarter milk samples of cows in one Italian dairy herd. A total of 304 quarters were classified as healthy (H) (negative CMT score, normal milk and no clinical changes of the udder), subclinical mastitis (SCM) (positive CMT score, normal milk and udder), clinical mastitis (CM) (positive CMT score, modified milk and udder). Milk samples were aseptically collected in a sterile tube, stored at 4–8°C, plated within 24 h onto malt extract agar added with biphenyl 0.1% and gentamicin 0.1%. Plates were incubated at 25°C and daily examined over a 21 day period. Yeasts' identification was achieved on the basis of macro- and microscopic features of colonies and by means of a technique of carbohydrate assimilation using commercial galleries (ID32C). Yeasts prevalence was calculated. A Chi-square test was applied for H and SCM+CM in relation to presence/absence of yeasts. Statistical significance was set at $p < 0.05$. The H quarters were 65.5%, the SCM 27% and the CM 7.5%. Yeasts were isolated in 5% of H, 2.3% of SCM and 0.7% of CM quarters. The prevalence of fungi was: *Candida* spp. 5.6% (*Candida curvata*, *Candida famata*, *Candida rugosa*, *Candida catenulata*, *Candida guilliermondii*, *Candida sphaerica*), *Rhodotorula* sp. 2.6%, other yeasts 1%. Chi-square test was not statistically significant. Our results confirmed that fungal species usually associated with mastitis are present also in normal milk.

P 26 | Changes in the productive and reproductive parameters of growing gilts induced by supplementing the api byproduct drone brood

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The nutritional value of drone larvae is well known for thousands of years. Recent studies have proven that this product is a donator of the hormones: prolactin, estradiol, progesterone, testosterone and can be used for growth promotion in animal husbandry instead of banned anabolic compounds. The present study aimed to analyze the effect of supplementing drone larvae to gilts diet on the productive and reproductive parameters. Twenty Large White gilts at 35 days age from the experimental base of Penza State Agricultural Academy were randomly divided in two groups. The control group received a standard diet. The experimental animals' diet was supplemented with 25 mg/kg/forage dry powder of drone brood till the age of 170 days. The productive parameters as well as the histological and immunohistochemical estimation of the gilts' ovaries after slaughtering were assessed. The treated animals manifested a higher body weight and loin eye area (with 7% and 9%, respectively), but had a delay in puberty onset compared to the controls. The ovaries of treated gilts showed an enhanced immunostaining for the growth factor GDF9. In conclusion, the application of this supplement should be carefully used and the dose should be limited in the replacement gilts due to its androgenic effect. (The research supported by grant N458ГC1/9751 from Foundation for Assistance of Small Innovative Enterprises.)

P 27 | Effect of sodium hyaluronate (HA) on liquid stored and cryopreserved boar semen

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The study was performed to determine the effect of sodium hyaluronate (HA) on quality of liquid and cryopreserved boar semen. The experiment was divided into two series. In first serie seven ejaculates were liquid preserved in Biosolwens Plus (BP)- control extender (E1) and BP supplemented with 0.5% HA (E2), 1% HA (E3) and 2% HA (E4). In second serie ten ejaculates were cryopreserved in egg-yolk lactose extender (LEYG) supplemented with: 0.5% HA (E5), 1.0% HA (E6), 2% HA (E7). Only ejaculates with >80% progressively motile (PM) sperm and 80% morphologically normal spermatozoa were used for experiment. The quality of fresh, 6 day stored and cryo-preserved semen was verified based on % of viable sperm with intact acrosome (PNA-/PI-), % of live sperm with the apoptotic-like changes (AnV+/PI-) and % TUNEL+ sperm nuclei. A statistically higher ($p \leq 0.01$; Duncan test) % of PNA-/PI- and lower AnV+/PI- sperm was observed in E2 (63.3 ± 1.4 ; 6.3 ± 1.0) compared to E1 (50.9 ± 3.5 ; 14.2 ± 2.3), E3 (54.2 ± 1.8 ; 12.6 ± 1.7) and E4 (55.1 ± 2.3 ; 11.8 ± 1.4). After freeze-thawing statistically lower % of PNA-/PI- and higher AnV+/PI- was identified in E5 (46.7 ± 3.2 ; 15.2 ± 2.4) compared to E6 (65.4 ± 2.6 ; 8.2 ± 0.9), E7 (63.7 ± 1.5 ; 9.0 ± 1.3). The TUNEL-positive sperm nuclei were ranged from 1.9 ± 0.3 to 2.1 ± 0.5 in all extenders and did not differ significantly. Our study demonstrates that extender supplemented with 0.5% HA resulted in the highest quality of liquid preserved spermatozoa at day 6 of storage. While the addition of 1% or 2% HA to LEYG cold overcome cryodamage and improve viability and acrosome integrity after freeze-thawing. (The study received funding from the National Centre for Research and Development under project agreement No.297267/14/2016 BIOSTRATEG 2).

P 28 | Two natural antioxidants and boar frozen semen: surprising effects

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Previous studies showed that 2 μ M resveratrol (R) and 50 μ M epigallocatechin-3-gallate (EG) exert positive effects on in vitro fertilizing ability of boar sperm if added after thawing. This research was aimed at studying the effects of the combination of the two antioxidants on sperm motility, viability (SYBR 14/PI), acrosome integrity (PSA-FITC), lipid peroxidation (BODIPY), DNA integrity (SCSA), and IVF in frozen thawed boar sperm after 1 h incubation post-thawing. Sperm parameters were evaluated by flow cytometry and CASA on

4 groups: CTR, R, EG and R+EG. The same groups were tested for IVF. No difference in viability, acrosome integrity and lipid peroxidation were found. A significant decrease in total and progressive motility was observed in R and R+EG groups (TM %: 43.7 ± 11.8 , 39.0 ± 12.3 , 8.3 ± 4.5 and 8.5 ± 5.4 ; PM %: 17.8 ± 4.2 , 15.3 ± 4.8 , 1.8 ± 1.5 , 2.2 ± 2.1 in CTR, EG, R, R+EG respectively; $p < 0.05$), as well as an increase in DNA damage (COMP %: 12.9 ± 6.3 , 12.5 ± 3.4 , 45.2 ± 25.5 , 48.5 ± 28.7 ; $p < 0.05$). A significant increase in the penetration rate was evident in EG, R, R+EG groups, (46.7 ± 12.5 ; 64.2 ± 14 ; 74.7 ± 12.4 , 79.2 ± 7.3 , $p < 0.01$); no synergistic effects were observed. These results demonstrate that the association of the two natural antioxidants exerts a positive effect on in vitro fertilizing ability of boar spermatozoa, with a negative effect on both motility and DNA integrity in R and R+EG groups. (Supported by "Fondazione del Monte di Bologna e Ravenna".)

P 29 | Use of a multi-color flow cytometric assay for the evaluation of bull fertility

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Our study aimed at the evaluation of a four-color flow cytometric assay for the characterization of bull fertility. Cryopreserved semen of five Brown Swiss, two Swiss Fleckvieh, two Red Holstein and one Simmental bull housed in station A ($n_A = 10$), and of 20 Holstein-Friesian bulls housed in station B ($n_B = 91$) was used. The 56-day non-return rate (NRR) of the bulls ranged from 50% to 79%. Bulls were assigned to two equal groups (fertile vs. sub-fertile) based on the population's mean NRR \pm SD criterion. After 0 (0 h) and 3 h of post-thaw incubation, sperm kinematics and DNA integrity were evaluated with computer-assisted sperm analysis (CASA) and SCSA™, respectively. A flow cytometric panel including propidium iodide, calcein violet AM, Fluo-4 AM and DiIC1(5) was used for the quantification of sperm sub-populations with intact plasma membrane, high esterase activity, low intracellular Ca^{2+} levels and high mitochondrial activity, respectively. The importance of sperm traits as predictors of NRR was explored with all-subsets regression. Sperm traits assessed with multi-color assay (MC) at 0 h and 3 h explained up to 40.33% and 24.72% of the total NRR variance, respectively; the inclusion of CASA and SCSA™ data increased the respective ratios to 61% (0 h) and 44% (3 h). Among MC traits, the percentage of sperm simultaneously showing high esterase and mitochondrial activity, or intact plasma membrane, high mitochondrial activity and low Ca^{2+} levels (0 h) were the most important determinants of NRR. CASA, SCSA™ and MC traits at 0 h and 3 h were not related to the breed of the bull ($p > 0.05$). In conclusion, the applied multi-color panel offered additional information on sperm quality not gained through other methods and contributed to the determination of bull fertilizing potential.

P 30 | The use of toluidine blue in the evaluation of chromatin morphometry and condensation in epididymal spermatozoa of cats

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Sperm evaluation for assisted reproduction rarely considers the integrity of DNA, which is crucial for embryonic development. Toluidine blue staining (TB) allows for simultaneous evaluation of chromatin changes and sperm morphometry. The method has been described in several species, however not in cats. The objectives of this study were to verify the applicability of the TB technique in evaluating DNA abnormalities of epididymal spermatozoa (caput, corpus and cauda) of 30 cats, and to investigate the correlation between the variables: DNA condensation and morphometry of the spermatid head. For this purpose, DNA modification indicators obtained by TB and acridine orange (AO) techniques were compared, and a correlation of 65.38% ($p < 0.001$) was observed. Correlation between DNA integrity and morphometry was noted. A significant decrease in sperm head size was identified during the crossing of the three-epididymal regions ($p < 0.0001$). The percentage of sperm cells with condensed chromatin increased significantly from the caput region to the cauda of the epididymis (73.64%, 84.31%, 96.62%, respectively, $p < 0.0001$). When comparing both techniques, we obtained a higher proportion of spermatozoa with chromatin alteration evidenced by TB (caput: 26.36%, corpus: 15.69% and cauda: 3.38%) than by AO (caput: 7.94%, corpus: 3.84% and cauda: 2.06%) regardless of the epididymal portion. This occurs because TB induces metachromasia, even when bounded to a few molecules of the DNA-protein complex, identifying therefore, more subtle changes. In conclusion, the TB staining technique can be employed in cats, allowing for the analysis of sperm DNA condensation, being more sensitive than the acridine orange staining.

P 31 | Role of heat shock protein 90 (HSP90) during heat stress in boar sperm

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Heat Shock Proteins (HSP) are present in all organisms and its activation is triggered by high temperatures to prevent protein denaturation. The aim of this work was to study the role of HSP90 in boar sperm subjected to heat stress using 17-AAG, a specific HSP90 inhibitor. To this purpose, boar sperm were incubated in non-capacitating medium (TBM) for 24 h or in capacitating medium (TCM) for 8 h at 38.5°C (Control) or 40°C (Heat stress) in the presence of 5 or 20 μ M of 17-AAG. After incubation, the parameters evaluated were: total motility

(TM), progressive motility (PM), viability (SBYR+/PI-), acrosome status (PNA+/IP-), mitochondrial membrane potential ($\Delta\Psi_m$) (JC-1), phosphatidylserine externalization (Anexin+/IP-) and membrane lipid organization (merocyanine+/Yopro-). A one-way ANOVA was used to compare values. In TBM, TM (22.7 ± 4.1 vs. 1.9 ± 1.1 ; % mean \pm SEM), PM (3.1 ± 0.9 vs. 0) and high $\Delta\Psi_m$ (19.5 ± 2.2 vs. 11.8 ± 0.8) decreased significantly at 40°C in presence of 20 μ M 17-AAG (control vs. 20 μ M 17-AAG, respectively; $p < 0.05$). However, at 38.5°C only a mild decrease in TM was observed (48.7 ± 3.1 vs. 32.1 ± 4.8 ; control vs. 20 μ M 17-AAG, respectively; $p < 0.05$). In TCM at 38.5°C a decrease in high $\Delta\Psi_m$ was observed (50.5 ± 3.6 vs. 33.4 ± 3.7 ; control vs. 20 μ M 17-AAG, respectively; $p < 0.05$). The rest of parameters analyzed remained unaffected. In conclusion, HSP90 is involved in the maintenance of boar sperm motility and $\Delta\Psi_m$ during prolonged heat stress in non-capacitating conditions, and in the maintenance of $\Delta\Psi_m$ under capacitating conditions.

P 32 | Treatment efficacy for experimentally induced ascending placentitis in mares

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The overall goal of this study was to assess the effectiveness of various therapeutic combinations of a long-acting estrogen (estradiol cypionate; ECP) and a long-acting progestin (altrenogest; ALT) in addition to a basic treatment for placentitis with trimethoprim-sulfamethoxazole and flunixin meglumine (TMS+FM). Pregnant mares (300 d gestation, $n = 46$) were randomly split into a control group (CONT, $n = 8$), and experimentally induced ascending placentitis groups ($n = 38$). Ascending placentitis was experimentally induced via intra-cervical inoculation of *Streptococcus equi*. Thereafter, induced mares were randomly assigned into: TMS+FM ($n = 8$); TMS+FM+ALT ($n = 8$); TMS+FM+ALT+ECP ($n = 6$); TMS+FM+ECP ($n = 6$); and no treatment (INOC, $n = 10$). Treatments were started 48 h after bacterial inoculation and carried out for 10 d. Continuous data were analyzed by ANOVA, and categorical data analyzed by Fisher's exact test. Mares in the TMS+FM+ECP (346 ± 5 ; 46 ± 4 d) and CONT (335 ± 5 ; 35 ± 5 d) groups had the longest gestation lengths and induction to delivery intervals. However, gestation length for these groups was similar to TMS+FM+ALT+ECP (330 ± 11 ; 22 ± 6 d). Foal survival at parturition and 7 d post-delivery were similar across treated groups (66.7–100%), and CONT group. Similar to CONT, TMS+FM+ECP had no high-risk foals; other treated groups had higher incidences (50–75%) ($p < 0.05$). The inclusion of ECP in the treatments resulted in foals with body weight similar to CONT group ($p > 0.05$). In conclusion, mares with experimentally induced ascending placentitis benefited from estrogen supplementation, but progestin supplementation did not appear to make a difference in outcomes. (Funds: CNPq, FAPERGS, and CAPES (Bolsista – Brasil #99999.005570/2015-08).)

P 33 | First case of XX-SRY positive DSD in an Italian saddlebred horse

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A 15 month old Italian saddlebred horse was submitted to clinical examination due to the presence of some abnormalities to his external genitalia as well as stallion-like behaviour. The physical exam highlighted the absence of the scrotum and a slight mammary gland development. A small penis of 11 cm in length was present in the ventral perineal region. Urination occurred through a urethral fossa at the distal end of the penis. The transrectal ultrasonography to visualise the internal genitalia was unrewarding. Under general anesthesia, two bilaterally symmetrical hypoplastic testes were removed from the inguinal canals using a standard closed orchiectomy technique. The testicles were submitted for histopathological examination. A blood sample was collected to perform a cytogenetic and molecular analyses, which revealed a disorder of sexual development (DSD) with a normal female karyotype ($2n = 64$; XX), the presence of SRY gene with normal sequence and the absence of ZFY gene. The XX-SRY positive DSDs have rarely been diagnosed in domestic animals, up to now it has been reported in a tortoiseshell cat and a Holstein cattle. The horse was diagnosed as a XX-SRY positive DSD and, to our knowledge, this is the first time that this type of DSD is found in equine species.

P 34 | Increased free fatty acids concentration impairs bovine endometrial epithelial cell proliferation in culture

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Negative energy balance (NEB) and increased plasma concentrations of non-esterified fatty acids (NEFA) are associated with ovarian and uterine dysfunction in postpartum cows. Previous studies showed that oleic (OA), palmitic (PA), stearic (SA) and their combination compromise proliferation of bovine granulosa and theca cells in vitro. This study aimed to investigate impacts of NEFA on proliferation of bovine endometrial epithelial cells (bEEC) in culture. Post-primary bEEC from 3 cows (passage 4) were cultivated in 96-wells plates. bEEC were exposed to 150, 300 or 500 μ M of individual OA, PA, SA and their combination (150 μ M of each NEFA) in 200 μ l of new medium, and 0.5% final concentration of ethanol (vehicle for NEFA) was used as control. At 0, 6, 24 and 48 h after NEFA exposure, 20 μ l WST-1/ECS solution (Quick

cell proliferation test, Abcam®) was added to each well and incubated for 4 h. Cell proliferation was measured from absorbance at 450 nm with a microplate reader. Experiments were performed twice, with two replicates measured per time X treatment. Cell proliferation ratios were calculated and compared to control at each time point and effects of treatment and time analyzed by ANOVA. bEEC proliferation was significantly decreased after exposure to 300 µM of PA and 500 µM of three single NEFA when compared to respective control ($p < 0.05$). Results did not differ from controls when 150 µM of their combination was used at any time point. At 6, 24 and 48 h, after NEFA exposure the decrease in cell proliferation was higher with 500 µM of OA and PA than with SA. These results indicate that NEFAs impair cell proliferation in a dose- and time-dependent way, and that NEB may affect endometrial cell function and epithelia remodeling in the postpartum period.

P 35 | Sperm chromatin integrity in stallions with *Lepidium meyenii* (Maca) dietary supplementation

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Stallions used for reproduction are selected for their appearance, performance, pedigree and lineage. It is often necessary to improve semen quality and preserve it for assisted reproductive technologies using safe and effective methods. *Lepidium meyenii* (Maca) is a traditional Andean crop that grows in the Peruvian Highlands. It has fertility-enhancing and antioxidant properties. In this study, the effect of dietary supplementation with Maca on chromatin integrity of fresh (at D0, D1, D2, D3, D4) and cooled-stored stallion sperm (after 24, 48 and 72 h) was assessed using the TUNEL assay. Animals were divided in: control (C; $n = 5$) and a treated group (M; $n = 5$). Animals from both groups received the standard diet but the M group received additionally daily food supplemented with 20 g of Maca for 60 days (from May to June 2015). The highest percentage of DNA strand breaks was found in cooled-stored sperm from animals belonging the C group in comparison to M group (D0: 10.04 ± 4.1 and $12.1 \pm 2.5\%$, respectively, and D4: 10.7 ± 3.2 and 5.9 ± 1.8 , respectively, $p < 0.05$). This percentage increased during the storage time, but significantly lower in M respect to C (D4 C: 33.2 ± 4.1 ; 64.3 ± 3.6 ; 70.6 ± 4.8 at 24, 48, 72 h, respectively; D4 M: 15.6 ± 2.9 ; 20.4 ± 5.3 ; 30.2 ± 4.5 at 24, 48, 72 h, respectively; $p < 0.05$). In conclusion, oral administration of Maca improves semen quality in stallions and enhances cooled storage.

P 36 | Effects of extremely low frequency electromagnetic field on swine oviductal epithelial cells

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All living beings are exposed to different electromagnetic fields (EMFs), either of natural or artificial origin. In particular, extremely low frequency-EMFs (ELF-EMFs) are classified as possible carcinogens (class 2b) by IARC but data on their potential negative effect on reproduction are still not conclusive. Based on our previous studies, here we investigated the effect of an ELF-EMF (sinusoidal wave; 0.75 mT intensity; 50 Hz frequency) on morphology, ion balance and mitochondrial function of swine oviductal cells. We in vitro cultured cells under either control conditions (sham) or exposing them to the field. After the exposure, we assessed cell phenotype (Hematoxylin-eosin staining), actin filaments and microtubule organization (FITC-phalloidin and immunofluorescence, respectively), mitochondrial activity (MitoTracker), and intracellular $[Ca^{2+}]$ (Fluo3-AM). We found that field exposure didn't exert any detectable effect on doubling time, cell morphology, tubulin organization; while it induced stress fibers formation in a higher percentage of cells (sham 15% vs. 70% exposed, $p < 0.05$). Intracellular $[Ca^{2+}]$ and mitochondrial activity were affected (exposed 58% and 63% compared to sham, respectively; $p < 0.0001$). The field didn't induce cytotoxic effects, but it could affect important metabolic function of the oviductal epithelial cells, thus suggesting a possible interaction with reproductive function.

P 37 | Cryopreservation of stallion sperm using sucrose as alternative to glycerol: preliminary results

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The aim of this study was to assess sucrose-based extenders as an alternative to glycerol for freezing stallion semen in terms of post-thaw plasma membrane integrity and acrosomal status. Semen samples were collected from 4 stallions by artificial vagina. Thereafter, semen was divided in 2 aliquots and centrifuged at $600 \times g$ for 10 min. The sperm pellets were resuspended with commercial Gent extender for stallion sperm freezing (Minitübe, Tiefenbach, Germany) containing glycerol (G) or adding 1% BSA and sucrose at final concentration of 0.1Molar (S) instead. After that, sperm were frozen-thawed using a standard protocol. Plasma membrane intact (PMI, %), acrosome-intact (AIS, %), reacted (ARS, %) or denuded (ADS, %) sperm were assessed by epifluorescence microscopy. Post-thaw sperm parameters were compared between extenders by ANOVA. The results were expressed as mean \pm standard error of the mean. No significant differences ($p > 0.05$) were found for PMI (69.00 ± 2.94 vs. 73.25 ± 1.25) and ARS (17.00 ± 4.38 vs. 8.58 ± 3.28) between G and S extenders. Additionally, S extender obtained higher values ($p < 0.05$) for AIS in comparison to G (27.25 ± 8.01^b vs. 66.13 ± 8.39^a). Moreover, G extender showed significant higher values ($p < 0.05$) for ADS in comparison to S (55.75 ± 10.39^a vs. 25.3 ± 5.55^b). In conclusion, sucrose

can be used as an alternative to glycerol for freezing stallion semen in terms of protecting plasma and acrosome membrane integrity. Further studies are needed including assessment of a larger number of samples and sperm parameters.

P 38 | Effect of anti-gonadotrophin-releasing hormone vaccination on the weight gain and testicular ecotexture of bull calves

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The objective of this study was to evaluate the effect of the anti-GnRH immunization on the testicular echogenicity (TEC), the average daily (DWG) and total weight gain (TWG) of pre- and peripubescent calves. For this study, 18 Girolando calves at an average age of 5 months were selected. The calves were divided into 3 groups: control (CG, n = 6), animals not subjected to the vaccination; prepubescent group (G1, n = 6), 1 ml of anti-GnRH Bopriva[®]/sc vaccine once a month for 3 months, started on 5 month old animals; peripubescent treatment group (G2, n = 6) vaccination of animals from 9 months up, treated at the same interval as the G1. All of the calves were evaluated monthly through testicular ultrasonography and weighing during a period of 9 months and data were analyzed by GLM model. There was increase in the TEC of all animals from 6 months of age, regardless of the experimental group. The animals of G1 presented a decrease in testicular echogenicity after the 3rd dose of the vaccine, however, reestablishing the echo pattern at the end of the experiment. Animals in the three groups presented positive values for DWG. However, the CG presented less TWG (126.17 ± 26.6 kg) as compared to G1 (168.17 ± 21.72 kg) and G2 (164.14 ± 42.79 kg). In conclusion, the increase in TEC was a result of the development of the testicular parenchyma, phenomena related to puberty, which occurred regardless of the experimental group. Nevertheless, anti-GnRH vaccination was responsible for the temporary decrease in TEC when used in prepubescent animals. The transitory interference in gonadal development and sexual repression in treated animals resulted in greater total weight gain, which supports the theory of better productive performance in animals submitted to repression of sexual activity.

P 39 | Fragmentation index of DNA sperm in llama semen (*Lama glama*)

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DNA fragmentation of spermatozoa is an important indicator of seminal quality and fertility. The quality of the genetic information

contained in the DNA molecule transmitted by the spermatozoa is important for the proper development of the embryo. In humans it has been shown that there is a significant correlation between sperm DNA damage and oocyte fertilization, embryo quality, development to blastocyst and embryo implantation. The objective of this study was to analyze the fragmentation of DNA sperm in llama semen collected by post-copulation vaginal aspiration. The collection of 4 llamas was performed on three occasions per animal. The volume, motility, concentration, vitality, sperm membrane functionality assessed with the Integrated Semen Analysis System (ISAS). The DNA fragmentation index was tested with the Halomax kit (Halotech DNA, Spain). Volume, motility, vitality and sperm concentration respectively were 1.3 ± 0.8 ml, $12.30 \pm 2.02\%$; $64.69 \pm 11.39\%$ and $183.55 \pm 28.48 \times 10^6$ sperm/ml. The functionality of the sperm membrane was $64.95 \pm 11.63\%$ and sperm DNA fragmentation index $11.58 \pm 13.25\%$. The values of these different parameters indicate the high quality of the collected semen.

P 40 | Effect of ZnO:Eu nanoparticles on kinetic sperm parameters and apoptosis in mice testis

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There's limited data about nanoparticles (NPs) in reproductive biotechnology. The main problem with NPs application is their cytotoxicity, which is specific for each NP. Thus, influence of each material on specific organ, tissue or cells should be investigated. Therefore, the aim of our study was to determine the effect of biodegradable Europium-doped zinc oxide nanoparticles (ZnO:Eu) on kinetic sperm parameters and cell apoptosis in mice testis. The suspension (0.3 ml) of ZnO:Eu in water [10 mg/ml] was administrated by gavage to mice at age 3–6 months. For control group pure water was administrated. Mouse (n = 21) were sacrificed after 1, 7 and 14 days after NPs administration. Sperm was isolated from vas deferens and analyzed in Sperm Class Analyer 5 (Microptic S.L, Spain). We observed no significant differences within groups in sperm concentration, motility and straightness (STR), but there was significant increase of straight-line velocity (VLS) and linearity (LIN) 14 days after ZnO:Eu application. In order to investigate whether ZnO:Eu nanoparticles cause apoptosis in testes, we performed microscopic evaluation based on immunofluorescence (staining for active caspase-3 and p53 protein expression). Compared with control group we observed no increased expressions of either between experimental groups. Above results suggest, that ZnO:Eu nanoparticles exhibited neither toxic, nor pro-apoptotic effects in male reproductive system. Furthermore, no negative effect on sperm concentration and motility was observed. In addition we observed positive effect of ZnO:Eu on VLS and LIN on 14 day after distribution. (Acknowledgments: NCN grants: UMO-2012/05/E/N24/02994 and 20/0139/N/ST3/04189).

P 41 | Correlation between systemic oxidative stress markers and semen parameters by sperm class analyzer (SCA) in dogs

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Artificial insemination is becoming more common in domestic dog breeding and in the ex-situ conservation programs for endangered wild canids. Sperm cells under aerobic conditions produce reactive oxygen species (ROS), mostly originated from normal metabolic activity. The aim of this study was to investigate the correlation between oxidative status in dogs and sperm quality assessed by Sperm Class Analyzer (SCA). Ten male dogs, 7 fertile and 3 infertile, aged 1 to 7 years old were included in this study. Each animal was submitted to clinical examination and semen collection and evaluation. Oxidative status was evaluated by d-ROMs test and Oxy-Adsorbent test in blood samples and the ratio between ROMs and OXY (%) was calculated and used as index of plasma oxidative state (OSi). Comparison between fertile and infertile dogs performed by Kruskal-Wallis analysis of variance ($p < 0.05$) showed significant differences between the two groups for the following kinetic parameters: VAP, VSL, BCF, STR, LIN, MOT%, PROG, RAPID, MEDIUM, SLOW, STATIC, OSi. Correlations between each kinetics parameter and OSi were evaluated separately in each group by Spearman test (high correlation: $\rho = 0.6-0.9$, $p < 0.05$): in infertile group a high positive correlation were demonstrated between OSi and VCL, SLOW, STATIC. A significant negative correlation was present between OSi and MOT and RAPID. In fertile group a significant positive and negative correlation were evaluated between OSi and SLOW and between OSi and PROG respectively. This study reports, for the first time, a direct correlation between oxidative stress and the main parameters of the fertility test in both fertile and infertile dogs. These results open new diagnostic, prognostic and therapeutic insights for canine male infertility.

P 42 | Effect of the blood chimerism in the reproductive tract: preliminary results in eight twin foals

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Chimerism is a genetic condition in which an individual carries two or more cell lines with different genetic background. In mammals, this pathology was described in two forms: 1) true chimerism, which originated from an early fusion of two embryos, 2) blood chimerism, which is produced by the placental fusion between fetuses. This condition was barely reported in horses since twinning is a rare condition.

Therefore, its reproductive effect is still not clear. In this study we analysed 8 foals (3 pairs of Pura Raza Española (PRE) and 1 pair of Criollo Argentino (CRA)) derived from twin deliveries. Animals, which ranged from 6 months to 1 year at the moment of the analysis, were cytogenetically characterized using classic and molecular methods. Karyotyping and C-banding were performed on metaphases obtained from whole blood cultures. Additionally, 7 STR markers (five located in ECAX and 2 located in ECAY) were determined in DNA obtained from blood and hair follicles separately. Karyotype showed two different cell lines only in 6 of the individuals (2 PRE and 1 CRA pairs). C-banding determined that those differences were located in the sex pair (64,XX/64,XY). Those results were confirmed by molecular analysis, showing results compatible with blood chimerism in 6 individuals. The remaining pair was characterized as normal twin brothers. Internal and external reproductive organs were characterized as normal and in agreement with the age of the foals. Those findings suggest that blood chimerism has no effect on the development of the reproductive tract of horses, and agree with previous studies that suggested that only true chimerism can affect their fertility. This report also showed that blood chimerism is not present in all the foals delivered after twin pregnancies.

P 43 | A retrospective study on uterine and vaginal prolapse in female dromedary

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The objective of the present study was to investigate the incidence and clinical findings associated with both vaginal and uterine prolapse in female dromedary camel. A total of 65 female dromedary camels were admitted to the veterinary teaching clinic, Qassim University, central of Saudi Arabia (15 uterine prolapses and 50 vaginal prolapses) for the correction of a complete or partial prolapse of the genital tract ante or post partum (p.p.). The prolapsed part was treated by manual reduction followed by vulvar suture using a Gerlach needle and a suture tape of gauze. Broad spectrum antibiotics and anti-inflammatory drugs were parenterally administered. Results of the present study revealed that 61.5% of the vaginal prolapses took place in non-pregnant females. We found prolapses just p.p. (30.8%), less than 3 months p.p. (24.6%), 6-9 months p.p. (30.8%) and 1 year p.p. (15.48%), respectively. 24.6% of the camels affected had a history of dystocia followed by a prolapsed vagina or uterus and 20% of them had dead fetuses. The duration of the prolapse until admission was 12-36 h pp (uterine prolapse) and 2-15 days (vaginal prolapse). 15.4% of the reduced prolapses recidivated after correction within 1 to 3 days. Mortality rate was 15.4% in dams suffering from uterine prolapse, mostly due to rupture of the uterine wall, severe bleeding, exteriorization of the intestinal disc and subsequent peritonitis. It can be concluded that the prolapse of the vagina and the uterus is a serious problem which necessitates immediate intervention and most often occurs after parturition. Mortality is more frequent in dams suffering from uterine prolapse.

P 44 | Subclinical endometritis in repeat breeder female dromedary

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The objective of the present study was to investigate the microbial involvement in subclinical endometritis in female dromedary camels with repeat breeder syndrome. A total of 61 female dromedary (age: 6.92 ± 0.85 years; parity: 1.10 ± 0.02) with three or more consecutive matings (6.03 ± 0.45 ; period of infertility: 14.46 ± 3.31 months) without conception were included in this investigation. No vaginal discharge was found. History and signalment were recorded, transrectal examination including ultrasonographic examination and the sampling of uterine swabs were performed. All swab samples were primarily cultured on Blood Agar (BA) for bacterial growth and on Sabouraud-Dextrose-Agar (SDA) for fungal growth. Pure bacterial colonies were preserved in slants of either Nutrient Agar (NA) or Mueller-Hinton Agar (MHA) according to their degree of fastidiousness, while fungi were preserved in SDA slants. The following bacteria and fungi were isolated: *Staphylococcus spp.* (9.6%), Gram-positive *Bacillus spp.* (6.5%), Gram-negative *Bacillus spp.* (6.4%), *Candida spp.* (5.3%), *Staphylococcus aureus* (4.3%), *E. coli* (2.1%), *Corynebacterium sp.* (1.7%), *Pasteurella sp.* (1.7%) and other enterobacteria (1.7%). In conclusion, isolation and culture of endometrial swabs are important for the diagnosis of subclinical endometritis in repeat breeder female dromedary. *Staphylococcus spp.* and *Bacillus spp.* were the most prevalent bacteria found.

P 45 | Donkey sperm vitrification as alternative to conventional freezing: preliminary results

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Sperm vitrification in the absence of permeable cryoprotectant agents has been recently achieved using sucrose-based extenders and high cooling and warming rates in several species but not donkeys. Considering the higher sensitivity they have to permeable cryoprotectants, the aim of this study was to determine the effect of different sucrose concentrations (molar, M) for jack sperm vitrification. Ejaculates were collected from four Andalusian Donkeys by artificial vagina. Vitrification was performed using an extender for semen cooling (Gent Minitübe, Tiefenbach, Germany) used as control (C) and adding different sucrose concentrations: 0.1M (S1), 0.2M (S2), and 0.3M (S3). Thereafter, 30 μ l suspensions of cells were immersed directly into liquid nitrogen from each treatment. Thawing procedure was carried out by submerging frozen spheres in INRA-96[®] extender at 42°C. Permeable membrane integrity (PMI, %) was compared between treatments by ANOVA followed by Duncan's post-hoc test and expressed as mean \pm standard error. Results

showed PMI was significantly higher in the three sucrose extenders S1 (41.86 ± 3.65), S2 (39.18 ± 3.31) and S3 (38.37 ± 2.66) than control without sucrose (30.71 ± 3.21). Therefore, sucrose seems to protect the membrane sperm cell during vitrification in the absence of permeable cryoprotectant, although further studies are needed including the assessment of a larger number of samples and sperm parameters.

P 46 | Positive effect of semen collection training on *Melopsittacus undulatus* semen quality

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This study aimed at assessing whether training birds at semen collection improves semen quality. Nineteen healthy *Melopsittacus undulatus* males, aged 1 to 4 years, were divided in two groups, L) low semen quality (N = 9) and H) high semen quality (N = 10). Semen was collected into graduated microcapillary tubes, twice-weekly, in two following 30-day periods in full breeding season (February and March), using a modified massage technique. The ejaculates were directly evaluated for colour and volume, then diluted (modified TALP, pH 8.2, 37.5°C). Semen concentration, motility and kinetic parameters (VAP, VSL and VCL) were measured using a CASA system (CEROS; Hamilton Thorne Research Inc.). The training effect on semen quality was analysed by comparing the semen parameters values of the two periods of collection in the two parrot groups, using the Student's T test for normally distributed data and the Wilcoxon's Rank Sum test for non-normally distributed ones. Forty ejaculates of group L (17 of which in the first period) and 48 of group D (18 of which in the first period) were analysed. Comparing the two groups of birds, Group H showed significantly higher values of VAP, VSL and VCL in the first period and higher values of semen volume and VCL in the second period. A significant increase in semen volume, VAP, VSL and VCL was recorded in the second period in both groups. Group H showed also a significant increase in sperm concentration. This study underlines the importance of training the semen donors, both for increasing the performance of suboptimal birds and for improving the performance of the better donors.

P 47 | A case of cutaneous malignant melanoma of canine mammary gland

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Malignant melanoma (melanoma) derives from the pigment-producing cells (melanocytes), predominantly located in the skin, mucous membranes, eye and choroid. In dogs, melanomas most

commonly occur on the skin and in the mouth, but in the mammary gland are usually metastatic. The incidence of primary melanoma involving mammary gland is very rare, accounting for less than 5% of all melanomas in human. Melanoma of the mammary gland can be a primary melanoma of the mammary skin (cutaneous melanoma), a melanoma metastasis to the mammary gland, an in-transit metastases to mammary tissue and skin, or a primary malignant melanoma of the mammary gland. An 8-year female yorkshire terrier with a tumor in the 4th right mammary gland and without other clinical signs was presented. After mastectomy tissue samples were collected for histopathological examination by the Fontana method and immunohistochemistry for cytokeratin (CK) and Melan A. Histopathology revealed infiltrative growth composed of polygonal to spindle-shaped cells arranged in nests. Neoplastic cells have round to oval nuclei with prominent nucleoli, scant to moderate amount of eosinophilic cytoplasm that contains moderate amount of brown-black pigments (melanin). The neoplastic cells showed positivity for Melan A and Fontana staining, and hence diagnosis of malignant melanoma was given. Normal appearing mammary gland tissue showed CK positivity and connective tissue at the periphery of the tumor was noted. Careful examination of the skin and mucous membranes failed to reveal a malignant melanoma at other locations. Therefore this dog was diagnosed with a primary cutaneous malignant melanoma of mammary gland. Because of the rarity of such a mammary lesion additional studies are required.

P 48 | Differential diagnosis of hemorrhagic anovulatory follicles (HAFs) and the preovulatory follicles in mares assessed by color flow doppler sonography

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The Color-Doppler ultrasound has the potential to evaluate the vascularity of the follicle wall in mares to recognize future ovulation or lack of ovulation. The aim of this study was to assess the relationships between HAFs and normal follicle vascularity evaluated by color Doppler ultrasonography in mares. The ultrasound examinations were carried out on 25 Warmblood mares at -3, -2, -1 days before ovulation and 1, 2 days after ovulation. Follicles and HAFs size was determined by measurement of the Maximal Cross-Sectional area of Follicles (MCSF) and blood supply by the Maximum Colored Area of the blood Vessels (MCAV) from Doppler ultrasound images. There were no significant differences in MCSF ($p > 0.05$) between days -3, -2, -1 before ovulation in both groups of mares with normal ovulation and with formation of HAFs. Whereas at day -1 MCAV significant decreased ($p = 0.0004$)

in cyclic mares with normal ovulation and increased ($p = 0.003$) in mare with HAFs formation. The increase of the blood supply of the HAFs (MCAV) corresponded with vascularization at the apical area on day -1. The lack of vascularization at -1 day (normal follicles) was related to constant MCAV decreases (this sentence is not clear). HAFs formed from viable preovulatory follicles that did not differ from ovulatory follicles in diameter or gray-scale echotexture. Doppler ultrasonography may be used to estimate the follicles growing, maturation and ovulate better than standard ultrasound, because more accurately follicles aging as well as controlling or predicting the time of ovulation. Therefore Color Doppler ultrasound provides more quantitative information than the standard ultrasound in phases of the estrous cycle and ovulation.

P 49 | The different density of pacemaker cells in equine reproductive tract

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Interstitial Cajal Cells (ICC) are the gastroenteric pacemakers that generate and propagate electrical slow waves. Interstitial Cajal-Like Cells (ICLC) in the reproductive tract are also suspected of discharging slow triggering waves. Since regulation of uterine contraction processes is critical to equine reproductive success the density of ICLC become an important health indicator of uterus in mares. The alteration in ICLC density was estimated by immunofluorescent methods (IF) and particularly confirmed by Scanning Electron Microscope (SEM). The density of ICLC using the specific markers vimentine and c-kit/CD117 was evaluated by IF in uterine tissues from 29 healthy mares. The fresh whole thickness samples were fixed, IF stained using primary (anti-vimentine; anti-CD117) and secondary (conjugated with AF488; AF568, respectively) antibodies and quantified under scanning cytometer TissueFaxs PLUS. Corresponding samples were frozen in liquid nitrogen, sectioned and examined under SEM Phenom XL. Most ICLC displayed particular morphology: triangular, spindle-shaped or star-like body with two or more, very long, moniliform processes and a clear nucleus. The typical ultrastructure morphological features: triangular-shaped cell located between smooth muscle cells with electron dense cytoplasm, numerous mitochondria and tips of ICLC prolongations were confirmed in SEM. The significantly higher ($p < 0.001$) density (mean \pm SEM, %) of double positive vimentine/CD 117 cells were demonstrated in oviductal tip of the uterine horn (4.87 ± 0.39) compared to the middle of the uterine horn (0.48 ± 0.09) and corpus uteri (0.53 ± 0.14) with no differences between the latter. The accumulation of pacemaker cells in the uterine horn tip may play an important role in uterine contraction processes.

P 50 | Effect of follicle size and endometrial oedema on ovulation induction and embryo recovery in the Andalusian donkey

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The timing of ovulation is important to the success of embryo transfer. This study was conducted to determine whether follicle size and endometrial oedema at administration of ovulation-inducing agents would affect ovulation induction and embryo recovery in Andalusian donor jennies. Fourteen jennies aged between 3–13 years were used. Ovarian activity was evaluated daily during oestrus by transrectal ultrasound and ovulation was verified. Jennies were randomly assigned to receive 1500 IU hCG (Veterin Corion, Divasa Farmavic, Spain) i.m. ($n = 13$) or 0.75 mg Deslorelin acetate (Sincrorrelin, Ourofino Saúde Animal, Brazil) i.m. ($n = 25$) during consecutive estrous cycles ($n = 38$). At time of treatment, the degree of endometrial oedema was graded (0–5) and follicular size was classified into 3 categories (35, 36–39, and ≥ 40 mm). Donors were inseminated and embryos were collected by non-surgical uterine flushing. Data were assessed by the chi-square test and the Kruskal-Wallis ANOVA. All donors responded well to the administration of both drugs. Time from treatment to ovulation was not affected ($p > 0.05$) either by treatment (hCG: 51.69 ± 3.69 h; Deslorelin acetate: 46.08 ± 3.37 h), donor, follicle size or endometrial oedema. Twenty-four embryos were recovered from 38 flushes (63.16%). Embryo recovery rate was affected ($p < 0.05$) by follicle size, being higher when treatment was given at follicles size from 36 to 39 mm (72.2%), and donor. Embryo recovery rate was not different ($p > 0.05$) for jennies with endometrial oedema of 0 (75%), 1 (71.4%) or 3 (83.3%), but was significantly ($p < 0.05$) lower for jennies with oedema of 2 (36.4%) or 4 (0%). These results demonstrate that follicle size and endometrial oedema have effect on embryo recovery in jennies treated with hCG or Deslorelin acetate.

P 51 | Metabolic profiles and hormonal status are not different between primiparous and multiparous dairy cows diagnosed with inactive ovaries (true anestrus)

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The present study aimed to compare the metabolic profiles and hormonal status of primiparous and multiparous dairy cows diagnosed with inactive ovaries (true anestrus). A total of 105 animals that had not been seen in estrus by the farm personnel by day 60 after

parturition were included in the study. The animals were blocked by parity - primiparous ($n = 46$) and multiparous ($n = 59$), bled and examined using trans-rectal ultrasonography. Of those, 33 primiparous and 22 multiparous cows (overall, 30.1% and 19.8%, respectively) were identified as having inactive ovaries (absence of CL and follicles > 8 mm, and $P4 < 0.5$ ng/ml). The hormonal status ($P4$ and $E2$ concentrations) between the primiparous and multiparous cows were not significantly different (0.248 ± 0.031 ng/ml and 0.360 ± 0.076 ng/ml) and (2.818 ± 0.176 pg/ml and 2.548 ± 0.268 pg/ml), respectively. Additionally, no significant differences were recorded between primiparous and multiparous cows concerning the peripheral level of total protein, albumin, cholesterol, phosphorus, magnesium and triglycerides. The primiparous cows had a numerically lower glucose (1.383 ± 0.116 mmol/l) but higher NEFA level (0.330 ± 0.040 mmol/l) in comparison to the multiparous ones (1.643 ± 0.171 mmol/l and 0.259 ± 0.034 mmol/l, respectively). Similarly, no significant differences in the BHB concentrations between the primiparous (0.77 ± 0.051 pg/ml) and multiparous (0.80 ± 0.105 pg/ml) cows were observed. In conclusion, our results show that there are no differences neither between the hormonal status nor the metabolic profiles between primi- and multiparous cows with inactive ovaries. However, primiparous cows have a higher incidence of being truly anestrus in comparison to multiparous cows. Further investigations are needed to explain this situation.

P 52 | Can inter-species (caprine-porcine) cloned embryos be able to complete their development to blastocyst stage?

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The current study prompted us to investigate whether hybrid (caprine-porcine; C/P) nuclear-transferred (hC/P-NT) embryos can be developmentally competent to progress to the blastocyst (B) stage under in vitro culture conditions. To create inter-species cloned (ISC) embryos, enucleated in vitro-matured pig oocytes were subzonally-injected with adult goat peripheral blood-derived fibroblast-like cells (AGPB-FLCs) that either had been epigenomically modulated via exposure to 350 nM scriptaid (SCPT) during their 24-h contact inhibition (Group I; GI) or had not been exposed to SCPT (Group II; GII). Successfully fused and electroactivated oocytes (C/P nuclear-ooplasmic hybrids) were cultured to morula (M) and B stages for 144 to 192 h. Among 150 ISC embryos assigned to GI, 108 (72.0%) were able to divide ex vivo. The percentages of embryos that reached the M and B stages were 37/150 (24.7%)^a and 15/150 (10.0%)^a, respectively. In GII, out of 119 ISC embryos, 82 (68.9%)^a underwent cleavage divisions under in vitro culture conditions (a,a: $p \geq 0.05$; Chi-square test), but 18 (15.1%)^b and 0 (0%)^b developed to the M and B stages, respectively (a,b: $p < 0.05$). In summary, ISC embryos that had been reconstructed with porcine enucleated oocytes and SCPT-treated AGPB-FLC nuclei were found to exclusively display the

capability to complete ex vivo development to the B stage. Conversely, their counterparts originating from porcine enucleated oocytes and SCPT-untreated AGPB-FLC nuclei did not exhibit such capability. SCPT-dependent epigenomic modulation of AGPB-FLCs brought about a significant increase in the M formation rate of hC/P-NT embryos, and also acquisition of developmental competence to reach the B stage due to presumptive improvement of donor cell nuclear reprogrammability.

P 53 | Early pregnancy diagnosis in dairy cows by ultrasound and determination of bovine-pregnancy-associated glycoproteins, bovine pregnancy specific protein B and progesterone

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The aim of this study was to compare the accuracy of the bovine pregnancy determined by ultrasound (USG) and bovine-pregnancy-associated glycoproteins (bPAG), bovine pregnancy specific protein B (bPSP-B) and progesterone (P4) tests for determination of early pregnancy in dairy cows. Our study included 58 Holstein cows in 5 different dairy farms. Blood samples were collected on 20th, 25th, 28th, 30th, 32nd and 40th day following artificial insemination (AI) for bPAGs-serum, bPSP-B and P4 tests. Milk samples were collected at the same days for bPAG-milk tests. Serum levels of bPAGs, bPSP-B, P4 and milk levels of bPAGs were measured using commercial ELISA test kits. Ultrasonography (ECM IMAGO® Veterinary; 5.0 and 7.5 mhz, linear probe, France) was conducted to all cows on 30th and 40th days following AI. 49 cows were found pregnant by USG on 40th days following AI. The positive predictive values for bPAG-serum and bPAG-milk tests were %100 at day 28 and 30. For bPSP-B tests, positive predictive values were 93.7% and 100% at day 28 and 30, respectively. Progesterone values were found different between pregnant and non-pregnant cows at the days of blood sampling except 40th day after AI. Specificity was found 100% for bPAG-serum and bPAG-milk, 96.7% for bPSP-B at day 28. Sensitivity values calculated 94.1% and 93.8% for bPAG-serum and bPSP-B, respectively. The area under ROC curve was 0.974 for bPAG-serum, 0.991 for bPAG-milk and 0.967 for bPSP-B on 28 day following AI. As a result, bPAG-serum, bPAG-milk and bPSP-B tests were found safe methods for pregnancy diagnosis in cows on 28 day following AI.

P 54 | Effect of a treatment without antibiotics on fertility of cows without a corpus luteum suffering from endometritis

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This study was performed to compare the effects of hormonal and intrauterine antimicrobial treatments in cows with a clinical endometritis and without a corpus luteum (CL). The clinical examinations were performed in 200 cows of 33 Swiss dairy farms. Each cow was screened for signs of endometritis between 21 to 45 d pp. Only cows with a CL < 20 mm as well as cows without other clinical diseases were included in the study. The 200 cows were randomly assigned to antibiotic (AB) and hormonal (H) treatments immediately after the clinical examination. Cows with ovarian follicles ≥ 10 mm received 500 mg Cefapirin i.ut. (group AB) or 500 µg GnRh i.m. (group H). If ovarian follicles were < 10 mm or ≥ 25 mm the cows received an intravaginal progesterone-releasing device for 7 days followed by 500 µg Cloprostenol i.m. The cows were checked again 21 ± 1 d after the first examination for vaginal discharge and for ovarian activity by using transrectal ultrasound. From this time on all cows were inseminated as soon as they showed clinical signs of estrus without pathological changes. Non-cycling cows were visited every two to three weeks and treated according to the clinical findings. Five and eight weeks after AI cows were checked for pregnancy by using ultrasound. Reproductive data were collected until 200 d pp. The choice of treatment (AB or H) did not ($p > 0.05$) affect the type of vaginal discharge and reproductive performance (days to first service, first-service conception rate, services per conception days open, cumulative pregnancy rate at 200 DIM, culling rate because of infertility). In conclusion the results show that an antibiotic treatment of cows without a corpus luteum could be replaced by a hormonal treatment without negative effects on reproductive performance.

P 55 | Effect of reduced glutathione addition to extender on quality and fertility of frozen goat semen

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The goal of this study was to evaluate the effect of reduced glutathione (GSH) on the quality and fertility of frozen-thawed goat spermatozoa. Saanen goat bucks ($n = 3$, 2–3-years old) were used as semen donors. In exp. 1, the effect of GSH in cryopreserved goats sperm was evaluated. The ejaculates ($n = 7$) were split and diluted 1:2 with Tris-glucose-glycerol-egg yolk (2.5%) diluent with GSH (0.02–0.08 mg/ml) or without GSH (control). The samples were placed into 0.25 ml straws and frozen in nitrogen vapour. The straws were thawed in water bath (37°C, 30 s). Post-thaw sperm progressive motility, acrosome and plasma membrane damages and viability were determined. In exp. 2, the effect of GSH addition to diluted semen (0.04 mg/ml) on the fertility results was evaluated. Goats in experimental ($n = 39$) and control ($n = 36$) group were inseminated twice in spontaneous oestrus (150×10^6 motile sperm/dose). Statistical analysis was made using a Students t-test and chi-square test. Post thawing evaluation revealed that extender with 0.04 mg/ml GSH yielded higher rates of sperm motility (by 8.9%) and acrosome integrity (by 10.5%) compared with control ($p < 0.05$). There were no differences in the percentage of intact plasma membrane and

viability of spermatozoa between the groups. The conception rate of the goats in experimental group was 8.5% higher compared to control ($p < 0.05$). These results indicate that addition of GSH to extender for goats sperm freezing at optimal concentration has a protective effect on some quality parameters of spermatozoa and their fertility.

P 56 | Immunohistochemical expression pattern of Desmin, Vimentin, α -Actin, Cytokeratin and Laminin in bovine endometrosis

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Bovine endometrosis (BE) is defined as endometrial, periglandular and/or stromal fibrosis with alterations of the affected glands. The histomorphological appearance of the involved periglandular stromal cells allows the classification of BE into active, inactive, and mixed fibrosis, which according to the integrity of the glandular epithelia, can show a destructive or non-destructive pattern. The objective was to describe the immunohistochemical expression patterns of intermediate filaments: Desmin, Vimentin, α -Actin, Cytokeratin, and Laminin in the different forms of BE. Uterine samples ($n = 67$) showing endometrosis were picked up from a collection of slides from University of Leipzig. The immunohistochemical expression pattern was quantified by estimating the percentage of positively labelled cells. Data was analysed for possible differences between destructive or non-destructive endometrosis. In both cases, the periglandular stromal cells showed a stromal coexpression of Desmin, Vimentin and α -Actin, which is a characteristic of myofibroblasts. A small percentage of glandular epithelial cells in destructive endometrosis reacted with Vimentin antibodies; this could be the expression of a differentiation to stabilize the cell or a sign of intensive (pathological) proliferation. The expression of Laminin in the basal lamina of altered endometrial glands within fibrotic foci was discontinuous, especially in the destructive endometrosis. To our knowledge, this is the first time this finding is reported in cows. In conclusion, immunohistological description of these cellular markers provides basic knowledge to understand pathogeny of BE.

(Supported by Leipzig University, UNAM PAPIIT IA204917 and PIAPI1649)

P 57 | Progesterone levels under feeding bovine with *Pittosporum undulatum* in vivo and their embryo production in vitro

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This study was performed to evaluate the reproductive performance of cattle fed *Pittosporum undulatum*. Progesterone levels were

monitored during the oestrus cycle and the capability of their oocytes to undergo in vitro maturation, fertilization and subsequent embryonic development were assessed. All heifers ($n = 8$) were fed for 5 weeks; the experimental group ($n = 4$) was subjected to a diet containing *P. undulatum* while the control group was not. During oestrus cycle, peripheral blood samples were collected every 3 days and progesterone levels were analyzed by enzyme-linked immunosorbent assay (ELISA). After slaughtering, heifers' ovaries were recovered and oocytes were collected, in vitro matured, fertilized and cultured for 7 days. The developmental rates of embryos were assessed every 2 days during this culture period. Results indicated that feeding heifers with *P. undulatum* significantly decreased ($p < 0.01$) plasma progesterone concentrations during the luteal phase of the cycle compared with the animals in the control group. Furthermore, for in vitro embryonic developmental rates, statistical differences were observed ($p < 0.05$) throughout maturation, cleavage and embryo developmental rates (78.3 ± 5.8 , 29.92 ± 4.31 , and 7.30 ± 3.1 for experimental animal group compared with 90.5 ± 3.0 , 41.86 ± 5.58 , and 21.88 ± 6.85 in the control group, respectively). We hypothesize that the adverse effects could be attributed to some *P. undulatum* compounds affecting directly or indirectly the cyclooxygenase-2 (COX-2) activation, which may diminish follicular development through the inhibition of prostaglandin synthesis and oocyte maturation and, consequently, reduce the ability of oocytes to be fertilized and subsequently develop.

P 58 | Positive effect of ethylene glycol on the refreezing of stallion semen

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Intracytoplasmic sperm injection has gained popularity due to the possibility to obtain embryos from mares with fertility problems or in sport activity. This technique requires few sperm and improves the fertility of stallions with limited or poor quality semen. To avoid waste of sperm, unused material is refrozen. Refreezing causes a decrease in semen quality. Several factors were evaluated to improve quality of refrozen semen however the effect of different cryoprotectants (CP) has not yet been investigated thus we compared ethylene glycol and glycerol. Six 0.5 ml straws from the same ejaculate were obtained for each of 7 stallions in 3 replicates. Each sample was diluted with EquiPlus[®] added with 4% egg yolk and 2% ethylene glycol or 4% glycerol, in order to create 3 straws for each CP. The straws were subjected to 1, 2 or 3 freezing-thawing cycles. The freezing procedure consists of 20 min at 4°C, 20 min over nitrogen vapor and final storage into liquid nitrogen. At each cycle, straws were thawed at 37°C for 3 min. Semen was analyzed for total and progressive motility (Hamilton-Thorne, Inc.), membrane functionality by hypo-osmotic swelling test (HOS test) and mitochondrial activity by tetrazolium reduction assay (MTT test). The refreezing significantly affected all endpoints studied, regardless of the type of CP used. However, at each cycle ethylene glycol showed higher progressive motility

compared to glycerol (20.84%, 11.71% and 4.70% vs. 15.19%, 8.31% and 2.61%, respectively) ($p < 0.05$). All other sperm parameters were similar among CP. In conclusion, for the production of frozen straws of equine semen, intended to repeated cycles of thawing and refreezing, the use of ethylene glycol as cryoprotectant is preferred.

P 59 | Effect of GnRH injection three days after PGF_{2α} on estrus synchronization

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The present study investigates the effect of GnRH injection three days after PGF_{2α} on estrus synchronization. Cows with a body condition score between 2.25 and 3.75, on average about 60 days post-partum and without reproduction problems were randomly allocated to two groups. All cows received two prostaglandin (25 mg lutalyse; zoetis) injections 11 days apart (day 0 and 11) and the treatment group ($n = 30$) was also administered GnRH (200 mcg cystorelin; ceva) 3 days after the first prostaglandin administration (PGP group). In the control group ($n = 29$), only two PGF_{2α} injections were given with 11 days interval (2XPG group). All cows were inseminated at the time of observed estrus. Blood samples were taken on day 0 and 11 and serum progesterone concentration was assessed. Serum estrogen concentration was measured three days after the second PGF_{2α}. Ultrasonography was used to check the ovarian status for the presence of CL and follicles when PGF_{2α} was injected. The estrous detection percent, estrogen and progesterone concentrations were measured and results were examined by SAS (chi-square, t-student). Estrous detection percent of the PGP group had a better performance than 2XPG group (70% vs. 57.17%, $p = 0.50$). The percent of cows which had progesterone levels above 2 ng/ml at the time of the second PGF_{2α} injection was numerically lower in the PGP group than in the 2XPG group (70% vs. 72.4%, $p = 0.84$), but effects of the ovarian status at the beginning of the protocol on that result was very significant ($p < 0.0001$). The pregnancy/AI in the PGP was higher than in the 2XPG (26.7% vs. 17.4%, $p = 0.42$). Finally, we suggest that if we don't see estrus three days after the first PG, administration of GnRH may be a good choice for having better estrus following the second PG.

P 60 | 3D ultrasound investigation of embryo-fetal development during the first pregnancy trimester in goats

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This study aimed to monitor the embryo-foetal development during the first trimester of pregnancy in goats by 3D ultrasound. Ten pregnant Bulgarian White milk goats, aged 2–4 years, 48–51 body weight,

artificially inseminated by fresh semen were used in the experiment. 3D ultrasound examination between Days 20 and 48 post insemination was made 7 days apart. For this purpose multi frequency linear probe (5–11 MHz) and transrectal and transabdominal approach were used. The obtained results showed 3D visualization of an embryo in a fluid filled uterine horn on Day 20, an enlarged embryo with amnion and spinal cord images on Day 27. The foetal structures (fore and hind legs, head, tail and umbilicus) were observed on day 34 of pregnancy. Clear 3D images of placentomas with typical shape were possible on Day 41. The detailed observation of foetal structures (skull, orbita, spinal cord, fore and hind legs and tail) was possible at the earliest on Day 48. In conclusion, 3D ultrasound examination provides monitoring of embryo-foetal development and gives detailed information for morphological changes during the first trimester of pregnancy in goats. The obtained results could be used as the basis of comparison for early detection of embryo-foetal pathology.

P 61 | Serum metabolome analysis in doe kids for the identification of biomarkers of sexual precocity

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Success of first breeding is a major concern for goat breeders, since failure to fertilize does increases unproductive time and breeding costs. Fertility rates after artificial insemination of young goats are highly variable and rather low. Breeders generally breed does that are older than 5 months and weight more than 32 kg. However, sexual precocity is highly variable between does. Up to now, there is no known biomarker for sexual precocity. A better characterization of the pubertal stage of maturity could help optimizing time for first breeding. Our objective was to analyze the serum metabolome of doe kids, just before the first breeding, in order to characterize the pubertal stage of maturity and identify biomarkers of sexual precocity. Weekly blood sampling was performed on twenty 6- to 7-month-old does born in February for 5 weeks before their first contact with bucks in September. Progesterone assays and metabolome analysis using 1H Nuclear Magnetic Resonance Spectroscopy were performed on the serum samples. No spontaneous ovulatory cycle was observed before breeding based on progesterone assays. All does had reached the pubertal stage of maturity at breeding since all got pregnant. Metabolome analysis allowed the identification of 109 spectral bins in sera. Between week 1 and 5 preceding buck introduction, 32 buckets showed significant variations (t-test, $p < 0.05$): i.e. inosine, formate, lactate and creatinine decreased, while threonine, tryptophan, isoleucine and trimethylamine oxide significantly increased. Metabolites with significant variations between the 5 considered weeks could be

biomarkers of sexual precocity; studies are in progress to identify them. (This study was funded by Région Poitou-Charentes and INRA.)

P 62 | Morphokinetic analysis of the development of mouse embryos from strains: C57BL/6W, 129s1/SvW and C3H/W by time lapse monitoring (TLM)

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The aim of the study was to determine the relationship between developmental potential of embryos and their morphokinetic parameters from mouse strains: C57BL/6W, C3H/W, 129S1/SvW. TLM was carried out on 128 embryos for 120 h using the Primo Vision™ Time-Lapse System - Vitrolife, Sweden. Embryos were cultured in medium CSC with 10% of SSS (Irvine Scientific, USA). Tables generated after the analysis of the videos of the time data, as well the morphokinetic graphs of the embryo development up to the stage: BL5/6 (expanded blastocyst), BL (blastocyst) and Err (morula) were analyzed. Statistical analysis was performed using one-way ANOVA for the comparison of time data of the subsequent embryo cleavages. Beside this, logistic regression analysis was used for studying the impact of the duration of the subsequent embryonic stages, fragmentation and degeneration on the developmental potential of the embryos. For all the tests it was set the confidence interval $\alpha = 0.05$. Results showed a significant difference between strains at first four cell divisions in the prognosis of developmental potential up to blastocyst (BL Er: $p = 0.0068$; BL5/6 Er: $p = 0.001$). A significant difference between the times of morula compaction between strains and their impact on the quality of the forming blastocysts was found: Err and BL5/6 ($p = 0.0006$); Err and BL ($p = 0.0051$). The BL group showed a significantly longer compaction time than the BL5/6 group. It means that the compaction process is decisive for further fate of the embryo. Morphokinetic parameters of the strain 129s1/SvW allowed to determine the time for properly developing embryos, so it can be used as a model for the study of factors affecting the morphokinetic parameters of mouse embryo development.

P 63 | Improving VET SKILLS: construction of a simulator for training caesarian section in bovines

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A caesarean section (CS) is an important obstetrical technique in ruminants to treat cases of dystocia. To perform a CS for training purposes

without a medical indication is a relevant welfare issue. Teaching CS is often restricted to theoretical explanations and video demonstration. The objective of this project was, to construct a simulator for a CS with which students can train 1) vaginal palpation of the obstetrical patient, 2) local anesthesia, 3) surgical techniques including incision and suture of the abdominal wall, 4) exteriorization of the uterus and extraction of the calf, and 5) incision and suture of the uterus. The scope of this simulator was to provide a realistic procedure of manual skills necessary for a CS. A life-sized cow used for decoration purpose was modified. Various pieces of the model were removed for the surgical approach in order to gain access to the abdominal cavity and to allow vaginal examination. A vaginal cavity and uterus was sewn with 8 mm neoprene. For simulating the abdominal wall a suture pad (50 × 50 cm) was casted with silicon and rubber foam to simulate the different layers of the abdominal wall. Overall costs for this were € 2100. A 25 kg dystocia calf model (VSI; Canada) is inserted into the uterus for the training session. The simulator is used in the clinical rotation of the Clinic of Animal Reproduction with groups of 9 students in their 5th year. All students described their learn experience as valuable and intense. The simulator helps them to understand the complex procedure of this obstetrical intervention. Furthermore, this is the first simulator that allows to train diagnosis and handling of the intrauterine calf and to exteriorize the uterus into the abdominal incision.

P 64 | Impacts of pathological alterations of the uterus and cervix on fertility in mares

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The aim of this study was to determine retrospectively the effects of the degrees of cervical formation, uterine edema, intrauterine fluid accumulation, oxytocin injection and uterus lavage on pregnancy rate (PR) in mares. From 166 mares 377 cycles were evaluated in 2013 and 2014. Gynecological examinations were performed one to three times per day. The mares were divided into three age groups (young: 3–11 y; middle age: 12–15 y; old: ≥ 16 y). Reproductive status was grouped as follows: maiden, lactating mares with a foal, primi- or pluriparous mares not mated in the last season and barren mares mated without success in the last season. Young mares had a higher ($p < 0.05$) PR than older mares. Barren mares showed the lowest PR ($p < 0.05$). Uterine fluid accumulation post breeding resulted in a reduced PR ($p < 0.05$) as did also cycles with a tight cervix compared to cycles with a more relaxed and dilated cervix ($p < 0.05$). A prominent endometrial edema led to a lower PR compared to cycles with mild endometrial edema ($p < 0.05$). The PR did not differ ($p \geq 0.05$) between cycles treated with oxytocin injections due to intrauterine fluid and cycles without injecting oxytocin, while cycles with a uterine lavage after breeding because of an intrauterine fluid accumulation > 2 cm showed a lower ($p < 0.05$) PR than cycles with less intrauterine fluid without an uterine lavage. In summary, older and barren mares and

mares with intrauterine fluid accumulations, a tight cervix and a prominent uterine edema are subfertile. Oxytocin injections increase the pregnancy rate in mares with intrauterine fluid accumulations, while uterine lavage does not.

P 65 | Identification of CLPTM1 gene mutation in domestic dog *Canis lupus familiaris*

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Cleft palate is one of the most common birth defects in domestic dogs. Currently, genetic background is considered the most important causative factor, the most likely candidate gene being CLPTM1 - Cleft Lip and Palate Associated Transmembrane Protein 1 in *Canis lupus familiaris*. Previous studies in brachycephalic dogs indicated 54 nucleotide substitution (C > T) of exon 12 of the CLPTM1 gene as the possible cause. The aim of this study was to design a rapid molecular test allowing identification of heterozygotes in the population. Biological material derived from healthy and affected animals (5 healthy and 10 affected French and 2 healthy and 6 affected English bulldogs) was used in our study. The isolated genetic material was amplified with polymerase chain reaction, followed by PCR-RFLP and the resulted product of 292 bp length was cut with specific HpyAV restriction enzyme. We found that both healthy and affected individuals of each breed were heterozygous (length of fragments 292 bp, 202 bp and 90 bp) for the synonymic C1599T mutation in exon 12 of CLPTM1 gene. The above results require further confirmation by sequencing the samples. Eventually, the test will make possible monitoring of the frequency of CLPTM1 gene mutation in domestic dogs.

P 66 | Quality of total RNA isolated from spermatozoa of boars with different semen freezability

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In this study the TRIzol/PureLink RNA protocol was used to extract total RNA from fresh spermatozoa of boars differed in semen freezability. The Neuman-Keuls post hoc test showed that boars with poor and good semen freezability were characterized by a significant reduction ($p < 0.05$) in CASA-analyzed sperm motility ($23.3 \pm 3.4\%$ and $45.9 \pm 3.2\%$, respectively), mitochondrial function analyzed by

the JC-1/PI assay ($33.9 \pm 4.3\%$ and $55.7 \pm 3.7\%$, respectively) and plasma membrane integrity assessed with the SYBR-14/PI assay ($39.0 \pm 2.1\%$ and $53.9 \pm 3.1\%$, respectively). All measurements of RNA concentrations were within the optimal absorbance ratio A260/A280 of 1.83 to 1.89. Spectrophotometric quantification resulted in total RNA yield of 4.6 ± 2.7 micrograms/100 to 150 million spermatozoa. The Bioanalyzer profiles showed wide variations in the quality of isolated RNA, comprising full-length intact RNA (2000 to 3000 bp), mainly from spermatozoa harvested from boars with good semen freezability. By contrast, boars with poor semen freezability displayed some degree of RNA degradation, comprising 25 to 200 bp fragments. Sperm RNA profiles from most of the boars with good semen freezability showed that the RNA integrity number (RIN) ranged from 2.4 to 3.0, suggesting high-quality RNA. It can be suggested that differences in the quality of total RNA isolated from spermatozoa of boars differed in semen freezability might have significant relevance in transcriptome study on RNA-Seq data. (Supported by a NCN project in Poland (2015/19/B/NZ9/01333).)

P 67 | *Simplicimonas*-like DNA in vaginal swabs of cattle cross-reacting in the real-time PCR for *T. foetus*

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Simplicimonas was described for the first time in 2010. It is a parabasalid genus in the class *Trichomonadea*. The organism was isolated from the intestines of different animal species. Up to now, no pathogenic effects were found. On the other hand, *Trichomonas foetus* is a venereal pathogen in cattle responsible for substantial economic loss due to vaginitis and infertility. Switzerland is free of *T. foetus* (notifiable disease). In September 2016, severe cases of vaginitis and vaginal discharge were observed in a herd of cows on a Swiss alpine pasture. After exclusion of BVDV and BHV-1, vaginal swabs of 34 cows were sampled. Real-time PCR was positive for *T. foetus* in 25 from a total of 34 assessed cows. However, the melting profiles of the probes targeting the diagnostic PCR products differed from the *T. foetus* positive control. Subsequent sequencing of the amplicons revealed 91% identity to *Simplicimonas sp.* sequences deposited in GenBank™. With the PCR presumed to be specific for the diagnosis of *T. foetus*, we found a high percentage of cross-reactions with *Simplicimonas*-like organisms. This is important to know, as diagnostic methods should be specific to avoid false-positive results leading to restricted animal movement, repeated samplings, and culling of animals in the worst case. In the cases described, no association between the detection of *Simplicimonas sp.* and the existence of vaginitis or fertility problems could be shown.

P 68 | Factors affecting pregnancy-associated glycoprotein (PAG) concentration in urine of dairy cows during early pregnancy

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Present knowledge indicates that the secretory function of the trophoblast is affected by many factors that can influence the PAG concentration in the blood and milk of pregnant cows. This study attempted to determine the factors affecting the PAG concentration in the blood and urine during the first 105 days of pregnancy in dairy cows. The study was performed on a group of 76 Holstein Friesian cows (in 1st–5th lactation). The animals were tested for PAG concentration in blood plasma and urine and tested for plasma progesterone and 17- β estradiol concentration determined by RIA. Sampling and ultrasound pregnancy diagnosis were performed on days 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105 of gestation. The correlation coefficient was used to assess a potential linear association between variables such as: cow's body weight, parity, milk yield, progesterone (P4) and 17- β estradiol (E2) concentration. Pregnant cows demonstrate a moderately positive relationship ($r=+0.51$) between the concentration of PAG and P4 in blood, while there was no correlation between the plasma P4 and urinary PAG concentrations. A strong positive linear relationship was demonstrated between concentrations of E2 and plasma PAG ($r=+0.73$), but no relationship was observed between E2 and PAG concentration in urine. Additionally, in cows over two lactations a statistically significant decrease in plasma PAG concentrations and increase in urine PAG concentrations in compare to the primiparous were observed. Factors like body weight and milk yield did not show any association with the mentioned blood and urinary concentrations. Results indicate a lower effect of the studied factors on the urine PAG in comparison to the plasma PAG concentration.

P 69 | The improvement of beef cows breeding management pregnancy control using PAG determination

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Beef producers often consider reproductive efficiency as the number of cows that produce a calf each year. The result in a breeding program based on the potential for a cow getting and maintaining

pregnant during a breeding season. The most prevalent methods for detecting early pregnancy in cattle (rectal palpation (RP) and USG examination) are difficult to perform in pasture-mating system. Pregnancy-associated glycoproteins (PAG) are secreted by the binucleate giant cells of the ruminant placenta and enter maternal circulation during placentation. PAG have been detected in serum as early as 22 days post mating and continue to increase throughout pregnancy until parturition. PAG concentration in maternal serum has been recognized as an indicator of pregnancy which may be useful in the reproductive management of cattle. The aim of this study was to compare of the serum PAG ELISA to RP and USG for pregnancy diagnosis in beef cows with previous bull exposure. Serum samples were collected over breeding season in 2016 from Limousin beef cows ($n = 95$) maintained in pasture-mating system. Cows were exposure to a herd bull and underwent pregnancy examination (RP, USG) between 1 and 7 month after mating. The presence of PAG in serum was determined using antigen-capture ELISAs. Pregnancy status of open and pregnant corresponded to serum SN values of <0.30 and ≥ 0.30 , respectively. When compared to RP and USG finding the performance of serum PAG ELISA was sensitivity of 95.6% (97.8–100.0%) and specificity of 100% (100–100%). The positive and negative predictive values were 100% (100–100%) and 55.6% (71.4–100%), respectively. We concluded the serum PAG ELISA was accurate in predicting pregnancy status and may be useful for breeding management in pasture-mating system of beef cows.

P 70 | Does total prostatectomy always cause postoperative urinary incontinence in dogs? Preliminary study

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Total prostatectomy is carried out in the cases of tumors, cysts or post-traumatic lesions of the prostate and associated with a high risk of postoperative urinary incontinence. This procedure is normally performed using access via the midline - prepubic laparotomy, or with osteotomy of the pubic symphysis. In the case of displacement of the prostate to the perineal hernia, this procedure can be performed using perineal access. In four uncastrated male dogs diagnosed with perineal hernia, enlarged prostate was localised in the hernial sac. All dogs showed benign prostatic hyperplasia with multiple small cysts within the prostate. In one dog a concomitant cyst outside the gland was present. The surgical access to the hernial sac and its contents (together with the prostate gland) was made through the perineal region. The decision of total prostatectomy was taken because of the difficulty in displacement of the prostate back into the abdominal cavity due to its large size, co-existing adhesions of the cyst and the prostate to the hernial sac, and the high risk of hernial recurrence. It was found (during 6 months after surgery) that the operated dogs had no symptoms of post-operative urinary incontinence. In one patient, a transient

episode of urinary incontinence, which lasted a month was observed. Performing prostatectomy via perineal enabled better access to the prostate gland and allowed for retaining more of the tissues in the region of the urinary bladder sphincter, as well as precise urethral anastomosis during surgery. Additionally this technique allows less traumatic the rest part of urinary bladder (without sphincter of bladder). The next study on the biggest group of patients is necessary to confirm our observations.

P 71 | Metabolomic profiling of the oviductal fluid across the estrous cycle in the cow

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Although crucial for gamete transport, fertilization and early embryo development, there is very limited knowledge on the metabolic content of the oviductal fluid (OF) and its regulation across the estrous cycle in domestic mammals. Bovine oviducts ipsi- and contralateral to the side of ovulation were collected at a local slaughterhouse and classified into 4 stages according to the ovarian and corpus luteum (CL) morphologies (n = 18–25 cows/stage): just after ovulation (postov), at mid- and late-luteal phase (mid-lut and late-lut), and before ovulation (preov). Concentrations of progesterone and 17-estradiol were assayed in pools of OF (3–4 pools/stage and side; 4–10 cows/pool) by GC-MS/MS and their content in metabolites quantified by nuclear magnetic resonance spectroscopy. Comparisons between groups were made with a two-way ANOVA followed by Bonferroni post-tests. A total of 38 metabolites were identified, among which the amino acid glycine and the energy substrates lactate and myoinositol were the most abundant at all stages. Little difference was seen in metabolite contents between ipsi- and contralateral OF. In ipsilateral OF, concentrations of glucose-1-phosphate were the most affected by the stage of the estrous cycle and were 5 to 6 times less abundant at preov and postov than at mid- and late-lut. At the opposite, glycine, alanine, valine, leucine and succinate were more abundant at postov than at mid- or late-lut in ipsilateral OF. This is the first metabolomic profiling of oviductal fluid in domestic mammals. These results may be helpful to optimize cultured media for gamete maturation, in vitro fertilization and embryo production. (This work was supported by APIS-GENE.)

P 72 | microRNA profiling in the bovine corpus luteum during the early pregnancy

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In mammals the establishment and continuation of early pregnancy depends on the ability of the Corpus Luteum (CL) to maintain its function. In cattle, it is the interferon-tau (IFNT) secretion from the conceptus that prevents CL regression and ensures progesterone production for the establishment of pregnancy. In addition to endocrine and paracrine signaling, a group of non-coding nucleic acids known as microRNA (miRNA) can also support CL sustainability. miRNA regulate gene expression post-transcriptionally and are shown to be involved in the modulation of CL function. However, the examination of miRNAs in corpus luteum function at the early pregnancy still remains uncovered. This study aims at profiling the expression of miRNA in CL during early pregnancy in cattle by comparing it to CL late in the cycle and to regressed CL. Corpora lutea were assigned in two different timely defined classes during the cycle (C13 class: days 13–16 and C18 class: day >18) and in a third class during the early pregnancy (P class: 1–2 month). A total of 9 corpora lutea from individual animals were included in the study, three corpora lutea for each CL class. miRNAs population was profiled using small RNA next generation sequencing and biologically relevant miRNAs were evaluated for their differential expression (DE) using the DESeq2-methodology. We show that 6 DE miRNAs (bta-mir-2890, -2332, -2441-3p, -148b, -1248 and -29c) are common to both comparisons, P vs. C13 and P vs. C18, while bta-mir-23a and -769 were unique miRNAs DE in P vs. C13, whereas forty-four unique miRNAs were identified as differentially expressed in P vs. C18. These data confirm that miRNAs are present in CL during early pregnancy and potentially regulate its function.

P 73 | Expression patterns of epigenetic chromatin modification enzymes in equine endometrium during early pregnancy

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DNA methylation and histone modifications in mammalian cells control the activity of chromatin modification and serve as inheritable epigenetic marks. Mechanism of DNA packaging and chromatin modification regulate gene expression depending on time and tissue/organ. Endometrial gene expression is tightly controlled, and has also critical influence on recognition of maternal recognition of pregnancy and embryo implantation. The objective was to investigate mRNA expression patterns of genes (total of 32 genes) from a set of families (Histone Acetyltransferase, Histone Methyltransferases, SET Domain Proteins, Histone Phosphorylation, Histone Ubiquitination, DNA/Histone Demethylases, Histone Deacetylases) of chromatin modification enzymes during the estrous cycle and early pregnancy. Biopsies were obtained from mares on day of ovulation (d0, n = 4), late diestrus (LD, n = 4, high progesterone [P4]), and after luteolysis in the beginning of the estrus phase (AL, n = 4, <1 ng/ml P4) of the cycle. Biopsies were also taken on days 14 (P14, n = 4), 18 (P18, n = 4), and 22 (P22; n = 4) of pregnancy. Relative mRNA expression levels were quantified using real-time quantitative RT-qPCR. Expression of all chromatin

modification enzymes were detected in equine endometrium during the estrous cycle and early pregnancy. Each gene family was observed to be regulated differentially either by cyclic changes during the cycle or by the presence of the embryo in the uterus. It may be inferred that chromatin modification enzymes are steadily expressed and regulated to some extent to establish well coordinated transcriptional activity with respect to epigenetic regulation in equine endometrium during estrous cycle and early pregnancy.

P 74 | Evaluation of the introduction of a CIDR device in a Cosynch synchronization protocol in dairy cows

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Synchronization of ovulation can reduce workload in dairy cattle reproductive management. Thus, we studied the effect of including a synchronization step (CIDR, progesterone) device to a Cosynch protocol, in the synchronization and pregnancy ratios of dairy cows. Cows (n = 90) received gonadotropin-releasing hormone (GnRH) on Day 0, prostaglandin F_{2α} (PGF_{2α}) on Day 7 and GnRH and fixed-time artificial insemination (FTAI) on Day 10. Between days 0 and 7, cows either received a progesterone intravaginal insert (Cosynch+CIDR group: 50 cows) or no insert (Cosynch group: 40 cows). Pregnancy was diagnosed on days 30–35, and non-pregnant cows were resynchronized immediately using the protocol that they had been assigned. The overall synchronization ratios were 79.7% (Cosynch group) and 72.9% (Cosynch+CIDR group). Cosynch cows had a higher pregnancy ratio than Cosynch+CIDR group at the first synchronization (40 ± 5.2% vs. 30 ± 4.8%). Nevertheless during the second synchronization the Cosynch+CIDR yielded better pregnancy ratio (19 ± 6.4% vs. 17 ± 6%). The total pregnancy rates per treatment were 45 ± 5.2% in the Cosynch group and 40 ± 5.2% in the Cosynch+CIDR group, with no significant differences. In conclusion, the introduction of a CIDR did not improve the pregnancy ratio in a Cosynch protocol in dairy cows. Nevertheless, Cosynch protocols, with or without CIDR have advantages such as only requiring three animal handlings, last only ten days and no labour investment for detecting the corpus luteum is required, with the consequent benefits in the farm management like in the reproductive ratios as the results obtained in our study are acceptable.

P 75 | Boar semen preservation at 4°C without the use of antibiotics in gilts

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The addition of antibiotics, either as a single molecule or in combinations, is the method universally utilized to control bacterial growth for swine semen production. In the context of growing concerns about the development of antibioresistance, the use of antibiotics for semen preservation should be challenged. The objective of this study was to develop an antibiotic free semen processing technique based on semen preservation at 4°C to avoid bacterial proliferation while maintaining sperm quality and not affecting the reproductive performance (pregnancy rate and prolificacy). Ejaculates from boars (N = 2) of proven fertility were collected and split into 2 equal parts to be preserved either in a standard semen extender containing a combination of 3 antibiotics (NutriXCell+©), packed in standard GTB bags© and kept at 17°C (Control Group, C) or in a new semen extender, packed in semen preservation bags manufactured with a plastic film with bacteriostatic properties (Bactibags©) and preserved at 4°C (Experimental Group, E). Sixty gilts (pietrain breed) were estrous-synchronized using Regumate® and randomly allocated to the E (n = 30) or C (n = 30) groups. Once in heat, gilts were inseminated every 12 h until the end of estrous. The numbers of embryos and corpora lutea were evaluated at 30 days after AI. Neither the number of embryos ($X^C \pm SE = 18.4 \pm 3.3$; $X^E \pm SE = 17.6 \pm 3.5$), nor the number of corpora lutea ($X^C \pm SE = 21.1 \pm 2.8$; $X^E \pm SE = 20.5 \pm 2.9$) differed between the two treatments (MW U test, p > 0.05). Semen preservation at 4°C, a temperature inhibiting bacterial growth, allows reproductive performance comparable to that of semen preserved at 17°C in a high performance semen preservation media.

P 76 | Expanded and compact equine cumulus-oocyte complexes exhibit different fatty acid and amino acid metabolism during in vitro maturation

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Equine cumulus-oocyte complexes (COCs) are classified as compact (Cp) or expanded (Ex) depending upon the degree of expansion of their cumulus. Around 30% of Cp oocytes mature during in vitro maturation while 60% of Ex COCs reach metaphase II. We have demonstrated consistently higher glucose uptake in Cp COCs compared to Ex, but no data regarding fatty acid (FA) or amino acid (AA) metabolism have been yet studied. We aimed to study FA and AA metabolism in Ex and Cp equine cumulus-oocyte complexes. Ex and Cp COCs were retrieved by follicular scraping and held overnight. Then, Ex (n = 99) and Cp (n = 17) germinal vesicles were separately placed in culture in 250 µl of TCM-199 with 25 mM bicarbonate, 10% FBS and 5 mU/ml of FSH in a humidified atmosphere of 5% CO₂ in air at 38.5°C for 30 h (7 replicates). The supernatants were frozen at -80°C and analyzed using proton Nuclear Magnetic Resonance (NMR). Cp COCs consumed more alanine (3.2 ± 1.8 mmol/COC; mean ± SEM) and acetate (2.8 ± 2.3 mmol/

COC) compared to Ex (0.02 ± 0.007 and 0.25 ± 0.05 nmol/COC, respectively; $p < 0.05$). Surprisingly, Cp COCs consumed high amounts of glutamine (132.5 ± 128.5 nmol/COC), while Ex COCs produced it (-1.2 ± 0.6 nmol/COC; $p < 0.05$). Our results show that Ex and Cp COCs differently use the fatty acids and amino acids present in the maturation medium and could be related to their divergent meiotic competence. Further studies are needed to fully unveil these mechanisms.

P 77 | Epididymal storage and N-acetylcysteine supplementation on chromatin status of cryopreserved stallion epididymal spermatozoa

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Epididymal spermatozoa is a post-mortem source of germplasm from valuable stallions. However, recovering can take place in the field, requiring the refrigerated storage of epididymes before sperm cryopreservation. Moreover, sperm DNA fragmentation can occur during the freezing/thawing process, being related to oxidative stress. Thus, we assessed the effect of refrigerated storage of epididymes and antioxidant supplementation in the post-thawing chromatin status of stallion spermatozoa. Epididymides were retrieved from 4 horses castrated for veterinary reasons and preserved for 24 or 48 h at 4°C; then, spermatozoa were harvested and frozen in INRA 96 (2.5% egg yolk, 2.5% glycerol, 2.5% dimethylformamide), added with 0, 0.1, 1 and 2.5 mM of the antioxidant N-acetylcysteine (NAC). After thawing, chromatin status was assessed by Sperm Chromatin Structure Assay (SCSA, flow cytometry), obtaining DNA fragmentation (%DFI) and chromatin immaturity (%HDS). The effect of male, refrigeration time and NAC concentration were analyzed by GLM (results as mean \pm SEM). %DFI was low ($2.12\% \pm 0.28$) and affected by male and NAC ($1.67\% \pm 0.23$ with 2.5 mM, $p < 0.05$), not being affected by refrigeration time. %HDS was also low ($2.99\% \pm 0.38$), and it was affected by an interaction male \times time, but not by NAC. Overall, %HDS decreased from 24 h ($3.80\% \pm 0.40$) to 48 h ($2.10\% \pm 0.41$). In conclusion, equine epididymal spermatozoa could withstand refrigerated storage up to 48 h with minimal chromatin alterations. Chromatin status seems to be defined by the male, and NAC at 2.5 mM could prevent DNA damage in the freezing/thawing process. Therefore, NAC might be considered for supplementing freezing media for horse epididymal samples, especially from males with susceptibility to sperm DNA damage.

P 78 | Honey bee may substitute egg yolk in ram semen extenders

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The objective of this study was to test whether the substitution of egg yolk by honey bee in the extenders for ram semen may preserve sperm quality during refrigeration at 5°C. This work was conducted in Gran Canaria, Spain (28°N). Semen was collected from 4 Canary rams using artificial vagina. Sperm concentration was adjusted to 400×10^6 cells/ml by adding the correspondent extender. After dilution, progressive motility (visual), plasma membrane integrity (eosin/nigrosine), functionality (HOST), and acrosome integrity (phase-contrast), were assessed. Firstly, egg yolk (20%) was replaced by honey which was added at: 10, 5 or 2.5%. Secondly, the inclusion of honey at 2.5% (i) without, or (ii) with egg yolk (20%) was tested. Diluted semen was slowly cooled to 5°C, and kept at that temperature for 48 h; sperm quality was assessed 24 and 48 h after cooling. Sperm variables were analysed for differences within and between treatments. Stage I. Motility and plasma membrane-function of spermatozoa at 24 and 48 h were similar in the control, 2.5 and 5% honey; 10% honey showed lower values ($p < 0.05$). There were no differences in the other variables. Stage II. Motility of spermatozoa at 24 and 48 h were similar in the control and 2.5% honey plus egg yolk; 2.5% honey without egg yolk showed lower values ($p < 0.05$). There were no differences in the other variables. In conclusion, honey bee (2.5 and 5%) may substitute both egg yolk and glucose in ram semen extenders during refrigeration for 48 h.

P 79 | Effects of FSH and nutrition on the luteal angiopoietin (Angpt) system in ewes

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The CL is a transient endocrine gland and one of the fastest growing and highly vascularized tissues. Its development and function are affected by various factors, including hormones and plane of nutrition. Recently, the effects of arginine (Arg) and diet plan on luteal vascular development have been addressed. However, their effects on one of the important complementary systems, i.e., Angpts, have not yet been investigated. Therefore, the aim of this study was to evaluate the expression of Angpt-1, -2, and the Tie2 receptor in CL of (Exp. 1) ewes administered with Arg or saline, and (Exp. 2) in animals treated with FSH, and fed a control, excess or restricted diet in both experiments. Samples were obtained from early, mid- and/or late-luteal phase of the estrous cycle. Immunohistochemistry (IHC) and qPCR were used. Localization of Angpt1 and Tie2 was observed in capillaries and larger blood vessels, while Angpt2 was present in larger blood vessels. Tie2 was also localized in small and large luteal cells. Protein expression of Angpt1 was affected by diet and luteal phase; Tie2 protein was luteal stage dependent. Angpt-1 and -2, Tie2, and Angpt1/Angpt2-mRNA ratio, were all affected by the luteal phase, but not by diet or Arg (Exp. 1), and by phase and diet in FSH-treated ewes (Exp. 2). When compared with saline treatment, FSH affected Angpt-1 and/

or -2, and Tie2 protein and mRNA expression, depending on stage and/or diet. Thus, expression of Angpt2 and Tie2 in the CL appears to vary during the luteal lifespan, and seems to be affected by diet and/or FSH treatment, indicating their regulatory role in luteal function in sheep. (Supported by USDA-AFRI grant 2011-67016-30174 to ATGB and DAR.)

P 80 | Clinical trial detectin estrus in a female dog with the help oa a temperature sensor placed intramuscular

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Body temperature is a tool helping to detect estrus and ovulation in different species like the cow but it is not used for female dogs. The objective of this work is to study the evolution of the temperature during estrus and ovulation of the female dog with the help of telemetric sensors. Eleven female dogs aged 1 to 8 years were included in the trial and 15 cycles were studied. The sensors were implanted under anesthesia between peritoneum and the right muscle of the belly. They measure the body temperature every 15 min. Their cycles were followed every 2 days after the beginning of the pro-oestrus as measured by vaginal smears and plasma progesterone levels. All dogs tolerated the temperature sensor very well. Six temperature curves could not be exploited during estrus due the equipment malfunction. A temperature peak was observed in an interval of 48 to 72 h before ovulation (+0.5°C in verage) and another within 72 h following ovulation. The increase of the temperature before ovulation could be linked to an increase of PGF_{2α} that follows the ovulatory discharge of LH and which is known for its effect on body temperature. The increase of the progesteron levels exceeding values of 10 ng/ml could be at the origin of the second peak in temperature. Thus in practice it could be possible to continuously follow the body temperature starting from the first signs of vulvar blood losses in order to time the moment of ovulation in the bitch.

P 81 | Molecular identification of the avian sex based on blastodisc DNA

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Molecular sex identification in many species of birds is generally based on the difference of the length of one of the CHD1 gene introns in Z and W chromosomes, obtained with PCR with P2/P8 primer set. The aim of this study was to propose an effective method of DNA

extraction from blastodisc and a diagnostic test, enabling to sex identification as early as possible at the stage of early embryonic development. All experiments were carried out with fertilized, freshly laid eggs of *Phasianus colchicus* (N = 266), which were collected in one day during its reproductive seasons (April and June). 189 blastodiscs were obtained from the surface of egg yolks (a few hours after eggs were laid, without incubation) with a sterile wooden stick (toothpick) and deposited in tubes in 70% ethanol. Each blastodisc was thoroughly fragmented and genomic DNA was extracted using column method. In PCR the initial denaturing step (95°C/5 min) was followed by 42 cycles at 94°C for 30 s, 48°C for 45 s, 72°C for 45 s and the final run at 72°C for 5 min to complete the program. PCR products were separated in 3% agarose gel and visualized by staining with ethidium bromide. The amplified fragments were: 332 bp CHD1-Z (ZZ male) and 358 bp CHD1-W (ZW female). It was possible to determine the sex of all 189 blastodiscs: there were 87 eggs with female and 102 eggs with male embryos. We conclude that we have developed an effective method for obtaining biological material (blastodiscs) and DNA extraction.

P 82 | Reproduction biology of the reed vole (*Microtus fortis*)

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The reed vole (*Microtus fortis* Büchner, 1889) is a small herbivore species (Cricetidae) inhabiting moist meadows of the forest and forest-steppe zones in Eastern Asia. In captivity, voles are used in scientific research, but also bred as forage for carnivorous species. Thanks to their mild temperament and ease of taming they become increasingly popular pets. In nature, reproduction lasts from April to November with up to 6 litters in the favorable season. Gestation period takes then around 20 days and the interval between litters is 40–45 days. The aim of this study was to examine the reproductive biology of the species under artificial conditions, with special emphasis on gestation and intervals between litters obtained in two periods. In this experiment 5 litters from voles kept 1:1 were observed in two mating periods: summer and autumn. Statistical analyses were performed using IBM SPSS Statistics 21 PL package. The first gestation lasted around 4.5 weeks after mating (late spring) of 3.5 months old animals and both parents raised their offspring until the birth of the next litter or up to 21 days max. The next two litters occurred on average in 19.5 days after the previous one without breeding pair separation. The same pair mating was repeated in autumn. The first autumn litter was delivered 22 days after parents mating, and the second one 21 days later. Autumn litters were smaller - counting on average 4 progeny, while in summer it was 6.7 (SD = 1.5). In addition, in autumn females reduced their litter size by killing 1–2 newborns during the first 10 days after birth. A strong negative correlation between litter size and progeny weight in subsequent rearing weeks was observed (–0.668, –0.614, –0.647, –0.556, from 0 to 3 weeks respectively, $p < 0.01$).

P 83 | Effect of estrogen imbalance during neonatal period on the expression of receptors for GDF9 and BMP15 in small antral follicle in pig

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Oocyte-derived GDF9 (growth differentiation factor 9) and BMP15 (bone morphogenetic protein 15) control follicle development acting via their cognate receptors (ALK5, ALK6 and BMPR2). Here we studied the effects of neonatal estrogen imbalance induced by chemicals with estrogenic/antiestrogenic activity on paracrine regulation of ovarian follicle development in pig. Animals were injected with 4-tert-octylphenol (OP, 100 mg/kg body weight [bw]), ICI 182,780 (ICI, 400 µg/kg bw), methoxychlor (MXC, 20 µg/kg bw) or corn oil with DMSO (control) between postnatal days 1 and 10 (n = 4). Small antral follicles (3–5 mm) were obtained from sexually mature gilts. To assess ALK5, ALK6 and BMPR2 expression immunohistochemistry (ALK6 and BMPR2 antibodies: a gift of Dr. C-H Heldin, Uppsala, Sweden), qPCR and Western-blot were performed. ALK5, ALK6 and BMPR2 were localized in granulosa and theca cells in all examined groups. ALK5 mRNA and protein expression were elevated in OP group and diminished in ICI group. ALK6 mRNA expression was elevated in MXC group, while MXC and OP treatment decreased protein level. MXC and OP treatment increased BMPR2 mRNA expression. Concluding, estrogen imbalance during neonatal period induces changes in ALK5, ALK6 and BMPR2 expression in porcine small antral follicles, which may affect follicular cell function regulated by GDF9 and BMP15. (Support: National Science Center, Poland (Grant No. 2015/19/B/NZ9/00420)).

P 84 | Comparison of two oestrus synchronisation protocols in lactating dairy cows: OVSYNCH protocol and a RESYNCH protocol 28 days after artificial insemination

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OVSYNCH protocol has been used for twenty-two years to synchronise oestrus. Several modifications were made to simplify it or to improve fertility results, one of them is the RESYNCH protocol. It consists of introducing a second ovsynch early, 28 days after artificial insemination (AI), even before knowing if the cow is pregnant after the 1st ovsynch. Its objective is to decrease the number of unproductive days in case of no pregnancy. The main trials were conducted in American herds, the European studies are few. In this study 214

dairy cows, of french herds were submitted to first AI 70–120 days post partum. 107 cows were submitted to ovsynch protocol (control group) followed by PGF_{2α} injection for non-pregnant cows, and 107 cows of unknown pregnancy status submitted to resynchronization 28 days after the first AI, with the Resynch protocol (R group). The success rate in the first ovsynch protocol was high, 41.6%. The level of milk production, particularly in high-producing cows, reduces the risk of pregnancy for cows inseminated following synchronization with an ovsynch protocol, as well as a fat-to-protein ratio greater than 1.5, indicating a major energy deficit. However, the pregnancy rate was highest in the control group 46.7% than in R group 36.4% with no statistically significant difference (p = 0.13), without improving pregnancy rate after resynchronization, 34.1% vs. 34.8% for R group vs. control group, respectively (after 2nd AI for the non pregnant cows), this protocol allows to submit more animals to second postpartum AI and to reduce the interval between a non-pregnant diagnosis and the fertilizing insemination.

P 85 | The use of cytopsin preparation (cyto centrifuge) technique cytopsin preparation for endometrium evaluation in embryo transferred cows

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The aim of this study was to evaluate the efficacy of a liquid base cytologic technique in the selection of recipient animals used in embryo transfer (ET) programs during the evaluation of the uterus cytologically and gynaecological. The research was performed on 45 cows from East Mediterranean Agriculture Research Institute farms in Adana. Cytopsin technique: Brushes were taken into fixative solution and cyto centrifuged at 1500 rpm for 15 min. Epithelial cells collected by the brush cytology technique were deposited onto poly L-lysine coated slide glasses by cyto centrifugation (Cytospin; Thermo Shandon Southern Ltd, Cheshire, England). Wet cyto centrifuged slides were drawn out, dried and stained with Hematoxylin-eosin (HE) and Diff -Quick. There was no statistical difference between milk yield, BCS and lactation counts in cows who were pregnant (22) and non-pregnant (23) (p > 0.05) and between pregnant and embryonic deaths groups 27.3% (6/22) (p > 0.05). Single cells were commonly seen, and the background was usually cleaner with less blood. Nuclear chromatin detail may be more easily discerned. The differential diagnosis of exfoliated endometrial cells includes abraded endometrium, inflammatory cells, young endometrial cells with cytoplasm and small round cell nucleus. The most common observed cells in cytological preparations were found to be endometrial in pregnant cattle (n = 20). In addition, leukocytes and supranuclear vacuolar cells were observed.

Neutrophils, macrophages, and lymphocyte concentrations were evident in non pregnant ($n = 5$) and embryonic deaths occur in cows ($n = 6$). The results indicate that further studies would be necessary to check the cytospin preparation techniques for embryo transferred cows in long term period.

P 86 | Effects of spermidine on in-vitro production of bovine embryos: preliminary results

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Polyamines play an essential role in mammalian physiology and participate in the modulation of chromatin structure, gene transcription and translation, DNA stabilization, signal transduction, cell growth, proliferation and migration, membrane stability and functioning of ion channels. Spermidine, a naturally occurring polyamine, has recently emerged as exhibiting anti-aging properties. The aim of the current study was to evaluate the effects of spermidine supplementation during in vitro maturation (IVM) of bovine oocytes on blastocyst rates after in vitro fertilization. Ovaries were collected from a local abattoir, and cumulus-oocyte complexes ($n = 273$, 91 per group) were matured in vitro in 3 different groups for 24 h, respectively. The maturation medium (mod. TCM199) was either supplemented with spermidine in 2 different concentrations (50 μmol or 5 μmol : experimental groups) or left unsupplemented (control group (CG)). After IVM, oocytes were fertilized in vitro for 19 h, and presumptive zygotes were cultured in vitro for 8 days. At the end of the culture period blastocyst rates were assessed. Results indicate that a supplementation with 50 μmol spermidine during IVM led to increased blastocyst rates (21.8% blastocysts) in comparison to the other groups ($p < 0.05$), since 5 μmol spermidine and 0 μmol (CG) gave rise to only 13.8% and 14.1% blastocysts, respectively. Further oocytes will be investigated for validation of these preliminary results. (*shared senior authorship).

P 87 | Massive chronic-infectious periorchitis and hydrocele in a stallion

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An 11-year old draft horse stallion, not intentionally used for breeding, was presented with fever ($>40^\circ\text{C}$) and a massively enlarged right (R) scrotum ($40 \times 30 \times 20$ cm) despite antimicrobial and anti-inflammatory therapy for 3 weeks. Otherwise, clinical examination was normal apart from fever (38.7°C) and a mild ventral edema. The entire scrotum was enlarged, hard, firm and warm, but not painful on palpation. Both testes were orientated properly. Ultrasonographic examination showed

anechoic fluid accumulation surrounding the R testis and captured in honey comb-like structures. Both testes had a homogenous pattern. A clear-yellow fluid could be aspirated from the vaginal cavity (500 ml) upon centesis. The cell count was increased with mainly neutrophils. Intracellular cocci were seen and the fluid cultured positive for *Strep. zooepidemicus*. Upon transrectal palpation the R spermatic cord was enlarged (approximately 4 cm). Transabdominal ultrasound and abdominocentesis were unremarkable. The stallion was treated with Na-penicillin, gentamicin, flunixin meglumine and pentoxifylline for one week; with minor improvement and a persistent fever. A bilateral open orchectomy with removal of the inflamed tissue was performed. Histopathology showed a chronic fibrinous periorchitis and severely fibrotic tunics. The R testis showed no spermatogenesis, while the left seemed functional. The stallion went home 2 weeks later with resolving swelling. An entry port for the infection has not been identified. However, causes could be secondary to trauma, orchitis or periorchitis, or idiopathic.

P 88 | Effect of insemination timing following 5 or 7 days CIDR Co-synch in Nilli Ravi buffalo heifers

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The aims of the present study were to compare the pregnancy rates in relation to optimal time of artificial insemination (AI) 5 or 7-days CIDR Co-synch in Nili Ravi buffalo heifers. Eighty Nili Ravi buffalo heifers were randomly separated into two treatments. First treatment ($n = 40$) was subjected to controlled internal drug release (CIDR) containing 1.38 grams progesterone for 5 days while the second treatment ($n = 40$) received CIDR for 7 days. On CIDR removal both treatments received 25 mg of prostaglandin intramuscularly hence further divided into two subgroups regarding timing of AI. In 5-day CIDR Co-synch ($n = 20$) animals were injected 100 μg of GnRH intramuscularly and inseminated concurrently at 72 h after CIDR removal and the remaining half ($n = 20$) were injected and inseminated concurrently at 84 h after CIDR removal. Similar treatments were given to the 7 days CIDR Co-synch group. Artificial insemination was done by using frozen thawed semen of a buffalo bull. Heifers were scanned for pregnancy within days 30–40 after AI. The follicular growth rate was tended to be high in 7-days as compared to 5-days CIDR Co-synch. Ovulatory follicle size was significantly ($p > 0.05$) higher in 7 than 5 days CIDR Co-synch. Ovulation rate was significantly higher in 7-day CIDR Co-synch as compared to 5-day CIDR Co-synch treatments (95% vs. 80%, $p = 0.043$). There was a non-significant difference in pregnancy rates between buffalo heifers inseminated either 72 h (30%) or 84 h (50%) in 5-day CIDR Co-synch treatment; whereas in 7-day CIDR Co-synch pregnancy rates were higher ($p < 0.05$), at 84 (65% odd ratio = 5.5) than at 72 h (25%). In conclusion the buffalo heifers treated with 7-day CIDR Co-synch at 84 h improved fertility.

P 89 | Dietary supplementation of tall oil fatty acid and resin acid in farrowing sows can affect the colostrum quality

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Colostrum plays an essential role in piglet survival and growth, providing the piglets with a source for both immunoglobulin (mainly IgG) and energy. The neonatal piglets lack IgG, which makes them dependent on colostrum as the sole source of antibody. The aim of this study was to examine whether tall oil fatty acid and resin acid (RA) derived as a co-product in pulp production, added to a late pregnancy diet affected colostrum composition and yield (CY) in sows. 44 sows were randomly allocated to two groups as follows: a negative control diet ($n = 21$) and the same diet supplemented with 5 ml RA/day ($n = 23$) during the last 1 week of pregnancy to whole lactation period. The RA used was Progres[®] (Hankkija Oy, Hyvinkää, Finland). Within the first 2 h from the beginning of farrowing, a 10 ml colostrum sample was obtained to check for nutritional composition (protein, fat, lactose, dry matter, with FITR analysis), and immunoglobulin content (IgA, IgM and IgG with ELISA analysis). All piglets were individually weighted at birth and 24 h later in order to calculate CY. Colostrum content of protein, lactose, fat and dry matter did not significantly differ between the two groups. While RA fed sows had higher level of IgG (86.37 ± 5.27 mg/ml vs. 70.94 ± 5.49 mg/ml) in colostrum ($p < 0.05$), IgA and IgM levels in colostrum remain the same in two groups. There were also no changes in the CY of the sows in the two groups. In conclusion, adding RA to late pregnancy diet in sows did not affect the CY and protein, fat and lactose content in colostrum, but contributed to higher IgG content. Therefore RA added to sow diet seems to increase colostrum IgG and ensure the better survival of neonate piglets.

P 90 | Effect of quercetin addition to murine morulae produced in vitro: changes in total cell count and hypoxia-inducible factor 1 α (HIF-1 α) expression

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Quercetin, a flavonoid with antioxidative properties induces the expression of the HIF-1 α in HeLa cells as well as trophoblast differentiation. We aimed to evaluate if embryo culture media (KSOM) added with quercetin increases the quality of murine embryos obtained by in vitro fertilization (IVF). B6D2 female mice were stimulated to trigger ovulation, cumulus-oocyte complexes were obtained and conventional IVF was performed; the zygotes were followed to the morula stage and 4 experimental groups were evaluated. IVFKSOM: morulae were cultured to the blastocyst stage, ($n = 20$); IVF5 μ M or IVF1 μ M: morula culture medium was added with 5 μ g/ml ($n = 25$) or 1 μ g/ml ($n = 30$) of quercetin for 2 h

and cultured in KSOM to the blastocyst stage. IU: in vivo produced blastocysts were obtained from pregnant females ($n = 16$). Blastomer number was assessed after staining with 2.5 μ g/ml of Hoechst-33342 and HIF-1 α expression was contrasted by indirect immunocytochemistry by fluorescence microscopy. Our results showed that the number of cells was 63.9 ± 2.5 for IU, 39.6 ± 3.2 for IVFKSOM, 37.8 ± 2.9 for IVF5 μ M and 38.9 ± 2.6 for IVF1 μ M (mean \pm SEM); Only IU derived blastocysts had a significantly higher number of cells ($p < 0.05$). The presence of HIF-1 α was consistently higher in quercetin-treated and in vivo blastocysts compared to control. Hence, supplementation of the culture medium with quercetin increases HIF-1 α expression but not the total cell number of IVF-derived murine blastocysts.

P 91 | Correlation between parameters of motility in frozen-thawed semen of goats

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The objective of the present study was to evaluate the relationship between sperm parameters that quantify the motility in frozen-thawed semen of 7 bucks goats (4 Alpine and 3 Saanen) located in Medellín, Department of Antioquia, Colombia. Statistical analysis was performed using a Pearson correlation. The results showed a strong association between curvilinear velocity (VCL) and mean velocity (VAP) ($r = 0.95$, $p \leq 0.001$), the correlation between linearity index (LIN) and straightness index (STR) was very strong ($r = 0.91$, $p \leq 0.001$). In contrast, the relationship between VCL, rectilinear velocity (VSL) and VAP vs. amplitude of lateral head displacement (ALH) was significant, but less pronounced ($r = 0.78$; $r = 0.45$; $r = 0.61$, $p \leq 0.001$). In relation to the frequency of sperm beat (BCF) vs. rectilinear velocity (VSL), VCL and VAP, mean correlations were observed. The variables that present the lowest correlation correspond to LIN vs. ALH ($r = 0.001$; $p \geq 0.07$), between ALH vs. STR ($r = 0.02$, $p \leq 0.001$) and between ALH vs. WOB (wooble) ($r = 0.05$, $p \leq 0.001$). The values corresponding to ALH not present a close correlation with the progression parameters in post-thawed goat semen, but it does have a correlation with the velocity. These results indicate that the rapid movement of the frozen-thawed buck sperm are associated with a greater lateral displacement of the sperm head.

P 92 | Expression of components of Ca²⁺ release-activated Ca²⁺ (CRAC) channel in the ovine corpus luteum during the early pregnancy and PGF_{2 α} induced luteolysis

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Calcium (Ca^{2+}) involves in several signal transduction pathways as the second messengers regulating physiological cellular events. Ca^{2+} is regarded as a mediator of prostaglandin F2 alpha ($\text{PGF}_{2\alpha}$) in cells. Ca^{2+} release-activated Ca^{2+} (CRAC) channel mediate entry of Ca^{2+} into cells and is composed of endoplasmic reticulum (ER)-located Ca^{2+} -sensing stromal interaction molecule (STIM 1–2) and the pore-forming subunit known as Orai 1–3. Objective was to evaluate expression of components of CRAC principally Orai1, Orai2, Orai3, STIM1, and STIM2 in the ovine corpus luteum (CL) during early pregnancy and at induced luteolysis. CL samples were obtained from pregnant (P) and cyclic (C) ewes of days 12 ($n = 4$) and 16 ($n = 4$). For the induced luteolysis model, ewes were injected with $\text{PGF}_{2\alpha}$ on 12th day of the cycle and luteal tissues were collected at 0 h (no $\text{PGF}_{2\alpha}$ injection, $n = 4$), 1 h (PG1, $n = 4$), 4 h (PG4, $n = 4$), and 16 h (PG16, $n = 4$) after injection. mRNA expression levels of CRAC components were quantified using RT-qPCR. Expression of Orai1 was decreased in PG1 while there was no change in PG4 and PG16. Expression of Orai2, Orai3 and STIM1 appeared to be steadily expressed in PG1. Orai2 was upregulated in PG16 while Orai3 and STIM were downregulated in both PG4 and PG16. While expression of STIM2 was decreased in PG1, it did not change in PG4 and P16. Expression of Orai1, Orai3, STIM1, and STIM2 was significantly upregulated in C16 compared to C12. Expression of Orai2, STIM1, and STIM2 were decreased in P16 compared to C16. Components of CRAC appeared to be differentially regulated in early pregnancy and during induced luteolysis. It may be concluded that CRAC involves in replenishing Ca^{2+} in luteal cells and endoplasmic reticulum during luteolysis.

P 93 | The evaluation of blood concentrations of Anti-Müllerian hormone (AMH) in dogs with cryptorchidism and neoplastic diseases

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The aim of this study was to compare the concentrations of Anti-Müllerian hormone (AMH) in intact dogs, dogs with cryptorchidism (unilateral, subcutaneous) and dogs with testicular tumour. In order to establish AMH concentration we used immunochemical methods of electrochemiluminescence analysis (ECLIA). The blood samples were collected peripherally from the v. cephalica antebrachii and locally from the plexus pampiniformis in both testes. Twenty four dogs of different breeds were divided into 3 categories (intact $n = 8$; cryptorchids $n = 8$; testicular tumours $n = 8$). Multiple comparison procedures were performed by the One Way Analysis of Variance (ANOVA), Student-Newman-Keuls method, using SigmaStat software (Jandel Corp. USA). Significant increase in AMH concentrations was found in cryptorchid group in both, peripheral and local venous blood compared to control. Significant increase in AMH concentrations in peripheral blood were observed in cryptorchid group ($54.98 \pm 30.07 \mu\text{g/ml}$) compared to control group ($6.49 \pm 3.24 \mu\text{g/ml}$). The same was observed in the case of local blood concentrations, being significantly higher in cryptorchid group (right testicle (RT) $51.92 \pm 30.59 \mu\text{g/ml}$; left testicle (LT)

$46.33 \pm 34.86 \mu\text{g/ml}$) ($p < 0.05$) and slightly higher in tumour group (RT $17.18 \pm 12.5 \mu\text{g/ml}$; LT $23.9 \pm 16.63 \mu\text{g/ml}$) in comparison with control group (RT $6.25 \pm 3.06 \mu\text{g/ml}$; LT $6.82 \pm 3.09 \mu\text{g/ml}$). Also the concentration in peripheral blood in tumour group ($28.13 \pm 26.7 \mu\text{g/ml}$) was higher than in the control group, but the difference was not significant. Histological typing of different kinds of testicular tumours will be part of our further study. (This study was supported by VEGA 1/0090/14.)

P 94 | Multiple periovulatory inseminations are associated with an increase of pregnancy rate in fertile mares without raising the risk for post-breeding endometritis

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Contrary to artificial insemination programs, under natural conditions a stallion mates a mare multiple times per estrous cycle. The objective of this study was to determine if multiple inseminations (MI) would result in increased pregnancy outcome, or if they are associated with an increase of post-breeding endometritis. Eighty-two estrous mares received 1.25 mg deslorelin to induce ovulation, and from 24 h later on, they were inseminated either twice (group DI), four times in short intervals (group MISI) or four times in long intervals (MILI), after division of only one commercial insemination dose into two or four portions each, respectively. Uterine bacteriology and cytology sampling were conducted directly before the first insemination and 24 h after the last insemination. Mares of the MI groups showed a significantly higher pregnancy rate than mares of the DI group. Bacteriological and cytological results showed no difference between groups. In addition, mares of the MISI group showed significantly less intrauterine fluid accumulation after the inseminations than mares of the DI and MILI group. We conclude that multiple periovulatory inseminations lead to higher pregnancy rates per cycle, and suggest, that they do not lead to impaired inflammatory reactions of the uterus in healthy fertile mares.

P 95 | Optimization and characterization a novel model for culture of equine oviductal epithelial cells

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Establishment an optimal in vitro culture model for equine oviductal epithelial cells could be a step forward to gain a deep insight of oviduct function and the early embryonic development in equines. Therefore, we optimized a new isolation method of oviductal epithelial cells and

cultured both (cells and explants) from follicular, early- and mid-luteal phases (5–6 independent replicates/group), using either fetal calf serum (10%FCS) or estrus mare serum (5%EMS). After culture, cells, explants and medium were collected at 0, 24, 48, 72 h, one week, and 10 days (cells), for total RNA isolation, and subsequent quantification of miRNAs and their target that are relevant to oviductal physiological function. Fresh collected tissue & cells served as control. Furthermore, oviductal explants were stained with haematoxylin-eosin (H&E) and measurement concentrations of prostaglandins (PGE2 & PGF α 2) in medium, by ELISA. Data analysis was done using two-way ANOVA. Epithelial morphology was maintained for 10 days, for both FCS & EMS, as shown by light and stereo microscope. Similarly, the presence of highly differentiated epithelium with basal nuclei and secretory granules were observed in H&E stained explants, until a week, for both FCS and EMS groups. MiR-155, miR-223, miR-17, miR-24, miR-532-5p, miR-181b, miR-21, and let-7a, as well as their targets; IGF1, OVGP1, PTGER2, CSF1, and VEGFA were differentially expressed according to the oestrous stage. Furthermore, the secreted PGE2 & PGF α 2 revealed different dynamic patterns according to the oestrous stage. Our in vitro model maintained the morphological and oviductal physiological function. So, it could be used as an excellent model for multi-omics study and deciphering the first cross-talk between mother and embryo in mares.

P 96 | Age-related alterations in the quality of sperm from the European bison

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The aim of the present research was to study the morphology, motility, DNA fragmentation and fertility characteristics of the epididymal sperm of the European bison, depending on the age of the bulls. Sperm was obtained following a forced slaughter (as a result of severe injury) of five bulls (3 bulls of 5–7 years old, 1 bull of 13 years old and 1 bull of 17 years old). After freezing and thawing, sperm motility was evaluated using CASA. DNA fragmentation of spermatozoa was assessed with the acridine orange test. The fertilizing capacity of sperm was determined using a heterologous IVF system (with sperm of bulls aged 7 and 17 years). The sperm was prepared by the swim-up method. In vitro matured bovine oocytes (n = 267 oocytes) were co-incubated with the sperm in TALP medium; the resulting embryos were cultured up to the blastocyst stage. The sperm motility of 5–7 years old bulls was higher than that of 17 years old bull (35.1 ± 3.4 vs. $21.7 \pm 0.6\%$, $p < 0.01$). Furthermore, the degree of DNA fragmentation of spermatozoa and the frequency of their abnormalities rose (at least $p < 0.01$) with increasing age from 5–7 to 17 years (from 7.6 ± 0.3 to $63.7 \pm 0.4\%$ and from 9.1 ± 0.2 to $14.6 \pm 0.9\%$, respectively). After IVF of bovine oocytes, significant differences were found in the rate of cleavage (76.6 ± 2.6 vs. $38.3 \pm 4.2\%$, $p < 0.001$) and blastocyst

formation (29.2 ± 3.0 vs. 9.1 ± 1.3 , $p < 0.001$) between sperm from young and old bulls. In conclusion, the quality of the European bison sperm declines with age at least to 17 years-old. (This study was supported by Program of Presidium of the Russian Academy of Science, project no. III.13.3.)

P 97 | Application of a new drug for chronic inflammation treatment in cows

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The objective was to develop the drug for endometritis treatment in cows and the method of its application. The complex drug contains antimicrobial components and silicon glycerolates with different functionality as the active substances, xanthan gum and thickener, distilled water as the solvent. The drug novelty is combining three bactericidal agents in one dosage form, which provides a synergistic effect due to different mechanisms of action on the microbial cell. The drug contains silicon dimethyl- and tetraglycerolates. Research and production tests were performed in 80 high producing cows with chronic endometritis (control and experimental). Treatment regimen based on a general stimulating therapy was assigned to the cows of both groups. Further, the cows from the control group were intra-uterinely injected with 40 ml of well-known industrial drug «Endometromag-T» 4 times every 48 h, and the cows from the experimental group – with the new drug (using the same dose and the same periods). Analysis of quantitative indicators of reproductive function showed a fairly significant reduction of the period from the start of treatment to the resumption of sexual cyclicity by 7 days, the period from birth to conception (service period) decreased by 29 days, and the insemination index – by a factor of 1.47 in the cows of the experimental group. The conception rate from the first insemination and the overall conception rate were higher in the experimental animals by 20% and 5%, respectively, in comparison to the control animals. Thus, the new drug possesses a wide range of antimicrobial action, a pronounced therapeutic effect, and it is more effective than its prototype. (This work was supported by the Russian Foundation for Basic Research, project 16-33-00376 mol_a).

P 98 | The CPC and BPH differentiation in multiparametric magnetic resonance imaging

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The canine prostate gland can be affected by several disease processes the most common being Canine Prostate Cancer (CPC) and Benign Prostatic Hyperplasia (BHP). Clinical signs associated with both conditions often overlap. Therefore it is important to reach a definitive diagnosis prior to initiating treatment. The purpose of this study was to evaluate the multiparametric Magnetic Resonance Imaging (mp-MRI) at 3.0 T as a method in differentiation of CPC from BHP. Two dogs (years 12, 13) with CPC and two (years 10, 9) with BHP confirmed with clinical and histological examination underwent general anaesthesia and mp-MRI based on PI-RADS™ v2 human protocols. Protocols were conducted with MR imaging on Discovery MR750w 3.0T, coil GEM Large Flex and consisted of axial T1WI, high resolution planar T2WI, diffusion weighted imaging (DWI) and dynamic contrast enhanced MRI (DCE). CPC appears as focal hypointensive against the background of the high-signal-intensity glandular tissue. CPC appears bright on DWI images and dark on apparent diffusion coefficient maps, indicating restricted diffusion. BPH gives a heterogeneous appearance to the transition zone on T2WI. BPH nodules demonstrate low T2WI signal intensity and mimic transition zone cancer. In conclusion, mp-MRI at 3 T may be useful for distinguishing CPC from BPH foci, with overlaid T2WI and DWI (ADC) images. The role of DCE imaging in differentiation between CPC and BHP in dogs is limited.

P 99 | Non-esterified fatty acids in early luteal bovine oviduct fluid mirror plasma concentrations

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Oviduct fluid (OF) provides the environment for the early embryo when it is at its most sensitive. Earlier research characterized OF, but information on its lipid composition is scarce. Specifically, non-esterified fatty acids (NEFAs) are highly relevant, as they are typically upregulated during lipolytic disorders and were shown to hamper in vitro embryo development. Therefore, using a novel ex vivo approach, we investigated whether and to which extent plasma NEFAs are reflected in OF. Plasma and OF from 49 Belgian Blue cows in the early luteal phase were sampled. Blood samples were obtained during exsanguination, and OF was collected using a method combining flushing and application of mechanical pressure. A tracer molecule, amaranth, was used to correct for differences in OF dilution, and post sampling glycolysis was inhibited with sodium fluoride. Plasma and OF NEFA concentrations were photometrically analysed. Data were expressed as mean \pm SD, and analysed using Pearson correlation and paired student's t-testing. Plasma and OF NEFA concentrations were 0.31 ± 0.14 and 0.29 ± 0.19 mmol/l, respectively. Plasma and OF NEFAs were not significantly different ($p = 0.13$), however, there was a trend for positive correlation (Pearson correlation coefficient = 0.56; $p = 0.07$). Results indicate that NEFAs occur in OF at concentrations

seen in plasma of healthy cattle. Furthermore, data suggest that elevated plasma NEFAs may alter the embryonic environment in the oviduct during maternal lipolytic disorders, which may contribute to the pathology of sub- and infertility.

P 100 | Sarcoma in newborn puppy, preliminary study

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Sarcomas in newborn puppies are very rare. Newborn German shepherd dog puppy with massive tumor on the right rear limb was presented without any other clinical changes. Six other puppies in this litter were normal clinically. The mass had been increasing size very intensively together with the puppy development for the next 7 days. The decision about surgical removal of the tumor was made however, during the procedure puppy died. Standard necropsy was performed, numerous samples of the mass as well as internal organs and blood samples were collected for histopathology and future genetic examination. Blood samples of other puppies in the litter, mother and father were collected as well. During post-mortem examination the only important lesion was dermal and subdermal mass which had poorly defined borders and soft consistency. Slides were stained with haematoxylin and eosin method. Microscopic examination revealed that subcutaneous tissue and skin were heavily infiltrated by mesenchymal, elongated and spindle-shaped cells, with poorly visible, scant, pale eosinophilic cytoplasm. Neoplastic infiltration was present also among adnexal structures and beneath epidermis. Nuclei of neoplastic cells were oval, elongated, with clear chromatin and visible nucleoli. Mitotic figures were common (2–4 mitotic figures per 1 hpf). Based on initial microscopic examination sarcoma was recognized. Detailed genetic investigations are in progress.

P 101 | Investigation of sensitivity of Fassis[®] BoviPreg test kit for early pregnancy diagnosis in cattle

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We aimed to reveal the sensitivity of Fassis[®] BoviPreg pregnancy test kit (PTK), based on determination of early pregnancy specific protein (PAG), on whole blood or serum of cows as early as 30 days (D30) of pregnancy. The current study was carried out on 41 cows. Blood samples were collected from vena cocygea for whole blood and serum on D30 following fixed time artificial insemination (FTAI). Pregnancies on D30 after FTAI were diagnosed with

transrectal ultrasonography (USG, as the gold standard method). Moreover Fassis[®]BoviPreg Test Kits (Fassisi, Gesellschaft für Veterinärmedizin und Umweltanalysen mbH, Göttingen, Germany) were used with whole blood in field condition (15–30°C) and with serum sample in laboratory condition (15–30°C) to determine pregnancies on D30. Pregnancy results from the PTK were compared with USG results. Pregnancies on day 50 were also checked again with USG and compared with results on day 30. Sensitivities of PTK (Pregnancy Positive Results/Pregnancies determined with USG on D30 following FTAI X100) in whole blood and serum were determined as 65.00% (13/20) and 75.00% (15/20), respectively. Specificity of PTK (Non-Pregnancy Results/Non-Pregnancy determined with USG on D30 following FTAI X 100) in whole blood and serum were determined as 76.19% (16/21) and 80.95% (17/21), respectively. Accuracies of pregnancy kit (All Accurate Results in PTK/Number of Animals in Study X 100) for whole blood and serum were 70.73% (29/41) and 78.04% (32/41), respectively. In conclusion, pregnancy results with Fassis[®]BoviPreg were more specific and sensitive for serum than that for whole blood. Nevertheless, the early pregnancy determination with kits was less sensitive, specific and also accurate than these with USG on D30 of pregnancy in cows.

P 102 | Castration influence on male and female rat body weight

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Castration is the most common surgical procedure in small animals. The aim of the study was to evaluate changes in body weight in growing rats caused by castration. Forty one 8-week-old Wistar rats (21 males and 20 females) were divided into two groups: study group (14 males, 13 females) and control group (7 males, 7 females). On day 0 study group rats underwent castration (orchidectomy in males, ovariectomy in females) in general anesthesia with the use of isoflurane. Control group rats underwent sham surgery. After the procedure animals body weights were measured. For 30 days rats from both groups were kept in culture conditions with 12-h day/night system and with the access to fresh water and fodder ad libitum. On day 30 body weight was measured again. On day 0 male rats from both study (218.9 g ± 23) and control group (235.7 g ± 22.2) were significantly heavier than females (160.6 g ± 22.4 and 173.1 ± 12, respectively) ($p < 0.05$). On day 30 this tendency has not changed. However, male rats from castrated group (330.4 g ± 22.1) were significantly lighter than those from control group (364.7 g ± 24.9) ($p = 0.004$), whereas castrated females (270.6 g ± 39.0) were significantly heavier than intact ones (226.1 g ± 13.6, respectively) ($p = 0.01$). In summary, castration seems to have significant influence on rats growth. Animals sex plays a crucial role in this mechanism. This phenomenon needs further research. A new study concerning sex hormones receptors in rat thyroid and adrenal glands is in progress.

P 103 | Surgical removal of follicular cysts during pregnancy in the bitch: a case report

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Ovulation disturbances often lead to follicular cyst formation in the bitch. Surgical removal is nowadays the most successful method of treatment. However, there is no available information about this therapy in pregnant patients. A 7.5-year-old German Shorthaired Pointer bitch was presented with a history of infertility for the last 3 years. The bitch has been showing signs of heat for 3 days. Clinical examination revealed no changes. Vaginal smear showed estrogenization. During ultrasound examination, growing follicles on both ovaries and one large cyst-like structure on the left ovary were present. It was decided to follow ovulation and development of the cyst by regular ultrasound examination. At the day of ovulation the cyst-like structure was not changed. The bitch was sent for mating 2 days later. Ten days after breeding blood serum estrogens level was 160 pg/ml. Ultrasound revealed one 13 mm follicular cyst on the left ovary. A decision of surgical cyst removal via laparotomy was made. Supplementation with exogenous progesterone was also performed. At day 25 postmating a diagnosis of multiple pregnancy was made. However, a new follicular cyst (11 mm) was found. Ultrasound control of pregnancy, progesterone supplementation until day 58 postmating and planned caesarean section at day 62 were recommended. Further pregnancy examinations showed no changes. In caesarean section with ovariohysterectomy 8 healthy puppies were excavated. In summary, surgical ovarian cyst removal combined with progesterone supplementation in the pregnant bitch can be a safe and successful method of treatment.

P 104 | Preliminary study on biomarkers for canine mammary tumors

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Preliminary study on biomarkers for canine mammary tumors
Canine mammary tumors (CMT) are the most frequently diagnosed neoplasia of bitches, therefore it represents a serious clinical problem. Due to its high prevalence, early diagnosis is necessary. Evaluation of specific serum and tissue biomarkers of CMT may be of a great use. In human medicine, CEA and CA 15-3 are the most frequently evaluated biomarkers of breast cancer. In case of CMT studies are scarce. We gathered 70 samples of CMT together with blood samples from veterinary clinics in Poland. All dogs had previously undergone blood tests as well as X-ray examination. During mastectomy, the great majority was spayed (60%). The average age of the bitches suffering from CMT was 9–12 years (65%). Breed

predisposition was seen, CMT being most common in mixed breed dogs (34%), followed by Yorkshire terriers (15%), Dachshunds (10%) and German Shepherds (8%). Among all samples, 68% represented malignant tumors (MT) and 32% were benign tumors (BT). Among MT, the most common was simple carcinoma (29%), followed by carcinoma arising in benign mixed tumor (20%) and complex carcinoma (17%). Metastases were found in 13% of the cases. Complex adenoma was the most frequent BT (17%), followed by benign mixed tumor (13%). We confirmed the presence of CEA and CA 15-3 in CMT in both serum and tissue samples. The preliminary results suggest a positive correlation between serum and tissue levels of CEA and CA15-3 and that its expression is higher in MT compared to BT. The research is ongoing and the results will soon be published.

P 105 | Histological investigation and karyotyping of amorphous globosus cases occurred in a dairy herd throughout one year observation

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Amorphous globosus (AG) or acardiac monster which is defined as a skin covered asymmetric spherical mass without any functional organs is a rare complication of twin pregnancies and has been reported in various species. During one year observation, 3 cases of AG occurred out of 1084 calving events (0.0027%) and 46 twinnings (0.065%). Regarding the morphology, the biggest one had a pumpkin like appearance (21 × 14 × 13 cm) while the other two cases were oval (16.5 × 10 × 6 cm) and spherical (12.5 × 13 × 12 cm). All of the 3 cases occurred in multiparous cows (third and fifth parity) and acardiac monsters appeared after delivery of a normal fetus without dystocia. In 2 cases, normal co-twins were alive and apparently healthy, while in one case it was stillborn. In human cases, the mortality rate of the normal co-twin has been reported to be 50–70% without therapeutic intervention, due to cardiac failure. Hence early diagnosis by color Doppler ultrasound and therapy by ligation of the umbilical cord through laser coagulation is essential. Histological examination of AG fetuses showed the presence of fibroblasts and dense connective tissue in the outer cortex of the spherical mass and loose connective tissue, adipose tissue and blood vessels at the central part. In two cases, fibrocartilaginous and osseous tissue were also found underneath the peripheral cortex. We used skin fibroblasts for karyotyping for chimerism detection. In two cases, karyotypes sex were similar to the normal co-twins (60 + XY and 60 + XX), while in one case it was opposite (60 + XY). Our result support previous reports about the possibility of a dizygotic origin of AG. Nevertheless, none of the born heifers did show clinical signs of freemartinism.

P 106 | Effect of age-associated lipidperoxidation on semen quality of Nili-Ravi buffalo bull

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The objective of the study was to compare Malondialdehyde (MDA) levels and quality of fresh and frozen-thawed semen in aged vs. young Nili-Ravi buffalo bulls. The hypothesis of the study was that age-associated increase in MDA will affect buffalo bull semen. Single ejaculate was collected on weekly basis for four weeks from aged (13.6 ± 1.0 years; n = 3) and young (3.4 ± 0.3 years; n = 3) Nili-Ravi buffalo bulls. MDA (nmol/ml) was estimated through thiobarbituric acid assay (TBA) in fresh and frozen-thawed semen. The quality of fresh and frozen-thawed semen was estimated through motility (%), viability (%), DNA and acrosome integrity. MDA (nmol/ml) did not differ (p > 0.05) between aged vs. young bulls in fresh (5.6 ± 1.4 vs. 5.2 ± 1.2) and frozen-thawed (53.1 ± 2.8 vs. 48.4 ± 2.6) semen, respectively. In fresh semen, motility, concentration, and viability did not differ (p > 0.05) in aged vs. young bulls; however, volume of fresh semen increased (p < 0.05) while DNA integrity decreased (p < 0.05) in aged vs. young bulls. In frozen-thawed semen, motility, viability and DNA integrity decreased (p < 0.05) in aged vs. young bulls. In frozen-thawed vs. fresh semen, MDA (nmol/ml) increased within young (48.4 ± 2.6 vs. 5.2 ± 1.2) and aged bulls (53.1 ± 2.8 vs. 5.6 ± 1.4) while motility and viability decreased (p < 0.05) within the age groups. In conclusion, MDA did not change due to age in buffalo bull semen. However, freezing and thawing cause increase in MDA production and decrease the semen quality in aged and young buffalo bulls.

P 107 | Bioactives from *Cynara scolymus* and vitamin E affect the superovulation and DNMT1 expression in mouse oocytes

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Recent data show the influence of superovulation on the epigenetic reprogramming in oocytes (Market-Velker et al. 2010; MacDonald and Mann 2013). Due to complexity of the ovulation mechanism, questions related to the effect of bioactive supplements on the superovulation success rate and oocytes' quality is still debatable (Velazquez 2011; Evangelista et al. 2011). The present study aimed at analyzing the effect of the feed additive, including artichoke (*Cynara scolymus*) extract and vitamin E, on the success rate of superovulation and DNMT1 expression in ovulated mouse oocytes. The experiment was conducted with two groups of superovulated Swiss white mice: control- stimulated by standard protocol (initial FSH-6.00I followed by LH-6.00 IU) and experimental - superovulation was combined with individual intake

of 1.5 µg/g bioactive additive for 30 days prior to hormonal treatment. The superovulation rate was estimated by stereomicroscopic counting of the oocytes extracted from the oviductal ampulla of the sacrificed animals. The mRNA expression of DNMT1 in ovulated oocytes was analyzed by RT-PCR. A greater average number of oocytes per animal was recovered in the group supplemented with bioactives (34.3 ± 3.0 against 25.8 ± 1.4 in control). However, the level of DNMT1 mRNA was 3 times higher in the oocytes of the treated animals. The data allow to suppose that during the extraordinary ovulation not all oocytes finish epigenetic reprogramming. (Research was supported by grant DKOST01/10 NSF-MES Bulgaria and COST Action FA1403.)

P 108 | Studies on the etiology, therapy and prognosis of uterine torsion in cattle

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Uterine torsion is a common cause of bovine dystocia. The aim of the study was to investigate factors which are important to predict neonatal survival and subsequent performance of the cow. The study presents 114 cases of uterine torsion documented under field conditions. The cows were examined during obstetrics, and 2 h post partum (p.p.), 2, 12, 21 days p.p., 3 and 6 months p.p. Whether the dam was well-prepared for parturition had a significant influence on the survival rate of the calf ($p = 0.006$): In cases of good preparation neonatal mortality was 14.85%, in cases of insufficient preparation it rose to 58.33%. When uterine torsion lasted > 12 h only 34.78% of the calves survived, while in cases with duration < 6 h and 6–12 h 85.71% and 92.21% of the calves survived, respectively ($p < 0.001$). In 82.46% of the cases treatment was made by manual rotation of the fetus and uterus per vaginam, in 17.54% by rolling the animal with the application of a plank. There were no significant differences between the treatments in respect of neonatal mortality or lacerations of the dam. The mean initial milk yield was 8.9 ± 2.84 kg; it declined with an increasing degree of torsion ($p = 0.010$). Cows with uterine torsion showed a high incidence of metritis: 2 days p.p. 25.89% and 12 days p.p. 40.37% presented signs of genital catarrh. In 81.90% of the cases a re-insemination was tried; 81.40% of these cows became pregnant (insemination index = 2.76). In conclusion, good periparturient monitoring and early intervention are of crucial importance in cases of uterine torsion. Because of the high incidence of metritis, a good puerperal monitoring has to be emphasized after uterine torsion.

P 109 | Suitability of dialysis as the method to remove phospholipase from the ejaculate of the buck

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Phospholipase A2 is the enzyme produced and excreted by the buck's bulbourethral glands. In the presence of egg yolk, the products from the activity of this enzyme are harmful to the spermatozoa. Their effect can be reduced by different approaches, i.e. to reduce concentration of egg yolk in extender, to replace egg yolk by other additives, to remove seminal plasma by centrifugation, etc. The main purpose of this study was to analyse whether it is possible to reduce the activity of phospholipase in buck semen by dialysis. In the experiment, 15 ejaculates from three Saanen bucks were included. The ejaculates were divided into three experimental groups. Semen in group Ce (Centrifugation) was washed twice by centrifugation for 20 min at 1.085xg. During this time the diluted control semen (Co) was left at room temperature. Semen in group D (Dialysis) was dialyzed using 300 kDa cut-off semi-permeable cellulose tubing, against buffer in the ratio 1:300 for six hours. The activity of phospholipase was measured in native ejaculate, in samples after centrifugation and after dialysis with the sPLA2 activity kit (Assay Designs, USA). Protein concentration was determined by commercial test (Bio-Rad Protein Assay, BioRad; Germany). The differences between samples were compared by one-way Anova. The activity of phospholipase was reduced to 21.34% after centrifugation and to 72.00% after dialysis when compared to native ejaculate ($p < 0.05$). In a further study, we intend to analyse the effect of dialysis on the quality of buck semen after conservation in egg yolk containing extenders.

P 110 | Effect of endocrine-active chemicals on plasma steroids level in neonatal pig

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This study investigated the effects of endocrine-active chemicals (EACs) with androgenic/antiandrogenic and estrogenic/antiestrogenic activity on plasma steroids level in neonatal pigs. Animals were injected with testosterone propionate (TP, 20 mg/kg body weight [bw]), flutamide (FLU, 50 mg/kg bw), 4-tert-octylphenol (OP, 100 mg/kg bw), ICI 182,780 (ICI, 400 µg/kg bw), methoxychlor (MXC, 100 mg/kg bw) or corn oil with DMSO (control) between postnatal days 1 and 10 ($n = 4$). Blood samples were drawn from 11-day-old pigs and the concentration of progesterone (P4), androstenedione (A4), testosterone (T), 17β-estradiol (E2) and estrone (E1) were measured with 3H-radioimmunoassay after extraction and analyzed using Mann-Whitney U-test. Higher levels ($p < 0.05$) of P4 and A4 was revealed in TP and FLU groups as compared with control. Additionally, plasma T concentration was elevated ($p < 0.05$) in TP group, while E2 level was diminished in FLU group. Both ICI and MXC caused a decrease ($p < 0.05$) in plasma A4, T and E1 concentrations. In OP group, plasma A4 and E1 levels were higher, but E2 was lower as compared with control ($p < 0.05$). Concluding, exposure to EACs during neonatal period led

to imbalance in plasma steroids level, which is mainly caused by EACs with estrogenic/antiestrogenic activities, and may further affect ovarian function. (Support: National Science Center, Poland (Grant No. 2015/17/B/NZ9/01457)).

P 111 | Modified bisection of zona-punctured blastocysts is a powerful tool used for experimental embryo duplication and subsequent production of genetically identical twin offspring in pigs

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In the modified approach to bisection of bovine and rabbit blastocysts, a one-point drilling in their zonae pellucidae is accomplished, which leads to assisted induction of their specific hatching process with a figure-of-eight pattern through zona perforation (Skrzyszowska et al. 1997). At last, two parts of the hatching blastocyst (the first inside and the second one outside the zona pellucida) are split into demi-embryos via performing the vertical midline incision downstream of the zona perforation and across a thin cellular cross-bridge connecting both embryonic compartments. The aim of the study was to use this modified method of embryo microdissection for generation of monozygotic twin piglets. A total of 434 embryos at the expanding or late blastocyst stages that had been recovered from uteri of 12 hormonally stimulated donor sows were subjected to the microsurgical puncturing of zonae pellucidae and selected to be extracorporeally incubated for 20 to 22 h. After the ex utero incubation had passed, 166 zona-punctured blastocysts that progressed to hatch according to a specific figure-8 pattern were manually bisected. As a result, 332 blastocyst halves were obtained. All the half-embryos were intended to be surgically transferred into the uteri of 30 recipient females. Pregnancies were detected in 5 recipients; 3 sows farrowed, delivering the litters that included an overall of 14 piglets. The genomic DNA isolated from blood samples that had been collected from all the piglets born was analysed to detect and profile the extent of consanguinity. In summary, the molecular genetic analysis has confirmed the generation of 1 pair of monogenetic twin piglets (sisters) that have been selected to undertake and apply preclinical biomedical research.

P 112 | Preliminary results: vaginal pH variations during oestrus cycle of the bitch

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An optimal uterine environment is important for a successful fertilization after an insemination. The spermatozoa mobility can be affected by the vaginal pH, which can compromise the fertilization and hence pregnancy. The objective of this study was to follow the vaginal pH of the bitch during the estrus cycle. In total, 11 estrus cycles of 7 Beagle bitches, aged between 3 and 7 years old, were studied. The estrus was triggered using a Deslorelin acetate implant (SUPRELORIN® 4.7 mg), implanted subcutaneously during anestrus – after termination of the metestrus using repeated subcutaneous injections (between 3 and 26) of a PGF_{2α} analog (Cloprostenol, ESTRUMATE®) at the dose of 2.5 µg/kg. The implant was removed 14 days after its implantation [1,2]. The estrus cycles were checked with vaginal smears and dosage of plasmatic progesterone with the Minividas® machine (Biomérieux). Vaginal pH was measured against the vaginal left wall, always at the same spot, using a surface pH electrode (SI analytics ScienceLine L39). The vaginal pH during anestrus is over 7 but drops sharply during proestrus, reaching pH values around 6. The vaginal pH remains low during ovulation and a few days (4 to 6) after (e.g: pH = 6.02 ± 0.16 when progesterone = 7.19 ± 1.31). Then it starts to increase progressively again, to reach similar pH values as those obtained during the anestrus (e.g: pH = 6.61 ± 0.22 when progesterone = 43.42 ± 5.68), highlighting the end of the fertile period. Conclusively, a sharp drop of the vaginal pH is observed during the proestrus and estrus of the bitch. Monitoring the vaginal pH could be a new method to follow the estrus cycle.

P 113 | Effect of low temperature on embryonic-larval period of development of zebrafish

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Temperature is a very important factor in controlling embryonic development. The purpose of this study was to investigate the effect of short-term exposure to low temperature (10°C) on further embryonic development and survival of the fish *Danio rerio*. Male and female fish (1: 2) were placed in the spawning tank at a temperature of 29°C for producing eggs. Embryos 1.5 h post fertilization were placed in cold water at a temperature of 10°C. Four experimental groups were identified: 30 min, 60 min, 90 min and 120 min of treatment. Embryos in 2–4, 24, 48 h post fertilization and larvae of 5, 6 and 7 days were studied. Analysis of these stages in both control and experimental groups showed normal and abnormal development of the forms and the availability of the main critical point of 24 h, when maximum embryonic mortality was recorded (the percentage of deaths in the experimental groups exceeded the control three times or more). It was shown that treatment of embryos at the blastula stage at 10°C resulted in a significantly ($p \geq 0.001$) lower number of embryos with normal morphology and an increase in the number of abnormal forms of development, which ultimately leads to death. However, 30-minute cooling embryos

to 10°C was less destructive when compared with longer (60, 90 and 120 min) cooling periods. This work can be applied in the study of stress in early embryos; in addition, it gives the possibility to extend the periods of the zygote stage and 2-blastomere stage to introduce genes and other agents in the earliest stages of development.

P 114 | Breed effects on cryotolerance of stallion semen

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Not all stallions provide acceptable quality of cryopreserved semen and pregnancy rates compared to fresh semen may be reduced. Cryotolerance of stallion semen has at least in part been suggested to be influenced by breed. In the present study, we investigated the quality of raw and frozen thawed semen in ejaculates collected from client stallions referred for production of cryopreserved semen. A total of 1250 ejaculates (n = 169 stallions) cryopreserved at Vetmeduni Vienna (Austria) were included into the study. Semen collection and freezing followed the same standardized protocol. Sperm total and progressive motility before freezing and after thawing as well as membrane integrity was assessed by computer assisted analysis (SpermVision; Minitube, Tiefenbach, Germany). Ejaculate characteristics were evaluated according to the stallion's breed (Warmblood n = 331; Quarter Horse n = 319; Arab n = 243; Standardbred n = 97; Icelandic Horse n = 75; Lipizzaner n = 46; Thoroughbred n = 43; other breeds n = 96). Statistical analysis was performed using analysis of variance (general linear model for repeated measures) with breed as between subject factor and age, month, year and individual stallion as covariates. Breed of the stallion influenced (p < 0.001) the difference in quality (total and progressive motility, membrane integrity) in frozen thawed in comparison to raw semen. This effect was influenced by year and month of semen collection. Age of the donor stallion had no effect on total motility and membrane integrity, but influenced progressive motility (p < 0.05). Sperm membrane integrity loss after freezing was most pronounced for Icelandic and Thoroughbred stallions compared to all other breeds (p < 0.05). In summary, the cryotolerance of stallion semen differs among breeds.

P 115 | Steroid levels in fluid of bovine follicles containing growing or fully grown oocytes

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Various components of follicular fluid (FF) including steroid hormones are suggested as biochemical predictors of oocyte quality (Carpintero et al. 2014, *J Hum Reprod Sci* 7:187–193). Brilliant cresyl blue (BCB) staining has been used for selection of the functional status of oocytes.

The aim of the present study was to determine the levels of estradiol (E) and testosterone (T) in the fluid of follicles (d = 6–8 mm) containing BCB+ or BCB- oocytes. Before culture oocytes were incubated in BCB solution (26 µM for 90 min) and were divided into BCB- (colorless cytoplasm, growing oocyte) and BCB+ (colored, fully grown oocyte). The level of T and E in FF was determined by enzyme immunoassay (photometer STAT Fax 2100 reagent kit firm "Hema" Ltd., Russia). Samples of FF from 267 follicles containing BCB+ oocytes and 108 follicles containing BCB-oocytes were centrifuged separately at 2,000 g for 10 min and the supernatants stored at -80°C. Five replicates were performed in experiments. The selected oocytes were matured in TCM 199 + 10% FCS and 10⁶/ml granulosa cells with 50 ng/ml prolactin. After IVM oocytes were fertilized in vitro and embryos were cultured by standard protocols (Alm et al. 2005, *Theriogenology* 63:2194–2205). Blastocyst development rate was significantly higher in BCB+ vs. BCB- oocytes [27% (72/267) vs. 9% (10/108), respectively, p < 0.01, χ^2 test]. We have not found significant differences in the level of (E) in FF from follicles with BCB+ or BCB- oocytes (7.43 ± 0.18 ng/ml and 6.56 ± 0.13 ng/ml). Level of T in fluid from the follicles containing BCB+ oocytes was higher than in fluid of follicles containing BCB-oocytes (12.43 ± 0.73 ng/ml vs. 8.01 ± 1.41 ng/ml, p < 0.01, Students t-test).

P 116 | Conditioned medium derived from amniotic progenitor cells and its therapeutic approach in bovine mastitis

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To date, the conditioned medium (CM) obtained from in vitro cultured mesenchymal stem cells (MSCs) has been proven to be sufficient to stimulate the structural and functional regeneration of cardiac, renal, spinal cord, and tendon tissues by paracrine action due to the action of bioactive factors. In this context, we examined whether administration of CM generated from amniotic mesenchymal cells (AMCs) was useful as mastitis therapy in cattle. The CM was prepared culturing bovine AMCs, derived from three different placentas, at passage three for four days in serum free culture medium. Twenty-four mastitic quarters were randomly divided in 2 groups: antibiotic group and CM group (treated with intramammary antibiotic or CM alone). The diagnosis of mastitis was based on somatic cell count and bacteriological evaluation of the milk from the affected quarter at day 0 of treatment. After milking, three ml of CM were used at each administration twice daily for two consecutive days. Treatment outcomes were assessed by clinical and laboratory evaluations. Somatic cell count was performed starting at day 0, and then at day 7, 14, and 30 of treatment. The treatment with only CM did not show statistically significant differences compared to antibiotic alone for acute mastitis neither for the recovery of the affected mammary quarters nor for the reduction of somatic cells (33% in both group with somatic cell counts less than 200,000; p > 0.05). However, CM alone was better than the use of antibiotics only for

the treatment of chronic mastitis (16.67% vs. 0.00%) that usually do not improve by antibiotic. This pilot study supports the hypothesis that CM, through release of yet unknown soluble factors, could be considered as a potential new tool for clinical applications in bovine mastitis.

P 117 | Canine semen refrigeration in a 2% liposome media: in-vitro and in-vivo fertility tests

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Previous experiments have revealed the liposome concentration of 2% enables the best conservation of dog sperm at 4°C for 96 h (Delay et al. 2015, *Reprod Dom Anim* 50(Suppl 3):27–32). The purpose of this experiment was test fertility of dog sperm in-vitro and in-vivo after refrigeration during 2 to 4 days at 4°C in a 2% liposome environment. First experiment: 10 sperm samples from dogs, aged between 4 and 7 years old, were used for in vitro-tests. HOS test, SYBR14-PI, FITC-PSA and Acridine-Orange (AO) tests were performed. After being preserved at 4°C for 4 days, in a 2% liposome environment or a 6% LDL environment, the results were 78.05 vs. 79.53% for HOS, 71.95 vs. 73.70% for SYBR14-PI test, 72.45 vs. 70.85% for FITC-PSA test and 99.1 vs. 98.4% for AO test. The results showed there is no difference between the 2% liposome environment and the 6% LDL environment when dog sperm is refrigerated for 4 days at 4°C. Second experiment: in-vivo fertility tests in a 2% liposome environment. Nine AI's have been performed on 5 beagle females. AI's have been performed when female dogs were in heat, naturally or induced. Three different procedures were done: 2 AI's in 2 days with sperm, cooled at 4°C for 2 days; 2 AI's in 2 days with sperm, cooled at 4°C for 4 days and 1 AI with sperm, cooled at 4°C for 4 days. The 2% liposome environment seems efficient: liposomes don't interfere with fertility: 3 out of 4 female dogs got pregnant after the first procedure. 1 out of 2 female dogs got pregnant after the second procedure. No bitch got pregnant after the third procedure. Conclusion: the 2% liposome medium seems to be a good alternative to the 6% LDL medium.

P 118 | The metabolic status of cows with different level of uterine contractility

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The aim of this work was to study the metabolism of dairy cows with a varying degree of uterine contractility on 30–40 days post partum. Metabolic status was evaluated twice: at the end of the first and second month of lactation. Total protein, glucose, triglycerides (TG), activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) were determined in blood serum. The

obtained data were analyzed using ANOVA (software SigmaPlot 12.5). The animals were divided into two groups. First group (GI): the uterus is in the pelvic cavity (n = 8). Second group (GII): the uterus is in abdominal cavity (n = 6). The uterus was measured by rectal examination. TG concentration was higher in GI than in GII (0.114 ± 0.015 mmol/l and 0.070 ± 0.011 mmol/l, $p < 0.05$, respectively) at the end of the first month of lactation. Total protein concentration was higher in GI (76.7 ± 1.1 g/l and 71.5 ± 2.0 g/l, $p < 0.05$) at the end of the second month of lactation. There were also different dynamic changes of biochemical parameters. ALT activity increased from 18.6 ± 2.1 μ /l to 26.4 ± 2.3 μ /l ($p < 0.05$), AST activity decreased from 96.6 ± 4.4 μ /l to 83.2 ± 1.7 μ /l ($p < 0.05$) in GI on the second month of lactation when compared to the first month. Only TG concentration changed in GII at the end of the second month of lactation (from 0.070 ± 0.011 mmol/l to 0.108 ± 0.007 mmol/l, $p < 0.05$). The 305 day milk yield of the prior lactation and the 100 day yield of the current lactation were higher in GI (10729 ± 390 kg vs. 9311 ± 534 and 4350 ± 151 kg vs. 3796 ± 116 kg, $p < 0.05$). The study shows that a good postpartum uterine contractility can be combined with a high milk yield and depends on the features of homeorhetic mechanisms of the animal in early lactation.

P 119 | Comparison of head morphometric measurements in semen of fresh and post-thawing in deers (*Odocoileus virginianus*)

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The deleterious effects of cryopreservation of deer semen on the morphometric measurements of the spermatic head have not been compared. This study aimed to compare the effect of two moments of fresh semen (F) vs. post thawed semen (PT). Semen samples (n = 30) were collected by electro ejaculation (Electroyac 5[®], Neogen Corporation, Lexington, KY, EUA) from five Colombia deer located in the North of Santander region, Colombia (L 7°54'N, L 72°30'W). Semen was centrifuged, diluted in commercial soy bean lecithin extender containing glycerol (AndroMed[®]). After that, semen was slowly cooled for 2 h at 5°C. Filled in 0.25 ml straws and frozen in nitrogen vapors. After thawing (37°C/30 s). One morphometric smear was made per sample, air-dried and stained with Diff-Qick. 100 sperm heads were analysed per software ISAS 1.2 (Proiser, Spain) and values in μ m for length (L), width (W), perimeter (P) and μ m² for area (A), were calculated. Morphometric parameters were compared between moments (F vs. PT) by ANOVA. The results are expressed as mean \pm SEM. Significant differences ($p < 0.001$) were found between F and PT for L (8.61 ± 0.78 vs. 8.43 ± 0.78), W (5.04 ± 0.41 vs. 4.99 ± 0.41), P (24.85 ± 2.35 vs. 24.25 ± 1.97), A (37.77 ± 4.64 vs. 36.60 ± 4.25). According to these results, the sperm head dimensions (SHD) in thawed semen samples

using AndroMed were smaller compared to fresh samples. In conclusion a reduction in SHD was shown due to cryopreservation procedures, a lower reduction of SHD after thawing could be related with better cryopreservation success. (Financial support: SENNOVA).

P 120 | Cryostorage time impairs motility parameters in frozen-thawed boar spermatozoa

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Freezing is the most efficient method for long-term preservation of spermatozoa for a future use in artificial reproduction technologies. However, there are controversies about the influence of cryostorage time (immersed in liquid nitrogen) on post-thaw sperm quality. This study aimed to reveal the effect of cryostorage time on motility parameters of frozen-thawed boar spermatozoa. Ejaculates ($n = 40$) from healthy and fertile boars were frozen using a standard 0.5 straws protocol, cryostored and thawed (37°C during 20 s) at two different times: two weeks after freezing (control) and 1–2 (short cryostorage time) or 3–4 years (large cryostorage time) later. Sperm motility (total and progressive and kinematics of motile spermatozoa) was evaluated using a CASA system at 30 and 150 min after thawing in samples stored at 37°C . The influence of cryostorage time on motility parameters was similar at 30 and 150 min post-thawing. Large cryostorage time decreased ($p < 0.01$) total and progressive sperm motility rates compared to the control (between 5 and 10% for total motility and 3 and 9% for progressive motility). Both cryostorage times slow down the sperm movement, making it more linear (low VCL and high LIN) and less vigorous (low WOB and BCF), which was more evident in samples cryostored during long than short periods of time. These results showed that the cryostorage time had a negative effect on motility parameters of frozen-thawed boar spermatozoa, which was more evident as cryostorage time increased. (Supported by Seneca Foundation, Murcia, Spain (19892/GERM/15) and CSC (China).)

P 121 | Morphometrical study of the lumen of the porcine cervix: nulliparous vs. multiparous

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Recently, post-cervical (PC) artificial insemination (AI) technique has overcome the traditional AI which deposited the semen directly in the cervix. However, the application of PCAI in gilts (nulliparous) is still limited due to the difficulty to traverse the cranial part of the cervix with the inner cannula. The aim of the present study was to compare the lumen of the cervix in multiparous vs. nulliparous sows. For

this purpose, silicone casts of the cervix were obtained from 19 nulliparous and 14 multiparous sows. The casts depicted a characteristic waved shape, which was morphometrically different in the two groups particularly in the cranial part of the cervix. The thickness of the casts (lumen), the average distance between consecutive ridges and the amplitude of the waves were higher in multiparous sows ($p < 0.05$), while the number of waves were higher in nulliparous ($p < 0.05$). No differences were found in the depth of the ridges and the perimeter of the waves. In conclusion, according to the casts' morphometry the lumen of the cervix is different in size and shape in nulliparous sows compared to multiparous sows, so the design of new strategies for AI and catheters should consider the particular morphology of the cranial cervix in gilts. (Supported by MINECO-FEDER AGL2015-66341-R.)

P 122 | Expression of PEBP4 in the oviduct during the estrous cycle of sows

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The oviduct undergoes changes under the influence of steroid hormones during the estrous cycle. PEBP4 is a protein secreted in the epididymis to the seminal plasma, and has been related with the promotion of sperm motility in pig (An et al. 2012). The aim of this study was to determine the expression of PEBP4 in the porcine oviduct during the different phases of the estrous cycle by RT-PCR and immunohistochemistry. Oviducts collected from a local abattoir were classified by follicular morphology: prepuber (follicles 1–2 mm in diameter), preovulatory (6–12 follicles 8–12 mm), postovulatory (6–12 hemorrhagic corpora) and luteal phase (6–12 corpora lutea). Total RNA of isthmus-ampullar was obtained and cDNA was synthesized with an oligo d(T) as primer. This cDNA was used as template in RT-qPCR amplifications using specific primers designed based on GenBank sequence for *Sus scrofa* (NM_001162888). Oviductal sections from the same samples were fixed in 4% paraformaldehyde, dehydrated, embedded in paraplast wax and histological sections were made. Sections were incubated with anti-PEBP4 antibody, goat anti-rabbit IgG-HRP antibody and visualized using DAB Kit. Analysis by qPCR revealed that the PEBP4 mRNA expression was more elevated in post-ovulatory compared to other phases suggesting its presence around fertilization time. Moreover, immunohistochemical assays detected PEBP4 protein at epithelium cell level in all phases (being superior in the prepubertal phase), suggesting its secretion to oviduct fluid. Is the first time that the presence of this protein has been described in the oviduct, where its function could be related with the sperm, as previously suggested. (This work was funded by MINECO-FEDER (AGL2015-70159-P and AGL2015-66341-R) and Fundación Séneca (19357/PI/14).)

P 123 | Polymyxin B but not flunixin inhibits the lipopolysaccharide-induced suppression of luteal function in isolated perfused bovine ovaries in vitro

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Escherichia coli lipopolysaccharide (LPS) suppresses bovine corpus luteum (CL) function in vivo. Two studies were conducted to investigate 1) the effects of LPS on luteal function in isolated perfused bovine ovaries in vitro, and 2) whether those effects are mediated directly by LPS or via LPS-induced release of prostaglandin (PG) F_{2α}. Therefore, impacts of PGF_{2α} and LPS were inhibited by a non-steroidal anti-inflammatory drug (flunixin) and an endotoxin-binding agent (polymyxin B), respectively. Bovine ovaries with a mid-cycle CL (n = 23) were collected after slaughter and perfused for 240 min. In study 1, LPS (0.5 µg/ml) was added to the medium of five ovaries after 60 min of equilibration, whereas six ovaries were not treated with LPS (control). In study 2, flunixin and polymyxin B (5 µg/ml of each) were added to the medium of six ovaries respectively after 50 min of equilibration, and all ovaries were treated with LPS (0.5 µg/ml) 10 min later. After 210 min of perfusion, ovaries in both studies were treated with 500 IU of human chorionic gonadotropin (hCG). In the effluent perfusate, concentrations of progesterone (P4) and PGF_{2α} were measured every 10 and 30 min, respectively. In study 1, treatment with LPS abolished the hCG-induced increase in P4 (p ≤ 0.05), and tended to increase (p = 0.10) the release of PGF_{2α}. In study 2, flunixin-treatment inhibited the increase of PGF_{2α} after LPS-challenge that was observed in polymyxin B-treated ovaries. After hCG-stimulation, P4 secretion increased (p < 0.05) in polymyxin B- but not in flunixin-treated ovaries. In conclusion, P4 responsiveness to hCG was reduced in LPS-treated ovaries in vitro. Although flunixin managed to inhibit PGF_{2α}, treatment with polymyxin B but not flunixin resulted in luteal responsiveness to hCG after LPS-challenge.

P 124 | Effect of calcium, bicarbonate, bovine serum albumin and medium pH on spontaneous acrosome reaction in equine sperm

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The acrosome reaction (AR) is an exocytotic event achieved after successful capacitation and which precedes fertilization. In the horse,

capacitation is still under study, as fertilization in vitro is not yet repeatable. We tested the effects of different components of a medium (Modified Whittens) on equine sperm spontaneous AR. Sperm were incubated in medium without bicarbonate, calcium or BSA (MW; pH 7.25) or in MW with 25 mM bicarbonate (MW+Bic), 2.4 mM CaCl₂ (MW+Ca) or 7 mg/ml BSA (MW+BSA) alone or in combination; complete medium (MW+++), was tested at pH 7.25, 7.9 and 8.5. Sperm (10 × 10⁶/ml) were incubated at 37°C for 4 h in 0.5 ml of medium in air (n = 12, 4 ejaculates/stallion); membrane integrity and AR were assessed by flow cytometry using 0.25 µg/ml FITC-Peanut agglutinin (PNA) and 1 µM of propidium iodide (PI). Only membrane-intact (PI-negative) sperm were considered. The percentage of membrane-intact sperm that underwent acrosome reaction in MW+Ca+Bic was significantly higher (26.33 ± 4.7%; mean ± SEM) than that for MW, MW+BSA, MW+Bic and MW+BSA+Ca (9.31 ± 1.16% to 10.71 ± 0.98%; p < 0.05), but was not different from any other treatment containing both calcium and bicarbonate. There was no effect of pH in MW+++ (14.7 ± 2.4, 17.5 ± 2.4 and 19.4 ± 2.1% for pH 7.25, 7.9 and 8.5, respectively). Our data indicate that bicarbonate and calcium, but not BSA are associated with AR induction in equine sperm; high pH does not further induce AR in MW+++.

P 125 | Percentage of apoptotic embryonic cells under exogenous porcine FSH dose effect in superovulation protocol of ewes

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The aim of this study was to quantify the percentage of apoptotic cells, using active caspase 3 technique, in evaluation of the quality of in vivo fertilised ovine embryos, under effect of different exogenous porcine FSH doses. Santa Inês ewes (n = 24) received an intra-vaginal progesterone device (CIDR[®]) on Day 0, which remained until Day 8. On Day 0 and 8, 0.125 mg of a synthetic analogue of PGF_{2α} (Sincrocio[®]) was also administered. Gonadotrophic treatment started on Day 6 when females were divided into three groups according to the total dose of exogenous pFSH (Folltropin[®]): G1 (n = 8) – 100 mg; G2 (n = 8) – 133 mg; G3 (n = 8) – 200 mg. FSH total doses were administered in eight injections given twice a day in descending order. On Day 6, all ewes also received 300 IU of eCG (Novormon[®]). Six days after natural mating following the superovulatory treatments, embryos were surgically collected and evaluated for morphology; grade I to III morulas and blastocysts were considered viable. Viable embryos were evaluated for percentage of apoptotic cells by active caspase 3 technique. Data were compared using Kruskal Wallis test and posttest Dunns (p < 0.05). Number of viable embryos was greater in G3 (3.88 ± 3.48) compared with G2 (1.5 ± 2.51) (p = 0.038) but

similar to G1 (2.63 ± 2.92). Percentage of apoptotic embryonic cells was greater in G1 ($5.00 \pm 1.96\%$) compared to G3 ($3.54 \pm 2.33\%$) and G2 ($3.24 \pm 2.29\%$) ($p = 0.012$). A pFSH dose of 200 mg was associated with the lowest percentage of apoptotic cells and best embryonic quality in Santa Inês ewes and is therefore recommended for hormonal ovarian superstimulation. (Financial support: Fapesp n°2014/04614-6, EMBRAPA n° 02.13.06.026.00.00, PROPE no TC1288/2015.)

P 126 | An attempt to produce lamb transplants via oocyte transfer

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An attempt on production of lamb-transplants via oocyte transfer Short generation interval increases genetic progress. In sheep it could be gained by oocyte transfer from immature ewe lambs. In farm animals, successful oocyte transfer was reported in swine and equine only. The purpose of the present study was to determine if transfer of oocytes to recipient-ewes followed by intra-uterine insemination would result in live offspring. The experiment was conducted on-farm in Southern Kazakhstan. During the breeding season 6-months old F1 crossbred White Suffolk (WS) x Kazakh semi-fine-wooled ewe lambs served as oocyte donors ($n = 7$). They were intramuscularly injected with 1500 IU of eCG at 8 am. At laparotomy performed 30–34 h post eCG injection, the donor's ovaries were exteriorised and follicles were aspirated with a 17G 350 mm Kitazato OPU needle. 2.5 years old Kazakh Fat Rumped (KFR) ewes in natural heat served as recipients ($n = 8$). They were drafted after the direct contact of the teaser rams (belly covered with sackcloth) in a fenced area daily in the morning for one hour. Only ewes showing behavioural symptoms of oestrus were separated. In total 20 good quality oocytes were transferred via laparotomy into the oviduct ipsilateral to the recipient's mature follicle. Recipient's own mature follicle was not aspirated. Just after oocyte transfer, 0.5 ml (approximately 100 million motile sperm cells) of fresh diluted WS semen was injected into the recipient's uterine horn ipsilateral to the oviduct in which the oocyte was transferred. Four recipient-ewes lambed and delivered 4 lambs with F1 WS x KFR phenotype, no lambs with 3/4 WS phenotype were born. It was concluded that production of lambs via oocyte transfer might be possible but needs further investigation.

P 127 | Thawing at 60°C is better than 37°C for dromedary camel spermatozoa

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Protocols for cryopreserving dromedary camel semen have not been optimized yet. Thawing temperature is known to affect post-thaw sperm quality in other species. Objective: to determine if thawing temperature

affects post-thaw dromedary sperm quality. Ejaculates were obtained from five dromedary males of known fertility (two per male), at the Camel Reproduction Center, Dubai. Semen was processed by repeated gentle aspiration to break down the viscous seminal plasma and was then centrifuged through a colloid to separate the spermatozoa [Malo et al. 2016, *Anim Reprod Sci* 169:123]. After resuspending the sperm pellet in Green Buffer (IMV Technologies, France) containing egg yolk and glycerol, the sperm samples were frozen and stored in liquid nitrogen. Straws were thawed at 37°C for 30 s or at 60°C for 10 s; sperm quality was evaluated by computer assisted sperm analysis at 0 h and 1 h, and for acrosome integrity at 0 h (eosin/nigrosine staining). Post-thaw motility was improved for the higher thawing temperature. At 0 h, total motility (TM; 34 ± 2 vs. $36 \pm 2\%$; $p < 0.05$), amplitude of lateral head deviation (ALH; 7 ± 0.08 vs. 7 ± 0.1 ; $p < 0.001$) and curvilinear velocity (VCL; 126 ± 2 vs. 131 ± 3 ; $p < 0.05$) were higher at 60°C than 37°C, and there was a trend for progressive motility (PM; 15 ± 0.8 vs. $16 \pm 1.0\%$, $p = 0.059$) to be higher. After 1 h PM ($8 \pm 0.8\%$ vs. $11 \pm 1\%$, $p < 0.018$) and straightness (STR; 66 ± 0.6 vs. 67 ± 0.8 ; $p < 0.02$) were higher for 60°C thawing temperature than 37°C. The proportion of spermatozoa with intact acrosomes was $22 \pm 1\%$ vs. $26 \pm 1\%$ for thawing at 37°C and 60°C, respectively ($p < 0.05$). In conclusion, a higher thawing temperature may be beneficial when thawing dromedary camel spermatozoa.

P 128 | Expression of nerve growth factor and its receptors in the pituitary and reproductive tract of rabbits (*Oryctolagus cuniculus*): possible NGF-mediated role to induce ovulation

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In the present study, we evaluated the expression of nerve growth factor (NGF) and cognate receptors, the neurotrophic tyrosine kinase receptor 1 (NTRK1) and nerve growth factor receptor (NGFR) in the anterior pituitary, ovary, and cervix of rabbits. Positive immuno-signals for NGF, NTRK1, and NGFR were detected in all tissues. RT-PCR confirmed the presence of mRNA for NGF, NTRK1, and NGFR in the same tissues, but not that of NTRK1 in the cervix. Present data confirm that seminal plasma NGF may induce ovulation in rabbits via direct endocrine mechanisms through binding to cognate receptors localized in the pituitary and/or ovary following adsorption from uterine/cervical mucosa. Blood samples were collected from 12 receptive rabbit does divided in two groups at the moment of artificial insemination (AI): control group ($n = 6$) were inseminated with saline, and raw semen group ($n = 6$) were inseminated with a pool of semen. In does inseminated with raw semen AI caused a higher ovulation rate. This was confirmed by an increase of serum progesterone. We infer existence of such a novel mechanism from the following results obtained in rabbits. Firstly, the

semen contains large amount of NGF, which is likely produced by the accessory glands. Next, the uterus and cervix express cognate receptors for NGF, which induces prostaglandin and nitric oxide (NO) release by uterine tissue *in vitro*. Finally, the presence of both NGF receptors in the cervix and of a higher ovulation rate in the raw semen group, provides the evidence for the existence of a possible NGF-driven mechanism that induces ovulation in artificial inseminated rabbits.

P 129 | Developmental capacity of bovine oocytes following JC-1 staining

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Mitochondrial functions are vital for oocyte and embryo development. JC-1 staining is commonly used to assess mitochondrial activity. However, to our knowledge, JC-1 has not been tested as a non-invasive marker for oocyte quality. We examined the effect of JC-1 when used at the end of *in vitro* oocytes maturation on subsequent embryo development. Bovine cumulus oocyte complexes (COCs, $n = 527$, 3 replicates) were matured for 22 h then randomly allocated to the following processing steps: Intact COCs (CUM), denuded oocytes (DEN), denuded and stained with JC-1 (5 $\mu\text{g}/\text{ml}$), with (CON) or without (JCE) confocal microscopy. All steps were done within 2 h in Hepes-TALP media at 37°C. At 24 h, oocytes from each processing step were fertilized (Day 0, Fert-TALP medium) and cultured (mSOF medium with 5% fetal bovine serum) in 384-well plates either individually ($n = 15$ –20/replicate, 30 $\mu\text{l}/\text{well}$) or in a group of 20–25 (75 $\mu\text{l}/\text{well}$). Embryo cleavage (Day 2) and blastocyst rates (D7.5) were recorded and analyzed by Wald-Chi square test and Bonferroni. In group fertilization and culture, cleavage rates in DEN, JCE, and CON were significantly ($p < 0.05$) lower than CUM (59.2, 41.3, and 38.2 vs. 85.7%, respectively). Blastocyst rate was not affected in DEN (18.4%) but significantly reduced ($p < 0.05$) in JCE (8.7%) and CON (8.8%) compared to CUM (31.0%). Similar effects were seen in individual culture; cleavage rates: 50.5*, 48.4*, and 40.5* vs. 84.1%, blastocyst rates: 15.2, 4.3*, 5.0* vs. 29.5%, respectively (* $p < 0.05$). Therefore, in addition to the reduction in fertilization rates due to removal of cumulus cells, mitochondrial staining with JC-1 significantly reduces the oocyte's developmental capacity. (W. Marei is supported by an FWO postdoctoral fellowship 1211417N.)

P 130 | The role of donkey seminal plasma (SP) proteins on the control of polymorphonuclear neutrophils (PMN)-spermatozoa (spz) ROS production

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The contact between spz and SP after semen collection is detrimental for spermatozoa survival. SP increases the low rate fertility

obtained by frozen/thawed semen in donkeys (Rota et al. 2008). A high endometrial inflammation was observed in several species (horses, donkeys, pigs). The role of SP seems to be very important in the modulation of this inflammation. The aim of this study was to separate donkey SP fractions based on the proteins molecular weight and analyze the effect on spz motility and ROS production when each fraction contacted with PMN-spz *in vitro*. PMN were isolated from jennies blood (Loftus et al. 2010) and concentration was adjusted to 100×10^6 PMN/ml. Simultaneously semen was collected by artificial vagina and diluted with Kenney extender. Sperm concentration was evaluated by Newbauer chamber. Semen samples were centrifuged to eliminate the SP and obtained pellet was resuspended in Kenney extender and adjusted to 500×10^6 spz/ml. A pool of SP obtained from 5 donkeys was fractionated by Amicon® filters as: <3 k/3–10/10–30/30–50/50–100/>10 kDa. Different treatments were prepared: PMN-500 $\times 10^6$ spz/ml and each SP fraction, PMN+500 $\times 10^6$ spz/ml, 500 $\times 10^6$ spz/ml+SP, 500 $\times 10^6$ spz/ml+Kenney and alone PMN. Then treatments were incubated at 37°C. Sperm motility was evaluated by CASA system (ISAS®) and H₂O₂ and peroxydes were measured by Amplex Red® Kit at 30', 1 h, 2 h, 3 h, 4 h. 30–50 and 50–100 kDa showed the best motility parameters along time and curiously showed low H₂O₂ and peroxydase levels. It seems that these fractions controlled the ROS production. It was observed too that PMN+500 $\times 10^6$ spz/ml (without SP) had an exacerbate H₂O₂ and peroxydase levels. In conclusion, SP controls ROS production and SP proteins between 30–100 KDa maintains the best spz motility.

P 131 | Preliminary results of the High-definition (HD) ultrasonography in evaluation of brachycephalic pregnant bitches

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The aim of the current study was to describe the preliminary results of the use of High-definition (HD) ultrasonography in evaluation of brachycephalic pregnant bitches. Ten pregnant bitches, brachycephalic, adults, multiparous and clinically healthy were selected for the study. Ultrasound examinations in HD were performed daily, from the 7th day after natural mating or artificial insemination until parturition, using the ACUSON S2000/SIEMENS with matrix and multifrequential transducer of 18.00 MHZ. The presence of gestational vesicles was verified at the 15th day of gestation using high-resolution ultrasound. In the embryonic phase we observed areas of placentation, large amount of fluid intra placental, umbilical cord, thoracic and abdominal structures formed, diaphragm, limbs buttons, skull structures, spine, spinal cord, ribs, heartbeat, and fetal movement, which was also observed to conventional examination. In the fetal phase the chambers and heart valves, aorta, kidneys, eyes, intestinal peristalsis were visible and detailed identification of bone structures of fetuses was possible. In addition, it was

possible to visualize organs not yet described as pancreas, adrenal, spleen, thyroid and ovary. The results obtained by of this preliminary study show that high-definition ultrasound presents had better image of quality and precocity for gestational diagnosis in the bitch when compared to conventional two-dimensional. These data seem promising for diagnosis of abnormalities and facts not yet elucidated in the area.

P 132 | Effect of melatonin on in vitro development of in vivo-derived 4-cell porcine embryos

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The addition of melatonin (MLT) to embryo culture medium supports embryo development and accelerates the timing of development of in vitro produced porcine embryos. Our goal was to evaluate the impact of MLT added to the embryo culture medium on developmental potential and kinetics of in vivo derived 4-cell stage porcine embryos and cryotolerance of the resulting blastocysts. Sows superovulated at post-weaning estrus were inseminated twice and subjected to laparotomy. An equal number of embryos from each donor was cultured in the presence (N = 70) or absence (N = 76; control) of 1 nM MLT in NCSU-23 medium for 5 days. Embryos were observed from day 3 to 5 of culture to assess blastocyst formation rates. Expanded blastocysts were vitrified using the Superfine Open Pulled Straw method and stored in liquid nitrogen for 6 months. Data were analyzed by Fisher's exact test. Blastocyst formation was similar between MLT-exposed and non-exposed embryos (97.1% and 100%, respectively). From the total number of blastocysts formed, 98.5% and 1.5% of embryos cultured in the presence of MLT achieved the expanded blastocyst stage or greater at days 3–4 and 5 of culture, respectively, and these values were different ($p < 0.004$) than values for group without MLT (85.5% and 14.5%, respectively). There were no differences in post-warming survival rates (78.4% and 86.0%, respectively) or total cell number in blastocyst (42.7 ± 3.7 and 39.4 ± 2.2 , respectively) between blastocysts produced in presence or absence of MLT. In conclusion, our results indicate that MLT accelerated the kinetics of embryo development, but did not affect the developmental potential of in vivo derived 4-cell embryos or the cryotolerance of the resulting blastocysts. (Supported by Seneca Foundation (19892/GERM/15).)

P 133 | Single Layer Centrifugation (SLC) as a predictive tool of boar fertility

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In artificial insemination (AI) programs, sub-fertile boars sometimes remain undetected using the traditional analytical techniques. When

used for breeding, they produce lower than average farrowing rates (FR) and litter sizes (LS). Therefore, it is of great interest to the industry to identify those boars at early stages, before they have fertility results on farms. Recently SLC has been shown to be a method for predicting the fertility potential in stallions (Morrell et al. 2014, *Theriogenology* 81:1005–11). As SLC may select the most robust sperm from boar ejaculates (Morrell et al. 2009, *J Reprod Dev* 55:547–52) it is possible that the recovery rates after SLC could be related to the fertility potential of the boars. The aim of this study was to evaluate the effectiveness of SLC using Porcicoll as a predictive tool for boar fertility. Aliquots (4.5 ml) of semen samples from 40 boars (one per boar) used in AI programs, were layered on top of 4 ml of Porcicoll and centrifuged at $300 \times g$ for 20 min. Sperm concentration, motility (M) and morphology (Mp) were evaluated before and after SLC using CASA. Data for FR and LS were collected from the commercial inseminations carried out with each boar. The relationship between M and FR and LS was analysed. M and Mp parameters improved after SLC ($p < 0.0342$). There was a positive correlation between M and LS (0.1375) and negative for FR (0.2254), which may enable sub-fertile boars to be identified. The current study does not give the answer for boar or ejaculate influence and more studies are needed before the implementation of this technique as a routine in boar semen quality control. However, these results are promising and indicate that SLC could be a choice for identification of sub-fertile boars.

P 134 | The inbred in management programs of Polish Warmblood mares participating in stationary performance tests

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Among the problems associated with Polish Warmblood mares management programs there is a lack of data from stationary performance tests. The relationship between foaling and conception rate and probability of offspring heterozygosity in horses, as well as the negative effect of inbred on the breeding indicators is known. Impaired fertility, reduced vitality, weaker growth and worse performance results has been shown. The aim of study was to estimate the relationship and inbreeding coefficient based on the pedigree analysis of the mares participating in performance tests. 1007 mares considered in stationary riding performance tests in years 2007–2012 included mares of Polish Warmblood (SP, n = 469), Malopolska breed (MLP, n = 224) and Wielkopolska breed (WLKP, n = 314). The Pedigree data were combined and analyzed using INBREED procedure of the SAS package and self-written software. The relationship coefficients (mean \pm SD, %) were: 0.72 ± 2.43 for SP; 2.64 ± 4.71 for MLP and 0.82 ± 3.01 for WLKP. No significant differences were found between breeds. The inbreeding coefficients were: 0.31 ± 0.99 (SP); 0.36 ± 1.43 (MLP) and

0.58 ± 1.47 (WLKP), with no differences between breeds. The 76% of mares were not inbred. The other indicated inbreeding coefficient at level: 1.41 ± 1.71 for 22% of SP; 1.68 ± 2.10 for 16% of MLP and 1.68 ± 2.10 for 34% of WLKP. In conclusion, the inbreeding coefficient in the population of Polish half-breed mares is maintained at a low level and including the relationship coefficients, it might be an indicator of breeding programs efficiency. We suggest a program of a low rate of inbreeding combined with selection for the results obtained in stationary performance as a management strategy for Polish Warmblood mares populations.

P 135 | Incubation of murine oocytes with N-acetylcysteine after thawing prevents mitochondrial depolarization

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Vitrification decreases the oocyte's mitochondrial membrane potential partly due to a Reactive Oxygen Species (ROS) imbalance. Our aim was to test the effect of N-acetylcysteine (NAC, a ROS scavenger) on the depolarization of the mitochondrial network of thawed oocytes. B6D2 female mice were stimulated to trigger ovulation and oocytes were denuded. Oocytes were equilibrated for 3 min in M2 medium added with 7.5% of Dimethylsulfoxide (DMSO) and 7.5% ethyleneglycol (EG; v/v) and vitrified in M2 medium with 15% of DMSO, 15% EG (v/v) and 0.5 M sucrose for 1 min; oocytes were warmed for 1 min at 37°C in M2 + 0.5 M sucrose and transferred to KSOM medium for 2 h in absence (G2; n = 11) or presence of 1 mM NAC (G3; n = 9). Also, fresh denuded oocytes were incubated in KSOM for 2 h (G1; n = 18). The degree of depolarization of the mitochondrial network was assessed using Rhodamine 123 and calculated by the spatial coefficient of variation (CV; standard deviation/average fluorescence). The CV values obtained were 37.70 ± 1.39 for G1, 27.29 ± 2.41 for G2 and 39.40 ± 1.56 for G3; CV ± SEM. CV decreased significantly after vitrification compared to fresh oocytes (G1 vs. G2; p < 0.05) and NAC prevented this decrease (G2 vs. G3; p < 0.05). Rhodamine 123 is a reliable indicator for the assessment of mitochondrial network depolarization and the addition of 1 mM NAC after vitrification prevents mitochondrial depolarization.

P 136 | The effect of susceptibility to mycotoxins in fodder on the breeding indicators in herd of sows

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In pigs fed with contaminated fodder a decline in breeding results is observed, increasing the number of abortions and stillbirths and obtaining lower litters. The aim of this study was to assess the effect of mycotoxins (MTx): zearalenon (ZEN) and ochratoxin A (OTA) both in fodder and blood serum on different breeding parameters. A total of 220 sows of Redon and Galia breeds were included in the study during the years 2013–2016. The following parameters were analysed: number of sows inseminated (NSi), confirmed pregnancies (NSp), farrowed (NSf), aborted (NSa); number of piglets: alive (NPa), dead (NPd), weaned (NPw); conception rate (CR), fertility of sows (FS), fertility of the herd (FH) percentage of: abortions (PA), stillbirths (PS), weaned (PW). MTx (mean±SEM) was analysed in fodder and in blood serum of the sows. Amounts of MTx obtained in autumn (October/November) were: fodder (7.21 ± 0.34 ppb ZEN; 5.97 ± 0.51 ppb OTA) and serum (2.70 ± 0.67 ppb ZEN; 2.02 ± 0.29 ppb OTA); and in spring (March/April): fodder (3.28 ± 0.87 ppb ZEN; 0.99 ± 0.26 ppb OTA) and serum (0.63 ± 0.12 ppb ZEN; 0.14 ± 0.08 ppb OTA). A significant increase (p < 0.05) in the concentration of MTx was found in autumn and spring in comparison to the rest of the year, where only trace amounts of MTx were found. NSa and PA (mean±SEM) were significantly higher (p < 0.05) in autumn, where the exposure to MTx was higher, in comparison to spring and the rest of the year (NSa: 4.89 ± 1.05 vs. 2.00 ± 0.89 vs. 2.21 ± 0.37; and PA: 18.11 ± 4.22 vs. 6.44 ± 2.74 vs. 8.73 ± 1.42, respectively). NSa in sows inseminated in the autumn was also significantly higher. In conclusion, the breeding factors analysed showed low reproductive efficiency in autumn, when the amounts of MTx were higher in both in blood serum and fodder.

P 137 | Comparison of four methods to assess plasma membrane integrity in fresh and frozen-thawed dog spermatozoa

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The objective was to compare four methods to assess plasma membrane integrity in fresh and frozen-thawed dog spermatozoa. Semen from 7 Belgian Shepherd dogs was resuspended in Tris-egg yolk (TEY) with 3% glycerol. Sperm were cooled from 22 to 5°C, and then TEY (this time 7% glycerol) was added to reach a final concentration of 5% glycerol and 200 × 10⁶ cells/ml. Sperm were packaged in 0.5 ml plastic straws; equilibration was done 16 h at 5°C before freezing. Straws were frozen over nitrogen vapours, stored in liquid nitrogen for one month, and thawed in water bath at 38°C for 30 s. Plasma membrane integrity (PMI) was assessed by (i) Eosin-Nigrosine – EN, (ii) Hypotonic Swelling Test – HOST, (iii) fluorescent stains – SYBR14/PI, and (iv) NucleoCounter[®]; EN was considered the reference method. Values of PMI from HOST, SYBR14/PI, and NucleoCounter were similar to that of EN (79.7%) in fresh sperm; however, values of HOST and SYBR14/PI were different from each other: 82.6 vs. 70% (p < 0.05), while there was no difference between NucleoCounter (74.1%) vs.

HOST and SYBR14/PI. In frozen-thawed sperm, values of PMI from SYBR14/PI (31%) and EN (31.9%) were similar, but values from HOST (38.8%) and NucleoCounter (25.2%) were different ($p < 0.05$); these two values were also different from each other. In conclusion, in fresh sperm HOST, SYBR14/PI, and NucleoCounter produced similar results than EN, but in frozen-thawed sperm only SYBR14/PI produced similar value than EN; HOST produced higher, and NucleoCounter smaller values than EN. (Supported by UNAM – PAPIIT (IN213815).)

P 138 | Anamniorrhexis (caul and en-caul birth) in stillborn dairy calves – the first case series

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This study focused on a unique un-researched cohort of calves – those born in the amnion. Normally the amniotic sac ruptures during stage two of labour (anamniorrhexis), releasing amniotic fluid and increasing contractions. However, in an undocumented proportion of calvings the amnion remains intact and the calf is born enclosed in part of (caul birth) or the complete amniotic sac (en-caul birth); anamniorrhexis ('born in the bag'; BIB). Such births have been associated with prematurity, primiparity, placentitis, bradytocia and malpresentation and as a cause of stillbirth. The objective of this study was to characterise the epidemiology and pathology associated with anamniorrhexis in calves. Sixty three cases (0.03%) of BIB were recorded in 2,232 perinatal mortalities (>260 days; 0–48 h) across 288 herd-years. The majority of all cases occurred in correctly presented (92%), singleton (81%), male calves (57%) in unobserved (52%) calvings of pluriparae (51%) close to term (278 days). In 92% of cases the farmer recorded the calf as dead at birth. Necropsy examination showed that 91% of calves had >50% pulmonary atelectasis and while 75% of calves died during (60.5%) or after (14.5%) calving, 25% were dead before calving. A minority of calves were congenitally deformed (lethal and non-lethal) (25%), infected (5%) or had goitre (3%). In conclusion, these findings suggest that contrary to current beliefs, this condition is not primarily associated with prematurity, bradytocia or malpresentation but primiparae (49%) are over-represented. In three-quarters of cases, anamniorrhexis preceded or was concurrent with calf death suggesting a possible causative role. This is the first descriptive study of anamniorrhexis in calves.

P 139 | Urine specific gravity serial analysis in Great Dane dogs from birth to 28 days of age

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Urinalyses, which is the most common biochemical test in human infants, could represent a suitable non-invasive clinical tool in puppies, too. Urine results in fact easier to be serially collected in newborn dogs than blood samples. Among urine parameters, urinary specific gravity

(USG), marker of hydration status, is lower in newborns than in adult and older puppies, due to renal immaturity, but no serial USG analysis up to 28 days of age in healthy dogs are available, to the authors' knowledge. Beside refractometer, the on-site dipstick was proven to be useful for urine rapid analysis both in humans and animals. The present study aimed to assess serial USG in 48 healthy Great Danes, up to 28-days-old. Urine samples ($n = 624$) were collected by manual stimulation daily from birth to 7 days, then twice a week up to 28 days, and directly evaluated by both refractometer and dipstick. No significant differences between refractometer and dipstick USG were found (T-test, $p > 0.05$); USG significantly increased with age ($p < 0.001$): lower at birth (mean \pm SD, 1014 ± 4.62 kg/l) until 10 days of age, when a significant increase was observed (1025 ± 8.09 kg/l); at 28 days, it (1027 ± 6.37 kg/l) was similar to adult one (2). The study confirmed the usefulness of dipstick for on-site USG assessment, and that newborn dogs kidneys are able to concentrate urine from the second week of age, with USG similar to adults at 28 days of life.

P 140 | Effect of birth weight, weaning weight and preweaning weight gain on fertility of Holstein heifers

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Holstein calves ($n = 767$) were used to determine the effect of birth weight (BW), weaning weight (WW) and preweaning daily weight gain (DWG) on reproductive performance of heifers inseminated with sex-sorted semen in a hot environment (25°N; mean annual temperature 23.7°C). Calves were raised under a conventional system that consisted of housing in separate calf open pens and manually feeding milk replacer twice daily until weaning at 8 week of age. DWG during rearing and fertility were monitored. BW was divided into three groups: <36, 36–39 and >39 kg. WW were classified as <66, 66–74 and >74 kg. DWG was categorized as <450, 450–520 and >520 g. Of the heifers initially bred, 7.8% failed to conceive with ≥ 5 services, with no differences among groups for BW, WW and DWG. Likewise, no growth traits did affect services/pregnancy (2.2 ± 1.3 for pregnant heifers only). Age at first estrus was shorter ($p < 0.01$) for the heaviest heifers at birth (390 ± 26 days) than for heifers whose birth weight was 36–39 or <36 kg (400 ± 41 and 400 ± 36 days, respectively). The proportion of heifers conceiving to first service was lowest (27.9%; $p < 0.01$) in heifers with the lowest birth weight compared to heifers weighing 36–39 kg (36.3%) and >39 kg at birth (40.3%). Neither BW, WW nor DWG significantly affected ($p > 0.10$) all-service conception rate. Categories for BW, WW and DWG did not influence abortion rate (3.5%). First-service conception rates decreased sharply ($p < 0.01$) when WW was <66 kg. Except for age at first estrus, DWG did not influence all other reproductive variables. It was concluded that the heavier the Holstein calves are at birth and weaning, the shorter the age at calving under the present conditions, but low birth and weaning weight did not hamper all-service conception rate.

P 141 | Isolation and cryopreservation of poultry primordial germ cells

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Primordial germ cells (PGCs) are precursors of all gametes and regarded as a valuable genetic material for preservation of the poultry gene pool. We have optimized methodical approaches for preparation and cryopreservation of avian PGCs in different poultry species. It was found that enzymatic treatment with 0.05% trypsin is an effective method for disaggregation of avian embryos in order to isolate foetal cells. The optimal age period for obtaining embryonic cells in culture with a maximum content of PGCs is from embryos of 5–6 days for chickens, up to 5 days for quail, from 6 to 7 days for geese, ducks and guinea fowl. Separation of different cell types based on their different ability to adhere resulted in PGCs culture maximally purified from other cell types. The maximum homogeneity of the cell population (78%) was observed at separation of cells through three-fold transfer of supernatant containing cells that did not adhere after one hour of culturing. Moreover single embryo fibroblasts remaining in a PGCs cell suspension after separation from other types of cells were an optimal feeder layer for culturing PGCs: formation of PGCs colonies was observed on day 3–4 of culturing. High viability of avian PGCs after cryopreservation was observed while using a medium comprising of 90% foetal bovine serum and 10% DMSO as cryoprotectant. (Supported by RSF (16-16-04104))

P 142 | Targeted supplementation of protein enriched *Opuntia cladodes* upon reproductive outcomes in anestrus goats exposed to the male effect: I. Estrus induction, estrus latency and ovulation rate

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The possible effect of protein enriched *Opuntia megacantha* Salm-Dyck cladodes supplementation on estrus induction (ESI, %), estrus latency (ESL, hours) and ovulation rate (OR, units) in goats during the anestrus season and exposed to the male effect, was evaluated. In early May, anestrus Alpine-Saanen-Nubian x Criollo adult goats (n = 38, 25.4° N) were randomly assigned to: 1). Protein-enriched *Opuntia* (PEO; n = 12; 44.5 ± 1.7 kg live weight (LW), 2.5 ± 0.14 units body condition score

(BC); 29.8% CP, 2.27 Mcal ME kg⁻¹), 2). Non-enriched *Opuntia* (NEO; n = 14; 41.9 ± 1.5 kg LW, 2.5 ± 0.1 units BC; 6.4% CP, 1.8 Mcal ME kg⁻¹), and 3). Control (CC; n = 12; 45.1 ± 1.5 kg LW, 2.5 ± 0.1 units BCS). NEO and PEO goats were individually supplemented with cladodes (160 g d⁻¹; 09:00–10:00 h), yet, PEO was enriched in a fermentation bioreactor (1% of *Scaromices cereveciae*, + 1% urea + 0.1% of ammonium sulphate). Transrectal ultrasonographic scannings (TRUS; n = 3) were performed in April to confirm goat's anestrus status. After an adjustment period of 10-days, the NEO and PEO continue the *Opuntia* supplementation another 20-days along exposure to testosterone-treated males of proven libido and fertility (Alpine-Saanen males, 2/group). Neither LW (p > 0.05) nor BCS (p > 0.05) differed among groups, yet, increased (p < 0.05) ESI % and ESL h (p < 0.05) occurred in PEO & NEO vs. CONT (100%, 57%, 42% and 62 ± 4.2, 60.0 ± 6.0, 32.0 ± 5.20 h, respectively). Besides, PEO had the greatest OR (p < 0.05) vs. NEO & CONT (1.3 ± 0.2, 0.7 ± 0.2 and 0.4 ± 0.2, respectively). Hence, peri-breeding targeted *Opuntia cladodes suppl.* in mix-breed dairy goats exposed to the male effect increased estrus induction, estrus latency and ovulation rate during the anestrus season. The last has the potential for application to marginal goat production systems.

P 143 | Influence of females body mass on selected features of the first and second egg in Wrocław Meat Pigeon hatch

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Wrocław Meat Pigeon is one of the Polish pigeon breeds. Females lay in average 10 pairs of eggs yearly. In each hatch the first egg is laid 7–10 days after copulation, the second 48 h later. Their incubation lasts for 16–19 days. The aim of this study was to assess the influence of female body mass (FBM) on selected features of eggs. The study was carried out on 9 females, each producing 2 eggs/hatch. Birds were kept indoors and caged in pairs, at 12 h/12 h light cycle and changing environmental conditions. Feeding and eggs collection took place twice a day, while water was constantly replenished. Eggs were weighted and measured. Additionally, the strength of shells was assessed. Spearman correlations between these features and FBM were estimated with IBM SPSS Statistics 23 PL and statistical significance of the obtained differences estimated with t Student Test. We found that average weight of second eggs was higher than of the first ones (23.52 g ± 1.61 g and 22.01 g ± 1.65 g, respectively). The second egg was in average 1.37 mm longer and 0.41 mm wider than the first one. It was found that all features, except strength, were higher for the second egg laid, yet in case of weight and length the difference was close to statistically significant. A highly significant, positive correlation was found between the weight and width (r = 0.867, p = 0.0025) of the first egg, as well as between the weight and length of the second (r = 0.833, p = 0.0053). There was no influence of FBM on egg weight.

P 144 | Extracorporeal developmental capacity of inter-species (caprine-bovine) nuclear-transferred (NT) embryos does not differ remarkably from developmental capacity of intra-species (caprine) NT embryos

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Our study was conducted to compare the rates of ex vivo development between inter-species (caprine-bovine; C/B) cloned embryos (Group I; GI) and intra-species (caprine; C) cloned embryos (Group II; GII). In GI, to generate inter-species clonal cytoplasmic hybrids (cybrids), enucleated in vitro-matured bovine oocytes (ooplasts) were reconstructed with the cell nuclei of adult goat peripheral blood-derived fibroblast-like cells (AGPB-FLCs) that had been subjected to contact inhibition. In GII, to create intra-species clonal cybrids (CL-CBDs), metaphase II-stage caprine ooplasts were reconstructed with the cell nuclei of contact-inhibited AGPB-FLCs. The inter- or intra-species CL-CBDs that had been efficiently electrofused and subsequently underwent delayed chemical activation were intended to be in vitro cultured. In GI, from among 137 cultured inter-species NT embryos, 104 (75.9%)^a were cleaved. The proportions of embryos that developed to morula (M) and blastocyst (B) stages were 36/137 (26.3%)^a and 22/137 (16.1%)^a, respectively. In GII, out of 128 cultured intra-species NT embryos, 103 (80.5%)^a were able to divide, but 38 (29.7%)^a and 23 (18.0%)^a reached the M and B stages, respectively (a,a: $p \geq 0.05$; Chi-square test). Summing up, the competences of inter-species (C/B) NT embryos to complete their development to the M/B stages did not vary significantly from those noticed for intra-species (C) NT embryos. This appears to be related to close taxonomic distance and phylogenetic consanguinity between donor specimens of somatic cells (*Capra aegagrus hircus*) and donor specimens of nuclear recipient oocytes (*Bos primigenius taurus*). Such characteristic features are observed in intra-family (Bovidae) and inter-genus (Capra-Bos) model of inter-species cloning of domestic goats.

P 145 | Computed tomography of the paranasal sinuses in 1-day-old puppies

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In dogs, the paired frontal sinus, the maxillary recess and the sphenoidal recess are found. Morphology of these structures undergoes significant changes, depending on the morphotype of the dog. This is important from a clinical point of view and during the interpretation of the computed tomographic image. The aim of the study was to determine the usefulness of computed tomography in the recognition and

assessment of the extent of the paranasal sinuses in newborn puppies. Also, it was an opportunity to evaluate the occurrence of any coexisting head disorders. The study involved 50 stillborn or euthanized 1-day-old puppies, from which 32 were dolichocephalic and 18 brachycephalic. CT was performed using a 16-slice helical scanner (Philips) with the following scan parameters: 120 kV, 200 mA, tube rotation time 0.5 s, slice thickness 0.5 mm, pitch 0.641, reconstruction interval 0.5 mm. The maxillary recess had mean 1.3 mm × 1.5 mm in height and width in the transverse plane. Air-filled frontal sinus was observed in 26 dolichocephalic and 8 brachycephalic dogs and its extent was markedly smaller in the brachycephalic morphotype. Four brachycephalic puppies, in which air-filled frontal sinus was not present, had coexisting cleft palate. The sphenoidal recess was not detected in any of the animals. Early diagnostic imaging, including computed tomography, gives the opportunity to detect skull abnormalities that are not recognized during clinical examination and additional tests used routinely in the veterinary medicine.

P 146 | *Chaetomium spp.*, a new agent identified in equine endometritis

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A 6 years old Andalusian mare was referred to our center in March 2004 because of infertility. The mare was born and bred in Spain, no previous foaling, but vagina and cervix were lacerated by natural mating. The mare was flushed, treated with antibiotics and mated repeatedly but with no pregnancy result. Ultrasound showed a hyperechogenic cervix (fibrotic) with 2 hypoechoic areas. Numerous PMN neutrophils and unidentified filamentous structures were observed by cytology. Endometrial swab culture evidenced the presence of *Streptococcus equi* Zooepidemicus and fungi *Chaetomium spp.* The case was considered a casual finding. But on May 2016, a Swedish Warmblood mare was referred due to infertility. The mare was born and bred in Sweden and had foaled 8 times. The last parturition, in 2013, was a complicated dystocia. Then mare was repeatedly flushed, treated with antibiotics, inseminated and mated without success. Ultrasound control showed intrauterine fluid during estrous and vaginoscopy evidenced an irregular and fibrotic cervix. Uterine cytology showed absence of PMN, and filamentous structures. By culture no bacterial growth but a lot of *Chaetomium globosum* fungi growth. Endometrial biopsy was classified as III stadium (Kenney and Doig, 1986). *Chaetomium* are fungi that grow in cellulose medium (perhaps stable floor), and this has never been described as endometritis agent. Obviously, *Chaetomium* was not the primary cause of endometritis, although the first mare had an acute endometritis, but with bacteria, the second one had an endometritis. Failure in uterine defense mechanisms may be a predisposing factor to colonize uterus as opportunist. But, can it survive in uterine medium?

P 147 | Combination of GnRH-prostaglandin and ram effect may enhance the reproductive performance of Karakul ewes during the non-breeding season

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The effect of a GnRH injection plus two injections of PGF_{2α} (10-days apart) combined with ram effect was studied in Karakul ewes during the non-breeding season. Progesterone (P4) concentrations, pregnancy rate, twin lambing and litter size were evaluated. Seventy ewes (2–4 years old) were divided into a treatment (n = 40) and a control (n = 30) group. Fifteen days before ram release (day -15), the treatment group was injected with GnRH (buserelin; 4.2 µg) intramuscularly followed by two injections of PGF_{2α} (D-Cloprostenol; 0.15 mg) on days -10 and 0. The rams were released into the ewe flock after the second prostaglandin injection (day 0). Blood samples of ewes were collected on days -15, -10 and 0 before ram release and days 20 and 70 after that. The treatment group had higher P4 concentrations compared to the control ewes on day -10 (3.50 ± 0.33 vs. 2.70 ± 0.35 ng/ml) though the difference was not significant (p > 0.05). Based on plasma P4 concentrations on day 70 (>2.5 ng/ml), a higher pregnancy rate was detected in the treatment group compared to the control ewes (92.5% vs. 73.3%; p = 0.04). Twin lambing rate was higher in the treated group compared to the control ewes (22.2% vs. 0.0%; p < 0.05). The litter size of the control and treated ewes was 1.0 ± 0.0 and 1.22 ± 0.10, respectively (p < 0.05). In the non-breeding season, the GnRH-PGF_{2α} treatment plus ram effect may enhance the pregnancy and lambing rates, twin pregnancies and litter size in Karakul ewes.

P 148 | Thyroid profiles during the postpartum period in primiparous dairy cows with different levels of the ovarian activity

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Hormones regulating metabolism are known to affect the female reproductive system. The aim of the present research was to study serum profiles of thyroid hormones during the postpartum period in primiparous dairy cows in relation to their ovarian activity. Blood samples from 47 Holstein cows were collected 2 weeks before calving and 1, 3, 5, 7, and 13 weeks after calving. After the 7th week postpartum, cows were divided into three groups according to their ovarian status: (1) normally cycling animals (CY, n = 26), (2) animals with a low ovarian activity (small ovaries having no corpus luteum nor large follicles; LA, n = 11), and (3) animals with inactive ovaries (small ovaries having no corpus luteum nor large/medium follicles; IO, n = 10). The diagnosis was confirmed by ultrasonography and progesterone analysis.

Hormonal levels in the serum were measured by ELISA. The serum content of thyroxine (T4) decreased after calving in all cows; however, this decrease was more profound in the IO group (1.7 times) than in CY or LA groups (1.4–1.5 times). In CY and IO cows, the level of triiodothyronine (T3) was maximal 1 week after calving and then declined 1.2 times (p < 0.05) and 1.6 times (p < 0.001), respectively, by the 5th week. Concurrently, the highest T3 level in LA cows was observed 3 weeks postpartum and its 1.7-fold reduction (p < 0.01) was found only at the 13th week. The T4/T3 ratio fell after calving and then rose in all groups. These changes occurred earlier in CY and IO cows than in LA ones and were more pronounced in IO animals. Thus, the level and duration of the shift in the postpartum balance between T4 and T3 may be related to suppression of the ovarian activity in primiparous dairy cows. (The study was supported by FASO Russia and RFBR (16-34-00875).)

P 149 | Molecular interactions between porcine CYP1A1 and selected dioxins may help to explain the high resistance of TCDD to biodegradation

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Polychlorinated dibenzo-p-dioxins are widespread by-products of human industrial activity that may adversely affect living organisms. Exposure to dioxins have toxicological effects on cells and tissues influencing, among others, reproductive system. It was found that TCDD affected progesterone (P4) and estradiol (E2) production by granulosa and theca interna cells in pigs, rats and humans. Dioxins are metabolized by cytochrome P450 enzymes e.g., CYP1A1. However, molecular mechanisms responsible for different resistance to biodegradation displayed by particular dioxins are unknown. In the present study, the molecular interactions between five selected dioxins and porcine CYP1A1 (pCYP1A1) protein were investigated by in silico approach. It was found that the ability of a dioxin to undergo pCYP1A1-mediated degradation is associated mainly with the number and position of chlorine atoms in the dioxin molecule. Among all examined congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) demonstrated the highest affinity (-33.32 ± 0.02 kcal/mol) to pCYP1A1 and the greatest distance (5.2 ± 0.3 Å) to the active site of the enzyme. In contrast to other dioxins, the binding of TCDD molecule to the pCYP1A1 active site caused a rapid and long-lasting closure of substrate channels resulting in the confinement of the molecule within the pCYP1A1 active site. Identification of specific aa residues involved in the TCDD-pCYP1A1 interactions and aa residues flanking the substrate channels may help to explain the extended half-life of TCDD molecule in living organisms. (This study was supported by grants No. 2012/05/B/NZ9/03333, UWM No. 528.0206.0806, and 2016/21/N/NZ9/02320. This research was supported in part by PL-Grid Infrastructure.)

P 150 | The potential role of Nodal in mare endometrosis

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Members of TGF β superfamily, as Nodal and TGF β 1, have an important role in mare's reproduction. Their dysfunction may contribute for uterine pathologies. Endometrosis is a degenerative process with a switch of normal endometrium to fibrotic tissue. The aim was to study: (i) how Nodal may influence the receptors of PGE2 (EP2; EP4), TGF β 1 (ALK5; TGFRII), and its own (ALK4; ALK7) mRNA level and prostaglandin (PG) secretion in equine endometrium; and (ii) estrous cycle and endometrosis influence on these vias. Endometria from follicular (FP; n = 6) and mid luteal phases (MLP; n = 6) were classified in Kenney's categories (cat) I and IIA (n = 7), or IIB and III (n = 5) and incubated (24 h; 37°C, 5% CO₂) with TNF, oxytocin or Nodal (0.1, 1; 10 ng/ml). The mRNA expression was assessed by qRT-PCR and ELISA was used for PG measurement. In cat I/IIA endometria, Nodal down-regulated EP2, EP4 and ALK4 mRNA expression and up-regulated TGFRII in both FP and MLP; and ALK5 and ALK7 only in MLP. In cat IIB/III, Nodal up-regulated mRNA levels of EP2, EP4 and ALK5 in FP, and ALK4 and ALK7 in MLP, whereas it inhibited TGFRII and ALK4 in FP, and EP2, EP4, ALK5 and TGFRII in MLP. Nodal (0.1 ng/ml) stimulated PG production, decreased PGE2 in FP (at 1 ng/ml) and increased PGF_{2 α} in MLP (at 10 ng/ml). In conclusion, Nodal may be involved in endometrosis in the mare, by impairment of anti-fibrotic PGE2 and pro-fibrotic TGF β 1 signaling pathways and increasing PGF_{2 α} production.

P 151 | Genes encoding mammalian oviductal proteins involved in fertilization are subjected to gene death and positive selection

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Oviductal proteins are known to play an important role in mammalian fertilization, as proteins are of seminal fluid. However, in contrast with the latter, the former's phylogenetic evolution has been poorly studied. The aim of the present work was to study in sixteen mammals the evolution of sixteen genes that encode oviductal proteins, which are known to be involved in at least one of the following steps: 1) sperm-oviduct interaction, 2) acrosome reaction, and/or 3) sperm-zona pellucida interaction. The analysis of the phylogenetic trees of these genes was made with Ensembl, the identification of pseudogenes was investigated with Genomicus, Mapviewer and tBLASTn analyses. The identification of positive selection was made using the PhyleasProg server. Most genes were present in all studied mammals, but some

genes were found as putative pseudogenes: ANXA5 and DMBT1 in tarsier, and probably PAEP in tarsier, mouse, rat, rabbit, dolphin and megabat, PTGDS in microbat and PLG in megabat. For 5 genes (DMBT1, HSPA8, NPPA, PAEP and PTGDS) positive selection was not found. For 4 genes (ANXA1, ANXA4, ANXA5 and HSPA5) branch site positive selection was found, whereas for 7 genes (ANXA2, LTF, OVGP1, PLG, S100A11, SPAM1 and SPP1) branch site model and model site positive selection were found. These results strongly suggest that genes encoding oviductal proteins that are important for gamete fertilization are subjected to positive selection during evolution, as numerous genes encoding proteins of mammalian seminal fluid. This indicates a potential selective pressure for processes such as speciation.

P 152 | Comparative effects of addition of superoxide dismutase and reduced glutathione on cryopreservation of Sahiwal bull semen

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The current study was planned to investigate the effects of antioxidants on quality of frozen-thawed Sahiwal bull semen. Semen was collected twice a week for 8 weeks by artificial vagina from six Sahiwal bulls kept at the Semen Production Unit Qadirabad, Sahiwal, Pakistan. The qualifying semen ejaculates were pooled and divided into 10 equal aliquots. Semen dilution was done in Tris citric acid extender (TCA), without antioxidants (control), or supplemented with either superoxide dismutase (SOD) (50, 100 and 200 IU/ml), or reduced glutathione (0.5, 1.0 and 2.0 mM) or combinations of SOD and reduced glutathione (50 IU/ml SOD and 0.5 mM reduced glutathione, 100 IU/ml SOD and 1.0 mM reduced glutathione and 200 IU/ml SOD and 2.0 mM reduced glutathione). Extended semen was loaded into 0.5 ml French straws, cooled from 37°C to 4°C, equilibrated for 4 h, frozen and stored in liquid nitrogen at -196°C. The post-thaw sperm motility, viability, acrosome and membrane integrity were significantly (p < 0.01) higher in samples treated with 100 IU/ml of SOD, 1.0 mM and 2.0 mM of reduced glutathione, or 50 IU/ml of SOD plus 0.5 mM of reduced glutathione. Thus, it was concluded that post-thaw semen quality of Sahiwal bulls may be improved by inclusion of SOD @ 100 IU/ml, reduced glutathione @ 0.5, 1.0 mM and combination of SOD and reduced glutathione @ 50 IU/ml plus 0.5 mM.

P 153 | Prevalence of anoestrus in dairy cows

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The aim of the present study was to determine the prevalence of postpartum anoestrus among dairy cows reared in three cattle farms.

The course of the postpartum period was monitored in 503 Holstein-Friesian cows. Clinical signs of oestrus were observed in 52.7% of the cattle. Oestrus was registered by the Heat-Time system. Cows without clinical signs of oestrus were examined on the 45th and 60th day postpartum via transrectal ultrasonography to detect the presence or absence of ovarian structures. Oestrus was present in 15.7% of primiparous and 37.0% of multiparous cows ($p < 0.001$). The highest relative proportion of anoestrus was demonstrated in cows from the group with follicle size >10 mm without corpus luteum (CL) (27.4%), followed by the group with CL (10.1%), hypofunction (5.2%) and follicular cysts (4.6%). The occurrence of anoestrus was different between the spring-summer (29.4%) and the autumn-winter period (17.9%; $p < 0.01$). As parity and season of calving were concerned, the highest relative proportion of anoestrus was observed in multiparous cows that calved in the spring and summer (19.9%), as compared to primiparous cows (9.5% $p < 0.05$). The results were subjected to statistical treatment program StatSoft. In conclusion, anoestrus was prevalent among a significant part of dairy cows, especially during the spring and summer periods, which necessitates the performance of additional examinations between the 45th and the 60th postpartum day and respective treatment for induction of oestrus and timely conception.

P 154 | Echographic parameters of the corpus luteum and uterine horn in cows under intrauterine growth retardation and embryo death

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Functional insufficiency of the corpus luteum (CL) is one of the dominating factors of embryonic development disorders in animals. The aim of the research was to determine various metric and functional parameters of CL during physiological formation of the embryo, and its role in embryonic growth retardation and death. The trial included 57 cows. Ultrasonic scanning of the reproductive tract was realized, blood concentrations of progesterone was tested during several stages in pregnancy (19th–23rd, 28th–32nd, 38th–45th days). According to the results of the last ultrasonic scanning the animals were divided into three groups: physiological formation and growth of the embryo (group I, $n = 26$), growth retarded embryos (group II, $n = 20$) and death embryos (group III, $n = 11$). Animals of group II demonstrated a significantly smaller CL diameter than cows of group I by 23.9–27.3%, and group III by 28.3–35.6% during the same period, the level of progesterone blood concentration – by 7.9–17.0% and by 12.0–43.3%, respectively, uterine horn cavity – by 22.0–37.8% and 44.8–69.0%. The results of the research allow to conclude that ultrasonic monitoring

of the CL and horn (uterus) parameters at early stages of pregnancy may be used not only for its diagnosis but also for predicting potential disorders of embryonic development for eventual treatment and prevention measures.

P 155 | The efficiency of ovarian responses to the estrus synchronization and superovulation of sheep in Kuwait

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The main disadvantage of multiple ovulation and embryo transfer (MOET) programs in sheep is the high variability of responses to super ovulation protocols and insufficient estrus synchronization of donors and recipients. The aim of the study was to evaluate the effectiveness of estrus synchronization and superovulation protocols on ovarian responses in sheep of local breeds in Kuwait. Twenty-three sheep received an intravaginal progesterone-releasing device (CIDR) for 14 days (day 0 – day14). A single i.m. injection of 0.25 mg cloprostenol (PGF_{2α} analogue) was given on the day of CIDR removal. On Day 12, a superovulatory pFSH (Pluset[®]) treatment was given to 4 ewes. Total pFSH (200 mg) doses were administered in eight i.m. injections (40 mg, 40 mg, 30 mg, 30 mg, 20 mg, 20 mg, 10 mg, 10 mg) given twice a day. On day 14, the CIDR was removed, and 200 IU of eCG and 0.25 mg cloprostenol administered. Ovarian responses were assessed by laparoscopy on Day 21. The work was carried out in December, the temperature in Kuwait was 10–15°C. There were 60.9% sheep with normal corpora lutea (CL), 13.1% sheep had follicles and 26.9% had inadequate luteal structures or both structures (CL and follicles). Three (75%) superovulated ewes had from 8 to 16 CLs. Thus, the used synchronization and superovulation protocol in sheep of local breeds in Kuwait has been quite effective. However, more research is needed to find even more efficient estrus synchronization protocols for sheep in the climatic conditions of Kuwait.

P 156 | Storage of in vivo-derived porcine blastocysts for 48 h in two culture media at 25°C or 37°C without controlled gassing

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This study aimed to design a liquid storage system for porcine blastocysts. Early (E; $N = 142$), full (F; $N = 189$) and expanded (Ex; $N = 153$) blastocysts were stored for 48 h at 25°C or 37°C, in two culture media without controlled CO₂ gassing (The chemically defined TL-HEPES-PVA and the semi-defined NCSU23-HEPES-BSA). Embryo viability

and embryonic development were morphologically assessed at 24 h and 48 h of storage. Then, embryos were cultured under conventional conditions into an incubator (NCSU23-BSA-fetal calf serum, 38.5°C, 5% of CO₂ and 95% humidity) for additional 48 h to re-assess viability. Blastocysts (E, F and Ex) cultured under conventional conditions were used as controls. The viability at 48 h and after culture was negatively affected ($p < 0.05$) by the storage in TL-HEPES compared with the controls; while viability of blastocysts stored in NCSHU23-HEPES-BSA was not affected and was close to 100% for all blastocyst stages. Embryo development at 48 h of storage was delayed ($p < 0.05$) in all experimental groups compared with controls. All blastocysts stored at 25°C for 48 h (E and F) or 24 h (Ex) maintained an intact zona pellucida. However, around 30% of Ex blastocysts were hatched at 48 h of storage in both temperature groups. This study demonstrated that liquid storage of blastocysts for 48 h (E and F) or 24 h (Ex) in NCSU23-HEPES-BSA at 25°C preserves embryo viability and maintains an intact zona pellucida, which makes this method useful for the transport of porcine blastocysts for ET. (Supported by MINECO (RTC-2016-5448-2 and AGL2015-69735-R) and Seneca Foundation (19892/GERM/15)).

P 157 | The histological analysis of spermatogenic epithelium twelve months after deslorelin implant administration in toms

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Suppression of the spermatogenic function was described as being very variable, even 7 months after the administration of the deslorelin implant (Novotny et al. 2015, *Theriogenology* 83:1188–93). The aim of this study was to evaluate the suppression of spermatogenesis in toms twelve months after GnRH agonist deslorelin implant insertion and to compare efficiency to that in dogs 5 months after azagly-nafarelin implant insertion (Goericke-Pesch et al. 2009, *Reprod Dom Anim* 44 (Suppl. 2):302–8). Five healthy, sexually mature toms were involved in the study. A deslorelin implant was administered subcutaneously. One year later semen was collected by electroejaculation in all toms and then all animals were castrated. Two hundred cross sections of seminiferous tubules were analysed by histological evaluation and the degree of suppression was assessed. The animals were assigned into groups according to the majority of tubules with the most developed germ cells observed: G0, spermatogonia; G1, spermatocytes; G2, round spermatids; G3, elongating spermatids and G4, elongated spermatids. The evaluation showed a high individual variation in the degree of suppression of spermatogenesis. Two toms were assigned to G0, one to G2 and two to G3. The collected semen contained no spermatozoa. According to the findings, the spermatogenic suppression in toms is unlikely to reach the degree that was described in dogs, i.e. to the level of spermatogonia/primary spermatocytes as the most developed germinal cells present. Nevertheless, due to the absence of spermatozoa in semen, all animals were considered as infertile.

P 158 | Luteal expression of the canine relaxin (RLN) system during pregnancy and at normal and antigestagen-induced parturition: hypophyseal localization and implications for luteotropic function

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RLN, acting through its receptors, RXFP1 and RXFP2, plays several roles during pregnancy in mammals, including indirect effects through stimulation of prolactin (PRL) release from hypophysis. In the domestic dog RLN originates from the placenta and is known as the only marker of gestation. However, the luteal expression of RLN-system has not been studied so far. Therefore, here the expression of RLN, RXFP1 and -2 was investigated in canine corpus luteum (CL) during pregnancy: pre-implantation (days (d.)8–12; $n = 5$), post-implantation (d.18–25; $n = 5$), mid-gestation (d. 35–40; $n = 5$) and at normal ($n = 3$) and antigestagen-induced luteolysis (Aglepristone, applied at mid-gestation; 10 mg/kg bw; 2×/24 h apart, $n = 10$). Immunohistochemistry (IHC) with custom-made canine-specific antibodies and TaqMan qPCR were applied. Moreover, co-localization of RLN-system and prolactin PRL was assessed in canine pituitary gland by IHC. The expression of luteal RLN was time-dependent, increased following implantation towards mid-gestation and decreased significantly at parturition. Antigestagen-treatment resulted in suppression of luteal RLN and RXFP2, but not RXFP1. RLN was localized predominantly in luteal cells, whereas RXFP1 and -2 were additionally strongly represented in luteal macrophages. All RLN-system members were found in canine hypophysis, with the most extensive signals for RXFP1 and -2. They were co-localized in pituitary lactotroph cells expressing PRL. In conclusion, RLN seems to be involved in auto/paracrine regulation of CL function in dogs in a time-dependent manner. The circulating, but also locally produced RLN, appears to act in adeno-hypophyseal cells providing PRL. (Research supported by The SNSF research grant nr 31003A_160251.)

P 159 | Effect of energy balance on cyclicity in primiparous Holstein and SRB dairy cows

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The objective of this study was to investigate the effect of two feeding levels during the antepartum and postpartum period on progesterone profiles in primiparous Holstein ($n = 22$) and SRB ($n = 22$) dairy cows kept in a loose housing system, in order to identify potential differences in the way these breeds respond to different energy

balance profiles after calving. The control group (HE, $n = 23$) was fed a diet for high-producing cows (target 35 kg/days energy-corrected milk, ECM). A lower feeding intensity (LE, $n = 21$) was achieved by giving -50% concentrate to target 25 kg/days ECM. Diets were implemented 30 days before expected calving. The HE diet group had later commencement luteal activity (CLA) than the LE diet group (3.1 ± 0.12 vs. 2.8 ± 0.13 days; $p < 0.01$). Moreover, the days to CLA increased ($p < 0.05$) as the BCS within Days -14 and 30 related to calving increased. Overall, the Holstein cows in the HE diet group tended ($p = 0.06$) to have the lowest probability (0.25 ± 0.13) for a normal progesterone profile (NPP), compared with the other three groups (Holstein LE; 0.60 ± 0.15 , SRB HE; 0.54 ± 0.15 ; SRB LE; 0.54 ± 0.15). A NPP was defined as a first rise of milk progesterone above 3 ng/ml followed by regular cyclicity for 45 or more days after calving. The total number of cows with early CLA (before Day 30) increased ($p < 0.05$) as the energy deficit at nadir increased. Further research is in progress to identify mechanisms that may explain the obtained results. (Financed by EU grant number: FP7-KBBE-2012-6, PROLIFIC).

P 160 | Bacterial count and quality of liquid stored boar semen with and without addition of antibiotics

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In regard to reduce antibiotic use in pig production the aim of this study was to compare bacterial count and sperm quality of liquid boar semen processed with and without antibiotics (AB). A total of 103 ejaculates were collected from Duroc and Premo[®] boars of two AI-stations. Equal parts of semen were extended (Androstar[®] Plus, Minitube, Germany) with and without addition of AB (Eurococktail, Minitube, Germany). Sperm characteristics and bacterial content were determined daily using CASA and flow cytometry during 6 days of storage (18°C). There were no differences ($p \geq 0.05$) in sperm characteristics in the first 2 days after collection and dilution of sperm with and without AB. At 2 and 4 days of storage moderate negative correlations ($-0.25 < r < -0.50$, $p < 0.05$) between bacterial count and the percentage of plasma membrane- and acrosome-intact sperm could be found in the group without AB, while in the group with AB there were no relationships ($p \geq 0.05$) between these parameters. From day 4 on bacterial count was higher ($p < 0.05$) in the group without AB. The abstinence of AB had no negative effects ($p \geq 0.05$) on motility and mitochondrial membrane potential of sperm during the whole experiment. In summary the abstinence of AB did not affect bacterial count and sperm quality in the first 2 days after preservation of sperm. However, afterwards the bacterial count increases and the sperm quality declines in sperm preserved without AB compared to sperm diluted with extender containing AB.

P 161 | Mammary squamous – cell carcinoma in a mare: clinical, cytological and immunofluorescent findings

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Mammary tumors are very rare in livestock animals, especially in mares. Sporadic cases of mammary gland tumors in the horse include carcinomas with metastasis and adenomas, whereas no squamous-cell carcinomas (SSC) have been reported. We described the clinical and cytological findings in a mare with mammary gland SCC, discussing the results of immunofluorescent (IF) for determining the expression of PRL receptor, CD 10, VEGF, Ki67 and p53. An 18-year-old thoroughbred mare was referred 10 days after parturition, for investigation of right mammary gland enlargement together with purulent discharge from the teat. The metastasis to the regional lymph nodes, vulva and vestibule of vagina were observed and locally aggressive biological behavior of the tumor was confirmed. Cytological evaluation of the mammary secretion revealed the prevalence of degenerated neutrophils, macrophages, giant cells and no presence of bacteria. Part of the cells demonstrated pyknosis and karyolysis, the others indicated polymorphism, anisokariosis and atypia. After euthanasia, samples were collected from mammary tissue (right and left) and fixed for histology and IF analysis. When compared to unaffected mammary gland (UM) the expression (%) of PRL R was significantly highest in right teat (4.94 ± 0.98), lower in left (1.88 ± 0.24) and the lowest in UM (0.20 ± 0.04). Similar pattern demonstrated expression of VEGF, p53 and Ki67 but no CD 10 with no difference between right teat (0.74 ± 0.05) and UM (0.74 ± 0.16). Significantly higher expression of CD10 (4.64 ± 0.70) was found in left teat. Mammary squamous – cell carcinoma in the mare demonstrates features of malignancy (Ki67, p53) and is able to create its own microenvironment (CD 10 and PRL R) including vessels proliferation (VEGF).

P 162 | In vitro development of porcine cloned embryos is stimulated by genomic DNA of blood-derived fibroblast-like cells to a lesser extent than by genomic DNA of bone marrow-derived mesenchymal stem cells

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The purpose of this study was to examine the impact of not only adult peripheral blood-derived fibroblast-like cell (APB-FLC) nuclei, but also adult bone marrow-derived mesenchymal stem cell (ABM-MSc) nuclei on the ex vivo developmental capabilities of cloned pig embryos generated using either type of genomic DNA donor cell. Oocytes that

had attained the meiotic maturity status under extracorporeal conditions were utilised as a source of genomic DNA recipient cells for the purposes of somatic cell nuclear transfer (SCNT). To form the ooplast-nuclear donor cell (NDC) complexes, the previously enucleated oocytes were subjected to microinjection of contact-inhibited/trypsinised APB-FLCs (Group I) or ABM-MSCs (Group II) under their zona pellucida. The ooplasts then underwent simultaneous fusion with NDCs and electrical activation. In Groups I and II, 141/163 (86.5%)^a and 123/135 (91.1%)^a oocytes were efficiently electrofused/electroactivated and classified for in vitro culture, respectively (a,a: $p \geq 0.05$; Chi-square test). Out of 141 and 123 cultured SCNT embryos assigned into Groups I and II, 88 (62.4%)^A and 114 (92.7%)^B exhibited cleavage activities, respectively (A,B: $p < 0.001$). The rates of embryos that completed their development to morula and blastocyst stages were 71/141 (50.4%)^A and 35/141 (24.8%)^A or 95/123 (77.2%)^B and 51/123 (41.5%)^B in Groups I or II, respectively (A,B: $p < 0.001$). In conclusion, porcine SCNT embryos reconstructed with APB-FLCs were characterized by significantly lower competences to undergo the cleavage divisions and to reach the morula/blastocyst stages as compared to those reconstructed with ABM-MSCs. (Presented work was financially supported by the National Centre for Research and Development (grant number BIOSTRATEG2/297267/14/NCBR/2016).).

P 163 | Evaluation of two intravaginal implants for the synchronization of estrus in criollo cattle in Peru

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The objective of this study was to compare the effect of two vaginal implants protocols on the follicular characteristics, the vaginal electrical resistance and the pregnancy rate of criollo cattle reared in high altitude (3100 m). All the cows ($n = 53$) were selected after manual palpation and ultrasound examination of their genital tract. All the cows had a body condition score between 2.5 and 3.5 (range 1–5). On day 1 group of cows ($n = 23$) was treated with 1.38 g of progesterone (Easi-breedTM CIDR[®] Zoetis) and the other group ($n = 30$) with 1 g of progesterone (DIB system Agrihealth New Zealand) for 7 days. All the cows were treated at day 1 with 2 mg of estradiol benzoate (im), at day 9 with 2 ml of prostaglandin (im) and at day 10 with 1 mg of estradiol benzoate. A timed artificial insemination was realized 54 h after the CIDR[®] or DIB removal. Pregnancy diagnosis was done by ultrasound 45 days later. Vaginal conductivity and diameter and perimeter of the preovulatory follicle were measured at the time of insemination. There were no significant differences ($p > 0.05$) between both treatments in the electrical resistance of the vaginal mucus, diameter and perimeter of the preovulatory follicle. The averages of these variables were 233 ± 36 ohms, 9.1 ± 1.9 mm and 24.6 ± 4.7 mm, respectively.

Pregnancy rates of cows treated with DIB and CIDR were 63.3% and 69.6%, respectively. No significant differences ($p > 0.05$) have been found. In conclusion, both treatments showed similar ovarian response, electrical resistance and pregnancy rate in criollo cattle reared in altitude.

P 164 | Changes in the proteome of porcine granulosa cells induced by TCDD

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Polychlorinated dibenzo-p-dioxins (dioxins) are by-products of human industrial activity. Due to its adverse effects as well as lipophilic character and low susceptibility to biodegradation, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is considered to be the most toxic dioxin. An exposure of living organisms to TCDD may cause numerous pathologies including reproductive disorders. The effects of TCDD on the proteome of porcine granulosa cells (AVG-16 cell line) were examined in the current study. The AVG-16 cells were treated with TCDD (100 nM) for 12 h and total cellular proteins were isolated and purified. The proteome was examined by means of two-dimensional fluorescence difference gel electrophoresis (2-D DIGE), and the proteins of interest were identified by MALDI-TOF/TOF tandem mass spectrometry. It was demonstrated that TCDD treatment affected the expression of multiple proteins. In silico analysis of data obtained from TCDD-treated cells revealed 87 differentially expressed proteins ($p < 0.01$, fold change ≥ 2.0 in comparison to control). Sixty one proteins were found to be up-regulated and 26 proteins were down-regulated by TCDD. Identification of proteins affected by TCDD will help to better understand molecular events involved in cell response to this highly toxic dioxin. (This study was supported by grants No. 2012/05/B/NZ9/03333 and UWM No. 528.0206.0806.)

P 165 | Elimination of bacteria from equine semen using colloid centrifugation before cool storage reduce H₂O₂ production and increase functional sperm

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Different procedures reduce microorganisms in semen during storage. The aims of this study were to: 1) evaluate techniques to eliminate total microflora load (TML) in cooled stored semen, 2) study

the relationship between microbial load and ROS production; 3) link microbial flora and functionality of cooled sperm. Ejaculates from 8 stallions were processed as follows: A. extended semen (ES); B. conventional centrifuged semen (CS) and C. Single layer centrifugation through Androcoll-E (SLC). All samples were preserved in INRA 96 at 5°C for 72 h. Aliquots of native semen and treatments were taken for bacteriological analysis at T0, T24, T48 and T72 h. The ROS production (dichlorodihydrofluorescein diacetate (H₂DCFDA) for H₂O₂ and Cell-ROX for total ROS), viability (YO-PRO-1-Ethidium) and lipid peroxidation (BODIPY) were assessed by flow cytometry. Bacteria isolated were *C. pseudodiphthericum*, *A. haemolyticum*, *Penicillium spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, and *Dermabacter hominis*. SLC removed significantly more bacteria ($p < 0.01$), 98.99% of microbial load (CFU/ml). TML correlated with dead sperm ($r: 0.60$, $p < 0.05$), live sperm ($r: -0.75$, $p > 0.01$), H₂O₂ production ($r: 0.74$, $p < 0.01$), and Cell-ROX production ($r: -0.47$, $p < 0.05$). H₂O₂ was reduced significantly in CS and SLC compared to ES ($p < 0.05$). Androcoll-E-processed samples showed higher ROS production (Cell-ROX+) at 48 and 72 h ($p < 0.05$), indicating superoxide production due to higher sperm metabolism rather than oxidative stress. To conclude, the bacterial load of semen increased H₂O₂ production and decreased the quality of cool-stored semen. SLC is the most efficient technique to remove bacteria from semen. (IJCI-2014-21671).

P 166 | Intra- and peri-testicular damage resulting in alterations in sperm morphology after testicular biopsy in a Holstein bull

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Testicular biopsy for evaluation of spermatogenesis can normally be performed in bulls with little adverse effects, but a 29 months old German Holstein bull was presented at the clinic with an increased percentage of morphologically abnormal sperms (>30%) as well as decreased sperm motility (55%). A testicular biopsy was collected to allow histological evaluation of spermatogenesis. During biopsy an increased bleeding was noted. Ultra-sonographic examination 6 days later revealed an intra-testicular hematoma along the needle tracts. Another hematoma was detected outside the testis between scrotal layers. Testicular changes and parameters were followed for 4.5 weeks by weekly ultrasonography and organization process of hematomas was documented. Furthermore, weekly semen collections revealed a doubling in morphologically abnormal sperms (mostly head and neck alterations) compared to initial values. Total motility did not exhibit considerable variations. Based on additional, to testicular biopsy unrelated issues, a decision was made for slaughtering the bull. Testes will be retrieved for histological analysis and confirmation of ultra-sonographic findings. We propose that testicular biopsy should

be performed after detailed imaging of greater testicular vessels in order to avoid hematoma induction to further increase the safety of the method. (Financial support was provided by the Association for Bioeconomy Research (FBF).)

P 167 | Preliminary findings on some reproductive indices following administration of Diclair® to rabbit-does

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Preliminary study of some reproductive indices on administration of daily double dose of 25 IU Dicclair® HP-FSH to 20 (16 Treated, 4 Control) crossbred adult rabbit-does (1.24 ± 0.26 kg), was conducted. Does housed 4/group in hutches were fed concentrates (20% CP) and water ad lib. Parameters were evaluated 12 h (h) post 2nd, 4th, 6th and 36 h post 6th injections (I). The differences (diff) between control (C) and treated (T) does in live-weight (1.19 ± 0.04 and 1.28 ± 0.48) kg as well as whole genitalia (3.52 ± 0.26 and 3.74 ± 4.21) g, respectively (resp.), were not significant ($p > 0.05$). The diff between C and T does in ovarian weight for the left (0.04 ± 0.01 and 0.12 ± 0.08) g and right (0.04 ± 0.01 and 0.14 ± 0.08) g ovaries, resp. were significant. The diff between C (7.26 ± 1.14) cm and T (8.26 ± 2.37) cm does in the lengths of the vagina was not significant. The diff between C and T does in the number of follicles present on the left (24.75 ± 6.02 and 44.88 ± 14.26) and right (23.88 ± 5.84 and 46.75 ± 14.67) ovaries, resp. were significant. The diff in the average number of follicles between 12 h post 2nd I (33.00 ± 6.93 and 35.50 ± 12.12) and 12 h post 6th I (62.50 ± 1.73 and 65.00 ± 3.46) as well as 36 h post 6th I (42.00 ± 6.93 and 47.00 ± 8.08) on the left and right ovaries, resp. were significant. Histological evidence of ovulation was seen at 12 h post 6th I (left side) and both sides at 36 h post 6th I. The mean areas for 2o, 3o and ovulated follicles were $1.41 \times 10^4 \mu\text{m}^2$, $2.37 \times 10^5 \mu\text{m}^2$ and $1.21 \times 10^5 \mu\text{m}^2$, resp. We conclude that Dicclair® HP-FSH induced follicle growth, with highest count and ovulation at 12 h post 6th I. These data will be useful in future superovulation studies.

P 168 | European beaver (*Castor fiber*) penile bone in computed tomographic and post-mortem examination

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In the European beaver the penile bone is used to determine age and sex. In this species, there is no obvious external sex characteristics, thus gender differentiation in live animals is difficult. Traditionally, it is made by palpation of the baculum or radiographic examination,

but both these methods can sometimes give equivocal results. 2 adult beaver cadavers were subjected to a radiographic and computed tomographic examination. CT scan was performed on a 16-slice helical scanner (Philips) with the following parameters: 120 kV, 235 mA, slice thickness 0.75 mm. In one of animals the penile bone was dissected for further evaluation. The topography and morphology of the bone was then described. In the first animal, the bone was clearly visible on radiographs, while in the second the visibility was significantly limited due to the mineralized content of the adjacent anal glands. CT scans clearly revealed the presence of the baculum, with a similar picture in both individuals. It was located caudoventrally to the ischiatic arch and had a macelike shape with a protuberance on the cranial end. Its overall length was 3.3 cm, 0.2 cm height and 0.6 cm width at the widest point. There was a groove for the urethra running in the median plane on the ventral surface. Computed tomography perfectly depicts the baculum and can be used to determine sex, especially when routine methods do not give a definite answer. It also allows detailed measurements that are used to assess the age of the animal.

P 169 | Histological findings from endometrium biopsies in jennies

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Nowadays, endometrial biopsy in mare is a key diagnostic examination to give a correct prediction for fertility. In jennies, there is scarcity of literature on endometrial histological changes. Therefore, the aim of the present work was to define the uterine histological pattern in sub/infertile jennies with references to the classification of Kenney and Doig, as well as correlating it to their ages. Fifty jennies were placed with jackasses of proven fertility. Thereafter, 14 jennies with fertility problems were examined by endometrial biopsies and obtained results were compared with biopsies from 12 healthy jennies in estrous and 6 h post IA. All biopsy samples were placed in 10% formalin and processed for histologic preparation. The number, types and location of the different immune cell type (PMN, eosinophils) were recorded, and an average of 5 fields was assessed. We observed that endometriosis grading is correlated with the age of jennies as reported in mares. (IIA 13 ± 3.9 years, IIB 14.6 ± 5.5 years, III 16.3 ± 1.5 years). Large numbers of PMN were observed in all biopsies with highly degenerative endometrium (19–26%) compared to healthy jennies in estrus (1.53%) but lower than in jennies with acute post insemination endometritis (87.74%). Moreover, there were significant differences between the values of eosinophils in jennies with endometriosis compared to jennies with healthy endometrium, demonstrating that these cells increased significantly in category IIA and IIB. Further studies are required to establish the role of these eosinophils in the inflammation and degenerative processes, and why PMN numbers are still elevated in most advanced endometriosis stages.

P 170 | A case of uterine hematoma in a mare after an endometrial biopsy

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Endometrial biopsy is an important, safe and painless procedure to assess equine uterine health. While minor bleeding at the site of sampling is a common condition, uterine hematoma is a rare complication. An endometrial biopsy was routinely performed on an unsedated 17 year-old welsh pony mare in estrus during a research protocol. From the next day on and during the following 7 days, a large amount of sanguineous fluid was observed in the uterine cavity during the daily ultrasound examination performed according with the protocol. No fever or any other systemic symptoms were noticed. Two weeks later, the ultrasound examination revealed an organised hyperechogenic mass compatible with a hematoma within the endometrial lumen. The mare was regularly controlled during the rest of the breeding season and no abnormalities in her cyclicity were observed. The size of the hematoma only began to decrease from the 3rd month after the biopsy onward and it disappeared completely 2 months later. This was the only complication following a biopsy in this study protocol that included a total of 70 biopsies on 49 mares and the very first case the authors got to see while biopsies have been common practice in their clinical and research activities for years. To our knowledge, this is the first time that the appearance of this condition is documented. The hematoma took 5 months to disappear at the ultrasound examination. During this time the mare could not be bred. Therefore, while endometrial biopsy should still be regarded as a safe procedure for the diagnosis of infertility in mares, the potential risk of a hematoma with its consequences for the breeding season has to be considered.

P 171 | Evaluation of selected gasometric parameters in piglets during weaning period

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The aim of the present study was to evaluate the selected gasometric and metabolic parameters during weaning period in piglets' blood. 120 healthy piglets at the age until 6 weeks, divided into 4 groups: I (n = 14) 7 days before weaning; II (n = 16) at the day of weaning; III (n = 46) one day after weaning; IV (n = 44) 7 days after weaning. Glucose, hematocrit (HCT) and oximetric parameters were assessed in whole blood with a critical points analyzer Siemens RAPIDPoint 500. At the day of weaning in group II HCT (median

(Mdn): HCT; II: 32.5% vs. I: 25.5%; $p = 0.004$), total hemoglobin concentration (Hb) (Mdn: Hb; II: 11.05 g/dL vs. I: 8.65 g/dL; $p = 0.003$) and deoxyhemoglobin concentration (HHb) (Mdn: HHb; II: 14.75% vs. I: 1.80%; $p = 0.034$) were higher than in group I. In group II methemoglobin concentration (MHb) (Mdn: MHb; II: 0.34% vs. III: 0.51%; $p = 0.036$) was lower than in group III. In group IV methemoglobin concentration (MHb) and carboxyhemoglobin (COHb) were lower than in group III (Mdn: MHb; IV: 0.37% vs. III: 0.51%; $p = 0.025$; Mdn: COHb; IV: 0.4% vs. III: 0.6%; $p = 0.032$). Glucose concentrations between groups I, II, III (Mdn: 165 mmol/l vs. 118.5 mmol/l vs. 95 mmol/l) were statistically different ($p \leq 0.001$). Data were analyzed with Mann-Whitney-Wilcoxon test. Weaning process in piglets causes oxidative stress, which was proven by obtained data including the increased of MHb concentration. This fraction is formed from the oxidation reaction of heme iron, which is accompanied by the creation of reactive oxygen species. Free radicals cause direct cell damage because they are able to omit most of the immunological reactions. The MHb drop in young piglets after weaning period (IV) is a proof for the ability to compensate that disorder.

P 172 | The use of strategic analysis as learning tool in the animal reproduction field

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Different learning strategies may be applied to stimulate the learning process and to engage students in deploying higher levels of knowledge. Such learning approaches aim to strengthen their critical thinking skills and the development of stronger clinical competences, if applied to medical education. The ability to present and discuss therapeutic options, to anticipate unexpected reactions, and to communicate are among the desired clinical competences in health professionals at day 1. In this work we discuss the usefulness of a common strategic analysis tool – the SWOT matrix – by describing and analysing the activities of students from the 6th and 8th semester of Veterinary Medicine Integrated Master at UTAD, in Animal Reproduction and Gynaecology and Obstetrics subjects. During a case-based activity, they were challenged to use the matrix to discuss available therapeutic options for cat contraception (6th sem.) or for a dog pyometra (8th sem.). After a short initial review (the students had worked with this matrix in the 5th sem.), students should fill out the matrix areas according to their understanding of the strengths, weaknesses, opportunities, and threats for the selected treatment, before exposing their arguments in face of the owner. Results show that although the strengths and weaknesses were easily identified by most students in both semesters, students consistently had problems in identifying opportunities and less so in the case of threats. The use of the SWOT matrix may be a useful tool to strengthen students' abilities to present and discuss a therapeutic procedure with the owner, as well as to anticipate any unexpected reactions of treatment.

P 173 | Assessment of plasma membrane integrity of donkey sperm using different staining protocols: preliminary results

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In a routine spermogram, plasma membrane integrity (PMI) should be assessed by a simple, quick and repeatable technique. There are different commercial kits, including combinations of propidium iodide (PI) and acridine orange (AO), but optimal concentrations of those fluorochromes have not been determined yet. The aim of this study was to compare the repeatability of different concentrations of AO combined with a standard solution of PI using a commercial kit as control. For that purpose, semen samples from three donkeys were frozen-thawed using a standard protocol. After that, samples were stained three times each with the following protocols: 1) Vital-Test® (Halotech DNA, Madrid, Spain) including unknown concentrations of AO and PI; 2) A1: 0.1 mg/ml AO; 3) A001: 0.001 mg/ml AO. Protocols 2 and 3 were combined with 0.1 mg/ml PI. At least 200 sperm were counted in each sample using an epifluorescence microscope. PMI (%) and coefficient of variation (CV, %) were compared between protocols by ANOVA. Results were expressed as mean \pm standard error of the mean. No significant differences ($p > 0.05$) were found between VT, A1 and A001 for PMI (49.28 \pm 1.76; 41.61 \pm 4.09; 32.83 \pm 5.26) and CV (14.74 \pm 6.35 vs. 7.24 \pm 1.08 vs. 17.45 \pm 4.92), respectively. In conclusion, all the staining protocols assessed were repeatable and so reliable for PMI evaluation in frozen-thawed donkey sperm. Further studies are needed including a larger sample size and comparisons with other techniques.

P 174 | MicroRNAs of equine amniotic mesenchymal cells-derived microvesicles and their involvement in anti-inflammatory process

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Cell-derived microvesicles (MVs) are a new mechanism of cell-to-cell communication. Our previous data showed that MVs are involved in downregulation of pro-inflammatory genes in LPS stressed equine tendon and endometrial cells. Aim of the present study was to evaluate whether MVs derived from equine amniotic cells (AMCs) contain selected micro-RNAs (miRNAs). Pool of AMCs derived from two amniotic membranes and their MVs were used. Following miRNA extraction and library preparation, deep sequencing was carried out on Illumina HiSeq 2000. Mirdeep2 on Illumina high quality trimmed sequences was used to detect known miRNAs from related species (sheep, cow and horse) and to support the individualization of novel miRNAs. Our

results showed that 1285 known and putative miRNA were identified and quantified both in AMCs and MVs. Between these miRNAs, 401 were classified as already known, 257 found for homology with other species and 627 were predicted candidates. Moreover, 146 differentially expressed (DE) miRNAs were identified, 36 of which were known and the remaining were novel. Among the known DE miRNAs, 17 miRNAs showed greater expression in MVs. Three of these DE genes were validated by RT-PCR: eca-miR-26, eca-miR-146a and eca-miR-223. Interestingly, specific DE-miRNAs found in MVs regulate different pathways related to inflammatory processes. These data stimulate further functional studies on the predicted target genes and pathways involved in the biological effect of equine AMCs.

P 175 | Bovine Pregnancy-associated Glycoproteins (PAG) in urine, milk, serum and 17 β -estradiol in cows with confirmed Late Embryonic Death (LED)

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In this work authors analysed PAG levels in blood, milk and urine as an indicator of trophoblast secretory function. Combined measurements of PAG concentrations in urine, milk and plasma, plasma 17 β -estradiol (E2) and transrectal ultrasonographic examination of the conceptus were performed. Data were derived from 30 multiparous dairy cows, which were divided into 2 groups: cows which demonstrated late embryonic death (LED; n = 14), and cows with undisturbed pregnancy (P; n = 16). Transrectal ultrasonographic examination, blood and urine samples were obtained on days 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105 of gestation. RIA was used to determine PAG concentration in blood, milk and urine. In group P a significantly positive correlation (Sp=0.73) between plasma E2 and plasma PAG concentration was confirmed. The derived data of E2 concentrations in the LED group were divided into two subgroups depending on the E2 concentration: the LE group (low levels of estradiol E2 < 2 pg/ml), and the HE group (high concentration of estradiol E2 > 2 pg/ml). Statistically significant differences were observed in the serum E2 concentration between LE, and HE groups, and between the LE and HE groups of the blood, milk and urine PAG concentration. It could be deduced that both indicators behave similarly in a situation of trophoblast secretory disorder. However a small negative correlation was observed (Sp = -0.35) between the blood PAG and E2 concentration in group LE and between urine PAG and E2 in LE (Sp = -0.41) and HE (Sp = -0.36). In the rest of the groups no correlation could be found. These results are consistent with the authors' previous reports and indicate the urine as an interesting indicator for undisturbed pregnancy.

P 176 | Influence of the insemination sheath on the semen retention rate in the AI gun

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The objective of this study was to evaluate the influence of the insemination sheath on the residual retention rate of semen in the AI gun. For this study, groups of 4 straws of cryopreserved bovine semen (n = 400 straws) were thawed in a water bath (37°C/30 s). Each group of 4 straws was used to re-pack 3 French 0.5 ml straws, composing samples with similar characteristics of weight and volume. Each dose of semen was individually weighed on a digital precision scale (A) and then mounted on one of three models of artificial insemination sheaths being tested (n = 100 semen doses/ tested sheath): leading brand in the global market (G1), leading brand in the Brazilian market (G2), and model identified as having a low residual retention rate of semen (G3; Evolution[®], Brazil). Each sheath was individually weighed before (B) and after placement of the straws containing the semen (C). The sheaths were mounted on universal applicators for AI in cattle and the content of each dose of semen was completely discarded through the pressure exerted by the plunger of the applicator. With the straws still coupled after the elimination of content, each sheath model was reweighed (D) and the seminal elimination percentage (SEP) was generated by the equation [(C-D/A) x100] and the retention rate (RR, %) was obtained by the equation [100-PES]. The data generated was analyzed by GLM model. The percentage of seminal elimination was 91.7^{ab} ± 3.01%; 90.6^a ± 12.54; and 96.5^b ± 2.42, respectively for groups G1, G2 and G3. The G1 and G3 sheaths presented lower RR than the G2 group (p < 0.05). In conclusion, there was a significant AI-sheath effect on the seminal retention and elimination rate in the AI gun, and this may compromise the conception rates of cows submitted to artificial insemination.

P 177 | Is Oviduct Specific Glycoprotein important in mare oviduct?

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Little knowledge exists on the role of equine Oviduct Specific Glycoprotein (eOSG) and on factors involved in its regulation. The goal was to study if (i) hormones, cytokines; or (ii) spermatozoa (Spz) affect eOSG production by mare oviduct. In Exp. 1, oviduct explants (infundibulum, ampulla, isthmus) from follicular, early and mid-luteal phases were stained for IHQ or incubated (24 h) in control medium or with E2 (10⁻⁹ M), P4 (10⁻⁷ M), OXT (10⁻⁷ M) or TNF α (10 ng/ml). In Exp. 2, follicular phase explants were cultured (24 h) in control medium (i), control + Spz, separated from explants by an insert (ii) and control + spz + explants in direct contact (iii). Even though eOSG was produced in

vitro by all oviduct parts, no treatment, oviduct portion or estrous cycle phase caused any difference. By IHC, eOSG mainly stained epithelial cells and scarcely connective tissue cells. In the isthmus, Spz either in direct or indirect contact with oviduct tissue, raised eOSG compared to Control and to the other portions of the oviduct ($p < 0.05$). In conclusion, this study shows that eOSG is mainly produced by epithelial cells in all portions of mare oviduct throughout the estrous cycle. Its synthesis and regulation might depend on other factors than ovarian steroids, OXT or TNF α . The stimulus of Spz in the isthmus to release eOSG might provide a unique microenvironment for fertilization and early embryo development in mares, as in other species.

P 178 | Semen collection and evaluation in reindeer (*Rangifer tarandus*)

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Semen of reindeer (*Rangifer tarandus*) is little studied due to the complexity of semen collection in taiga and tundra, and low-domestication of reindeer. The aim of the study was to collect and evaluate the quality of sperm in reindeer (*Rangifer tarandus*). Semen was collected from 10 males by electroejaculation in Taimyr, Evenkia and St. Petersburg. Males were fixed by rope on the legs and horns or sedatives – xylazine hydrochloride and analogs. Injection of drugs was made by darts of veterinary gun. Electrical impulses were at 2–3 s intervals and voltage increased from 0.5 to 15 volts by steps of 0.5. Ejaculation occurred after 6–10 electroimpulses at a voltage 4–8 volts, amperage 0.2–0.9 amps and frequency 40–60 Hz (depending on the individual features of males). Nineteen ejaculates were collected. The morphology of spermatozoa and acrosomes was assessed by phase contrast microscopy. The obtained data were analyzed using the software SigmaPlot 12.5. Volume varied from 0.28 to 0.90 ml, sperm concentration – from 0.28 to 1.1 billion/ml, motility from 40 to 85%. There were $11.5 \pm 1.24\%$ damaged acrosomes and $6.8 \pm 1.11\%$ injuries in the tail and neck of spermatozoa. It can be identify the following changes in acrosome: swelling (loosening), wrinkling and the absence of the acrosome. The improving of the semen collection method in reindeer and evaluation of sperm quality have to be done in accordance with the climatic, technological and traditional features of reindeer breeding, as well as low-domestication of reindeer (*Rangifer tarandus*). (Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01023.)

P 179 | The ovulation-inducing effect of chicken sperm

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The ovulation – inducing effect of seminal plasma was reported in camels, camelid species and rabbits. We aimed to find this effect in the hens of gene pool breeds. Brooding instinct, common among such breeds (particularly of Asian origin) affects egg performance. Part of hens stops laying, this results in narrowing of genetic variability in the next generation. The experiments were carried out on 88 laying hens of the Chinese Silkie breed aged 24 and 32 weeks of life (exp. I and II), kept in individual cages and artificially inseminated by native mixed sperm in dose 0.05 ml. One month prior to reproduction season there were inseminated 3 times at 4-day-intervals. The efficiency of the stimulation was evaluated by the restoration of egg laying in broody hens and by the level of egg performance (%). The brooding instinct missed in a part of hens (26% – exp. I, 28% – exp. II). Other hens (27% and 24%, respectively) did not react to the stimulation. A positive reaction occurred in 47% of hens in experiment I and 48% in experiment II. After the restoration of egg laying the number of eggs, laid in a cycle, increased from 4 to 16 eggs. Intervals between the cycles became shorter. This positively influenced the total egg performance. Before the stimulation it was 49% (exp. I), 38% (exp. II). A month after the stimulation, it increased to 58% and 45%, and later decreased to 42% and 40%. In floor group keeping this effect was not observed, because cocks and broody hens do not copulate. Egg performance level in the same months was 18%, 25%, 31%. So, we revealed an ovulation – inducing effect in hens. This effect can help to eliminate brooding instinct in layers. Injection of cocks sperm into the oviduct of hens enables ovulation process and increased hatching eggs output.

P 180 | Different methods for in situ collection of samples from the bovine oviduct

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It is well known that the oviduct exerts main impact on reproductive performance of cattle, but adequate clinical tools are missing for in vivo diagnosis. In total, thirteen none-bred and clinical healthy Simmental heifers aged between 14 and 16 months from a commercial dairy farm were assigned to one of the collection methods and used for 4 consecutive sampling sessions. Animals were synchronized (PGF_{2 α} and GnRH, 2x) and sampled at the day of heat (day 0) and at 10, 21, and 31. An endoscopic system (Karl Storz, Vienna, Austria) was inserted through the mid-dorsal area of the fornix vaginae providing access to the oviducts. Samples were collected from both oviducts using one of the following systems: i) a curved metal catheter connected to a perfusor tube with 1 ml PBS syringe for flushing (FLU-group), or ii) a curved glass capillary for desquamation of epithelial cells (CAP-group), or iii) a cytobrush (\varnothing 2 mm, Karl Storz, Vienna, Austria) (CB-group). Obtained material was applied onto slides, dried, fixed and stained (Hemacolor Merck, Darmstadt, Germany). Quality

and quantity (minimum of 300 cells) of endothelium cells and polymorphonuclear neutrophils (PMNs) were microscopically assessed. In total, 94 samples from 13 heifers revealed a mean proportion of PMNs in the FLU-group, CAP-group, and CB-group of 11 (17–61), 0 (0–3), and 4 (0–20), respectively. There was no effect of repeated sampling on the number of PMNs. In conclusion, each of the three techniques has been approved to be suitable for the cytological application and may be a good tool to serve for clinical diagnosis. Future studies aim at increasing the numbers of heifers, including dairy cows and performing microbial and molecular analyses.

P 181 | Follicular dynamics during the non-reproductive season in Miranda donkey

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More superficial reproductive seasonal effects in jennies and the need for better breeding results in breeds at risk lead to the study of ovarian activity during the non-breeding season. Using 15 jennies, the diameter in both ovaries was measured using transrectal ultrasound, every other day, from September to April, in two consecutive years. Only 5 jennies displayed seasonal anestrus, suggesting a less pronounced seasonal effect. Despite this, indications of reduced ovarian activity and smaller follicular diameters during the non-breeding season were corroborated by a considerably higher rate of follicles smaller than 18 mm in diameter in winter and detection of silent estrus and anovulatory dominant follicles, even in animals that kept cycling. During the winter season, in jennies of the anestrus group, the follicular pattern consisted in the emergence of consecutive minor waves while among the remaining jennies, the most common wave pattern was the emergence of only one major primary follicular wave, with either one or two ovulatory follicles. However, cycles with more than one follicular wave were also observed, both with two major waves or with a minor wave followed by a major wave, encompassing cycles during the non-reproductive season and the transition seasons. In the transition periods of autumn and spring, the jennies in the anestrus group showed ovulatory failure and variable estrus duration. The presence of both major and minor secondary waves was detected, both during non-reproductive season and transition seasons.

P 182 | Abortion in the mare

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The knowledge about the etiology of spontaneous abortion in Swedish mares is insufficient. Mare owners shall report to the breeding organizations the result of pregnancy, e.g. abortion but not any diagnosis.

Therefore, there is no available statistics about etiology of abortion among the breeding organizations. However, since abortions can be caused by infectious diseases, to find the etiology is important, especially if the abortion concerns contagious viral diseases. In Sweden, samples from fetal membranes and/or fetal organs from aborted mares can be sent in to the National Veterinary Institute (SVA). The laboratory then uses PCR to analyze samples for Equine herpesvirus, EHV1 and EHV4 and Equine Arteritis Virus, EAV. In this study, SVA data from the period 2007 – Sept 2016 about EHV-1, EHV-4 and EAV was extracted out from the data base in which analytical results are stored. Thereafter, only cases in which “abortion” and “mare” was mentioned were included in the data set. During this 10 year period, the total number of analyses of EHV-1, EHV-4 and EAV was 655, 647 and 643, respectively. The most common diagnosed cause for abortion was EHV1. The proportion varied significantly between years ($p < 0.02$), the highest (24.7%) was found in 2008 and the lowest (3.6%) in 2015, the mean being 12.2%. Also abortion caused by EHV4 was found, however with a much lower proportion. The mean was 2.0% for the whole period and the variation (NS) between years was 1.1% to 5.1% (2014). Only one case (weak newborn foal) was diagnosed being positive for EAV 2010. Since the number of abortion cases is much higher than the number of samples sent in to SVA, one conclusion is that mare owners should be informed and encouraged to send in abortion samples.

P 183 | Risk factors associated with insufficient colostrum quality and with failure of passive transfer in Swiss dairy calves

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Previous studies have revealed that failure of passive transfer of immunity (FPT) is common in Swiss dairy calves. The prevalence of poor quality colostrum and FPT as well as the risk factors associated with low colostrum gammaglobulin (Gg) concentrations and low Gg levels in the calves' serum were investigated in 141 dairy farms in the region of Berne, Switzerland. Colostrum and calf serum samples from 373 dam-calf pairs were analyzed for their Gg concentration via electrophoresis. Information about colostrum management was gained by means of a questionnaire. The results were statistically evaluated by use of univariable and multivariable models. Prevalence values of 15.5% for poor colostrum quality (<50 g Gg/l) and of 43.5% for FPT in calves (<10 g Gg/l serum) were determined. The main factors associated with poor colostrum quality included milk leakage prior to or during parturition and a time lag >6 h between parturition and first milking. The results confirmed that the occurrence of FPT in calves was mainly influenced by the quality of colostrum, the amount of ingested colostrum and the time between birth and first suckling. The assessment of practices used for colostrum management indicated a large potential

for improvement in colostrum harvesting as well as in colostrum feeding to the calves in the study herds. The risk factors for low colostrum quality and FPT identified in the study should be addressed in priority in recommendations provided to farmers to improve colostrum immunity in newborn dairy calves in Switzerland.

P 184 | True hermaphroditism in a Cocker Spaniel: a case report

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If pseudohermaphroditism is uncommon, true hermaphroditism, defined as presence of both male and female gonads either separately or within a single structure named ovotestis, is rare in domestic animals. A 10 month-old Cocker Spaniel is presented for delayed puberty and a mass protruding from the vagina. No heat has ever been observed by the owners and the "bitch" is as big as her brothers. External genitalia appears like that of a prepubertal female, with an enlarged clitoris protruding from the vulva. A baculum can be palpated. US examination shows tubular genital structures connecting to the vagina and leading to two gonads looking like normal testicles. The structures that are surgically removed grossly look like a normal uterus and two testicles. The uterus appears histopathologically normal. Both gonads contain atrophic testicular structures but a few ovarian follicles are observed in one and some rete ovarii in the other. Progesteronemia was basal. Testosterone levels were low compared to a normal intact male dog, while estrogens were high, like in a bitch with hyperestrogenism. Developmental disorders are mainly caused by genetic or chromosomal abnormalities or chemical exposure. Although no karyotype was performed on this case, the breed and a putative other case in the same family is suggestive of a hereditary condition. Sex determination is regulated by the foetal gonads' secretion of testosterone and its metabolites, and Anti-Müllerian Hormone (AMH) from Sertoli cells. The hormonal profile of this case is concordant with the observation that low testosterone and high production of estrogens or androgen resistance during pregnancy can be the origin of abnormal external sex phenotypes.

P 185 | Influence of the age of Nelore bulls on measures of accessory sex glands – preliminary data

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The aim was to define ultrasonographic measurements of the vesicular, bulbourethral, prostate and ampulla glands of the vas deferens in Nelore bulls of different ages. Forty-two bulls were used: G1 (n = 21) animals aged 14.90 ± 0.19 months, and G2 (n = 21) animals aged 33.93 ± 3.48 months. B-Mode ultrasound examinations were performed using Mindray Z5[®] equipment (Shenzhen, China), with a 7.5 MHz linear transrectal transducer. Dimensions of the accessory sex glands were determined by average of the cranio-caudal and dorsoventral dimensions for vesicular and bulbourethral glands and for body of the prostate; and average of the three dorsoventral dimensions for disseminated part of the prostate and ampulla of the vas deferens. For the paired organs the mean were calculated. The data were submitted to the least squares method through the GLM procedure of the statistical program SAS (mean \pm SEM, $p < 0.05$). There were difference ($p < 0.05$) between G1 and G2 in the size of the vesicular glands (2.19 ± 0.08 vs. 2.72 ± 0.08 cm), body of the prostate (0.76 ± 0.03 vs. 0.88 ± 0.03 cm) and ampulla glands of the vas deferens (0.41 ± 0.03 vs. 0.61 ± 0.03 cm), respectively. However, there were no significant difference ($p > 0.05$) between G1 and G2 in the size of the bulbourethral glands (1.22 ± 0.05 vs. 1.32 ± 0.05 cm) and disseminated part of prostate (1.02 ± 0.06 vs. 1.07 ± 0.06 cm), respectively. In conclusion, the vesicular glands, body of the prostate, and ampulla of the vas deferens of Nelore bulls increase in size with age.

P 186 | Quantitative elastography of the uteroplacental structure of pregnant bitches – preliminary results

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The aim of this study was to determine the shear wave velocity (SWV) values of the uteroplacental structure of pregnant bitches using ARFI (Acoustic Radiation Force Impulse). 3 brachycephalic bitches (6 placental units) were used with a weight of 10–15 kg. After conducting preliminary tests and checked the healthiness, animals were submitted to elastography ARFI, using the ultrasound equipment ACUSON S2000/SIEMENS and 9.0 MHz transducer. The measurements were performed daily from the 21st to the 55th gestational day and every 12 h from the 56th to the parturition. A minimum of 5 readings were obtained in each portion of the placental structure evaluated (proximal, lateral and distal) to obtain the mean of SWV. ANOVA test and Tukey's test were performed and the variable was correlated with the gestational days using the Pearson's test. The animals did not present clinical and obstetric abnormalities during gestation and delivery. The mean SWV was higher in the proximal region ($p < 0.01$). The mean SWV of the dorsal region (CI = 2.62 ± 0.23 m/s) and lateral (CI = 1.59 ± 0.14 m/s) was considered constant ($p > 0.05$) during weeks of gestation. For the ventral region of the uteroplacental structure (CI = 1.45 ± 0.14 m/s), values were significantly higher

($p < 0.001$) at the last gestational week compared to previous weeks; and correlates positively with gestational age ($r = 0.256$, $p = 0.010$). Elastographic analysis can be easily performed in uteroplacental tissue in bitches and it is expected that the evaluations proposed in this research may contribute to future studies in physiopathology processes and in other species.

P 187 | Exogenous FSH dose effect on progesterone concentrations profile in superovulated ewes

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The aim was to evaluate the profile of progesterone (P4) concentrations in early luteal phase under FSH doses effect in superovulatory protocol of ewes. Santa Ines ewes ($n = 29$) were used and received an intravaginal P4 device (CIDR[®]) on Day 0, remaining until Day 8. On Day 0 and 8 were administered 0.5 ml of PGF_{2α} analogue (Sincrocio[®]). On Day 6 began the FSH treatment, when the ewes were randomly divided into groups: G1 ($n = 9$), G2 ($n = 10$) and G3 ($n = 10$) with 200, 133 and 100 mg of FSH (Folltropin[®]), respectively. On Day 6, ewes also received 300 IU of eCG. Blood samples were collected daily from Day 11 to 15 (corresponding embryo recovery day), to analyze the profile of serum P4 concentrations measured by RIA technique. Statistical analysis was performed using R software, the serum P4 concentrations were compared between treatments and days by repeated measures ANOVA and Tukey post-hoc (mean±SD, $p < 0.05$). There was no interaction between both effects. The P4 concentration was similar between the FSH doses (4.2 ± 6.4 , 3.0 ± 4.1 , 3.3 ± 3.2 ng/ml to G1, G2 and G3, respectively, $p = 0.66$). However, this parameter increased gradually ($p < 0.001$) from Day 11 to 15 (0.6 ± 0.5^c , $2.1 \pm 2.5b^c$, 3.1 ± 2.3^{bc} , 4.5 ± 4.3^b , 7.7 ± 8.2^a ng/ml, respectively). In summary, the different exogenous FSH doses were able to promote similar effect in the uterine environment for the embryos. (Financial support: EMBRAPA n° 02.13.06.026.00.00, PROPE no TC1288/2015)

P 188 | Effect of the stage of seasonal anestrus upon estrus response and time to ovulation in goats treated with progesterone and human chorionic gonadotropin

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The aim of this study was to determine the effect of the stage of seasonal anestrus upon estrus response and time to ovulation in goats treated with progesterone (P4) plus human chorionic gonadotropin (hCG). The study was conducted at 26° N during April (early anestrus season; $n = 13$) and June (late anestrus season; $n = 12$). In both seasons, adult multiparous acyclic goats (40.3 ± 5.7 kg and 2.3 ± 0.3 body condition score) were used. Goats were treated with 20 mg of P4 by im route and 24 h later, goats received 100 UI of hCG im. At 12 h after the hCG administration, goats were checked for estrus behavior with an aproned male for 10 min during the next 120 h. At start of estrus, each goat was summited for transrectal ultrasonography every 6 h until all preovulatory follicles ovulated. The estrus response and ovulatory rate were analyzed with a Fisher exact test. The onset of estrus, time to ovulation after hCG administration and number of ovulations were tested with a T Student. The stage of anestrus did not affect the variables analyzed ($p > 0.05$). Certainly, the estrus response was 92.3% and 100% during April and June respectively ($p > 0.05$), and ovulation occurs in all goats showing estrus. The mean interval for onset to estrus and the time to ovulation was similar ($p > 0.05$) in April (59.5 ± 14 and 90 ± 13) and June (60 ± 8 h and 93 ± 11 h), respectively. While the number of ovulations per goat during April was 2.0 ± 0.0 for June was 1.5 ± 0.5 ($p > 0.05$). To conclude, the treatment of P4 plus hCG is able to induce and synchronize the estrous response and ovulation in goats during both the early and late stages of the anestrus season at this latitude.

P 189 | Cooled semen artificial insemination in donkeys: field results

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Semen cooling and artificial insemination (AI) in small populations and endangered breeds allow a better gene distribution reducing inbreeding. Data on field fertility after AIs with cooled donkey semen are scarce. The aim of this study was to evaluate retrospectively the results obtained in the field after AIs performed by several clinicians with cooled semen obtained from five Amiata donkeys and produced by the Semen Production Centre of Region Tuscany between 2004 and 2014. Overall, from 63 ejaculates, 68 semen doses were obtained to be used in 40 females (31 jennies and 9 mares). Semen doses contained between 1×10^9 and 3×10^9 spermatozoa with >80% total motility and >55% progressive motility (CEROS 12.1 Hamilton Thorne Research, Beverly, MA), diluted in either INRA82EY ($n = 5$) or INRA96[®]. Jennies were inseminated for a mean of 1.52 cycles/season and in 8 cycles two semen doses were requested. The following per-cycle and per-season pregnancy rates (39.6% and 61.3%) and per-cycle and per-season foaling rates (34.9% and 51.7%, respectively) were obtained in jennies. With a single AI/cycle, the number of spermatozoa per semen dose ($\leq 1500 \times 10^6$ or $\geq 2000 \times 10^6$) had not a statistically significant effect on per-cycle pregnancy rate (25.0% and 38.5%, respectively). Two AIs/cycle resulted in a 50% pregnancy rate.

Transport time (used on the same day or shipped overnight) had no effect on pregnancy rate (33.3% and 44.4%, respectively). In mares, per-cycle and per-season pregnancy rates (63.6% and 77.8%, respectively) were not statistically different from those observed in jennies ($p > 0.05$). In conclusion, cooled donkey semen can be used in the field for AIs of both jennies and mares with acceptable results (Funding: Ente Terre Reginali Toscane).

P 190 | Routine B-mode ultrasound evaluation of mammary tumors in dogs

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Ultrasound B-mode scanning is an important clinical tool for routine breast screening: it is a noninvasive exam that can describe the tumor growth, differentiating between masses with well-defined contours or invading the surrounding normal tissues. The diagnostic value in differentiating benign and malignant mammary tumors has not been demonstrated in dogs. Aim of this study was to retrospectively assess whether the routine ultrasound evaluation of mammary tumors in dogs has a diagnostic value. Forty-two mammary tumors from 31 bitches, aged 9.8 ± 2.4 years, were examined by conventional ultrasound before surgical excision. The following descriptive parameters were recorded: size (smaller or larger than 1 cm), shape (spherical or irregular), margins definition (circumscribed or indistinct), invasiveness in surrounding tissue, echotexture (homogeneous or heterogeneous), presence of cysts, hyperechoic foci, acoustic shadowing. Histologically, the tumors were classified as benign or malignant, with a 1–3 malignancy degree. The association of each nonparametric ultrasonographic parameter to each tumor type was analysed using Chi square test. None of the parameters was significantly associated with either tumor type, meaning that any descriptive criteria cannot be a reliable diagnostic indicator of malignancy. Benign and grade I tumors were significantly more frequent in intact bitches ($p = 0.002$). Although ultrasonography is routinely performed before mastectomy to check regional lymph nodes and the whole abdominal cavity in order to exclude the presence of metastasis, the additional scan of mammary tumors does not help diagnosis in dogs.

P 191 | Progesterone concentration in plasma does not change during the preimplantation period of pregnant roe deer (*Capreolus capreolus*)

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Diapause is the temporary suppression of the embryonic development. In roe deer, a species which presents embryo dormancy at the blastocyst stage for around 4–5 months, little is known about the mechanisms involved. After delayed development, the growth rate raises rapidly, the embryo elongates and finally implants. All does show at least one active corpus luteum independent of their pregnancy status. This is in contrast to other diapause species, in which the corpus luteum shows reduced activity resulting in basal peripheral progesterone concentration until the resumption of embryonic growth. The changes of progesterone concentration in the peripheral blood of roe deer during the different pregnancy stages are controversial. Therefore, we analyzed plasma progesterone concentration of 93 pregnant does shot during the hunting season 2016 in north-eastern Switzerland and southern Bavaria. Blood samples were taken from the thoracic cavity after shot, and experimental groups were assigned based on size and developmental stage of the embryo found after uterus flushing: ≤ 1 mm (A), 1–2 mm (B), 2–3 mm (C), elongated embryo 5–100 mm (D), preimplantation-existing placenta, but no placentomes formed (E), implanted-placenta with placentomes (F). The progesterone concentration in plasma (ng/ml) as determined by ELISA was not significantly different between the groups: 5.09 ± 0.6 ($n = 20$), 5.09 ± 0.2 ($n = 36$), 6.11 ± 0.5 ($n = 11$), 4.32 ± 0.4 ($n = 12$), 4.90 ± 0.31 ($n = 11$) and 3.92 ($n = 3$) for the A, B, C, D, E and F embryonic stages, respectively. These results demonstrate that progesterone does not change over the period of diapause in roe deer and remains stable after embryo reactivation. We conclude that progesterone in roe deer does not play a key role in the escape of the embryo from diapause.

P 192 | Effect of maternal protein intake in the development of the bovine epididymis

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Maturation of the bovine spermatozoa is achieved only after transit through the epididymis. This complex maturation process involves alteration of the membrane lipids, chromatin condensation, migration of the cytoplasmic droplet and changes in the acrosome. These modifications require the interaction of the spermatozoa with proteins synthesised and secreted by the epididymal epithelium. It is widely accepted that environmental perturbations during pregnancy may negatively affect the developing fetus. Numerous studies have focused on reproductive deficiencies produced in offspring following under- or over-nutrition in utero. Dietary protein is an important gestational dietary component in ruminants with its deficiency impacting the quality of the sperm later on in life. In this large scale, farm based experiment we studied the impact of either, low or high dietary protein in nulliparous yearling heifers ($n = 360$) during the peri conception (60 days before artificial insemination (AI) to 23 days post conception (dpc)) and/or first trimester of gestation (23dpc to 98dpc) upon the development

of the epididymis in their 20 month old male offspring (n = 40). Size, number and proportion of the epididymal tubules and blood vessels, as well as the amount of collagen surrounding the tubules were measured. In addition the proteomics of the epididymal fluid were analysed. This study provides an insight into the epididymal structural and proteomic alterations in male adult offspring consequent to differing in utero protein environments. The observed effects have the potential to reduce fertility via alterations to the sperm maturational process within the epididymis.

P 193 | Silencing of aryl hydrocarbon receptor gene in porcine granulosa cells by using short interfering RNA (siRNA)

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RNA interference is a biological process in which expression of target transcript may be inhibited by siRNAs. The RNA interference induced by synthetic siRNA has become a powerful tool to examine gene functions. In the current study, siRNAs were used to silence aryl hydrocarbon receptor (AhR) gene expression in porcine granulosa cells (AVG-16 cell line). AhR is known both to mediate the adverse effects of many chemicals present in the environment and to play a significant role in the regulation of physiological processes in animals. The current study was performed to develop a method of AhR gene silencing in AVG-16 cells by using siRNA. We tested various: 1/ transfection reagents, 2/ siRNA sequences and 3/ experimental conditions. DharmaFECT and TransIT-X2™ Dynamic Delivery System, but not Lipofectamine 2000 (a widely used transfection reagent), efficiently penetrated cell membranes and introduced siRNA into AVG-16 cells, but neither was successful in AhR silencing. The effective transfection of the cells was achieved only with the use of combination of three different siRNA sequences and Viromer Blue transfection reagent. This approach enabled us to effectively (55–80%) silence the AhR gene expression in AVG-16 cells (This study was supported by grants No.2012/05/B/NZ9/03333 and 2016/21/B/NZ4/00202.).

P 194 | Vasectomy of European beaver *Castor fiber L.1758* – selected morphological and clinical issues

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European beaver is the largest Eurasian rodent. The population of this species on the territory of Poland was recently threatened with extinction. As a result of species protection, its population was rebuilt and even in some areas of the country, there are too many specimens of this species. Beavers cause significant economic losses as a result of their normal activities. Surgical vasectomy of this species may be a solution to this problem. Post-mortem examination was conducted on two males of European beavers. Organs have been isolated and were measured: maximum width, length, thickness of testicles and diameter of the deferent ducts. Average dimensions were as follows: right testicle 30.7 × 18.7 × 8.53 mm, left testicle 27 × 17.5 × 7.3 mm and deferent duct diameter 2.9 mm. It was also suggested that the optimal surgical access to the deferent duct is on the linea alba 10 cm cranially from the edge of pseudo-cloaca. Access in this area enables severing and ligation of left and right deferent ducts. These data can be used to develop a scheme of beavers vasectomy. This treatment can be used to control the population of free-living animals as well as those in zoos.

P 195 | The estrous stimulation of based on melatonin and progesterone during the non-breeding season in sheep

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In this study, it was aimed to investigate the effect of melatonin and progestagen administration on reproductive performance based on estrous stimulation during the non-breeding season in Kivircik ewes. A total of 197 sheep and 10 rams were used (2–5 years old, at least one parturition). The animals were divided randomly into four groups (Melatonin [n = 48], Progestagen sponge [n = 49], Melatonin+Progestagen sponge [n = 50] and Control [n = 50]). Blood samples were collected before each treatment. Progesterone (P4) concentrations were measured in all sera samples via commercial ELISA kits. Estrous detection was observed clinically during ten days after sponge removal, and reproductive tracts were controlled ultrasonographically using a transrectal linear probe. Pregnancies were determined with transrectal or abdominal ultrasonography at the 30th and 90th day after breeding. Estrous expression rate was significantly ($p < 0.05$) lower in the control group (32%) compared to the Melatonin, Progestagen sponge and Melatonin+Progestagen sponge groups; 45, 51 and 67%, respectively. Similarly, pregnancy rate was significantly ($p < 0.05$) lower in control group (88%) compared to the other groups (100%) at the end of the breeding season. All of the animals were cyclic ($P4 > 1$ ng/ml) prior to breeding. It was concluded that melatonin and progestagen administration can have a positive effect on reproductive performance during the non-breeding season in Kivircik ewes (This study was supported by CEVA Animal Health, Turkey.).

P 196 | Expression pattern of TLR4 gene in ewe reproductive tract during oestrous cycle

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The uterus expresses most of TLR family genes of which some are involved in certain physiological and pathological processes. These genes affect steroidogenesis, apoptosis and cytokine production in granulosa cells, and stimulate the production of antimicrobial peptides. TLRs play a role in the capacitation of sperm in female reproductive tract, protection of the reproductive tract against diseases and mastitis. Twenty samples out of 107 ovine reproductive tracts collected from a local slaughterhouse were selected according to the phases of the estrous cycle. The relative expression of the TLR4 gene was evaluated by Real-time PCR after extracting RNA and cDNA synthesis. Statistical analyzes showed significant increase in TLR4 gene expression in the uterus and cervix during the luteal phase in comparison with the follicular phase. It seems that the uterus up regulates TLR4 gene in the luteal phase providing a sterile and suitable environment for sperm capacitation and newly fertilized zygotes implantation. As the opening of the cervix during the follicular phase increases the risk of uterine contamination, it is logical to see the increase in TLR4 expression during luteal phase. In addition, the increase in TLR4 expression in the uterus may help the sperm capacitation.

P 197 | 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) affects the transcriptome of porcine granulosa cells

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TCDD is a man-made chemical present in the environment. An exposure of living organisms to TCDD may result in reproductive pathologies. The aim of the current study was to examine the effects of TCDD on the transcriptome of porcine granulosa cells (AVG-16 cell line). To identify genes involved in the mechanism of TCDD action in AVG-16 cells we have used next generation sequencing (NGS). The cells were treated with TCDD (100 nM) for 3 h and total cellular RNA was isolated and sequenced. In TCDD-treated cells, we identified 87 (58 up- and 29 down-regulated) differentially expressed genes ($\text{padj} < 0.05$, \log_2 fold change ≥ 1.0). A functional classification of all genes which expression was significantly affected ($\text{padj} < 0.05$) by TCDD was performed using KEGG database. Since granulosa cells are important for follicular growth, development and functions, we focused on genes associated with the following metabolic pathways: the TGF- β signaling pathway, chemokine signaling pathway, MAPK signaling pathway and

cytokine-cytokine receptor signaling. The obtained results showed that TCDD may affect ovarian follicle fate by influencing granulosa cell cycle, proliferation and DNA repair. (This study was supported by grants No. 2012/05/B/NZ9/03333, 2015/19/N/NZ9/00680 and UWM No. 528.0206.0806.)

P 198 | Effect of three days progesterone injection treatment on follicular cyst in high yielding dairy cow

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The objective of this experiment was to evaluate the effect of 3 days administration of progesterone on the lifespan of ovarian follicular cysts as a part of treatment regime in high yielding dairy cows. Lactating dairy cows ($n = 113$), at least 45 days postpartum, with ovarian follicular cysts were identified by rectal palpation and the diagnosis was confirmed by transrectal ultrasonography. Large follicle (>20 mm) without any luteal tissue was the criteria for follicular cysts diagnosis. Blood sample also was obtained from coccygeal vein or artery for progesterone assay (<1 ng/ml) by ELISA method to confirm no luteal body at day of diagnosis and also at day of prostaglandin usage. Treatment group ($n = 67$) protocol consist of gonadotropin (day 0) followed by three consecutive days injection of 200 mg of progesterone and finally administration of prostaglandin $F_{2\alpha}$ (PG) on day 10. The control group ($n = 46$) receives no progesterone injection. The animals that come to heat following PG were artificially inseminated. Pregnancy diagnosis was performed 30 days post insemination via transrectal ultrasonography. Days from diagnosis of cyst to first service was shorter in treatment group (20.18 days) than control group (32.61 days) ($p < 0.001$). Days open were reduced by 22.59 days (92.47 days in progesterone treated cows and 115.06 days in control cows, respectively, $p < 0.01$). Number of insemination was not different between the two groups in relation to pregnancy. Parturition to first insemination was not different between tow groups. In conclusion, it seems that 3 days injection of 200 mg of progesterone extra to ordinary treatment of follicular cysts may improve fertility and reproduction performance in high producing dairy cows.

P 199 | Ex vivo development of porcine cloned embryos is promoted by scriptaid-mediated epigenomic transformation of adult dermal fibroblast cells

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The present study was carried out to ascertain the in vitro developmental competences of nuclear-transferred pig embryos derived from

adult cutaneous fibroblast cells (ACFCs) that had been epigenetically modified by treatment with non-specific inhibitor of histone deacetylases, known as scriptaid (6-(1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide). Before use for somatic cell cloning, the permanent ACFC lines (between passages 2 and 4) that had been established from the primary cultures derived from ear skin biopsies of a prepubertal gilt were exposed to 350 nM scriptaid during 24-h contact inhibition. Reconstruction of enucleated in vitro-matured oocytes (ooplasts) was accomplished by their electrofusion with epigenetically modulated ACFCs. Simultaneous fusion and electrical activation of reconstituted oocytes were triggered using two consecutive DC pulses of 1.2 kV/cm for 60 μ s. The percentages of dividing embryos (238/314; 75.8%^A), morulae (186/314; 59.2%^A) and blastocysts (97/314; 30.9%^A) that originated from ooplasts reconstituted with the cell nuclei of ACFCs undergoing scriptaid treatment were significantly higher than in the scriptaid-unexposed group (167/292; 57.2%^B, 125/292; 42.8%^B and 51/292; 17.5%^B, respectively) [A,B: $p < 0.001$; Chi-square test]. Altogether, the improvement is not only cleavage activity of porcine cloned embryos, but also their morula/blastocyst formation rates seem to arise from enhanced abilities for promotion of faithful and complete epigenetic reprogramming of scriptaid-treated ACFC nuclei in a host cytoplasm of reconstituted oocytes. (This work was financially supported by the National Centre for Research and Development (grant number INNOMED/1/17/NCBR/2014).)

P 200 | Fetal B-mode and Doppler ultrasonography in gestational evaluation of ewes

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This research aims to assess the efficiency of B-mode and Doppler ultrasonography on ewes gestational evaluation and propose new mathematical equations to stipulate gestational age. Ten Santa Inês ewes were examined weekly, from the 3rd until 21st week. MyLab™30 VET (Esaote S.p.A., Genova, Liguria, Italy) was used with 5–7.5 MHz transducers. Until week 8 linear transrectal transducer was used, thereafter examinations were made with a convex transducer transabdominally. On week 3 median length of the embryos was 8.7 ± 2.2 mm and heart rate 180.6 ± 12 bpm. No further specific B-mode evaluations were possible due to embryos insufficient development. At 12th week it was no longer possible to measure fetal length and some biometry variables were correlated with gestational age: heart rate (193 ± 12.2 bpm) $R^2=0.93$; biparietal diameter (30.5 ± 1.6 mm) $R^2=0.97$; toracic diameter (34.95 ± 4.7 mm) $R^2=0.98$; abdominal diameter (40.75 ± 5.2 mm) $R^2=0.98$. At 21st week: heart rate (127 ± 9.1 bpm); biparietal diameter (52.5 ± 0.9 mm); abdominal diameter (72.2 ± 7.4 mm). Due to fetal intense movements not all variables are acquired on each third of gestation. B-mode variable with the highest correlation ($R^2=0.98$) with gestational weeks

(GW) was abdominal diameter (AD mm) in a linear regression ($GW=4.1 + 0.2 \times AD$). Concerning the Doppler parameters, only resistance index (RI) of umbilical cord artery had high correlation ($R^2=0.54$) with gestational week in a linear regression ($GW=29.6-21.7 \times RI$). (Acknowledgment to FAPESP for scholarship 2014/15422-0).

P 201 | Improvement of fertility of cows using Prostaglandin F_{2 α} analogue

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The present study was conducted at Rajshahi, Bangladesh for improving the pregnancy rate in cows after using PGF_{2 α} analogue during the period from January 2013 to December 2014. The oestrous synchronization was performed by single and / or double injection of PGF_{2 α} analogue (Ovuprost® / Prostenol® 2 ml/cow) in 240 anoestrus dairy cows. The selected cattle were injected with a PGF_{2 α} analogue and the second injection of PGF_{2 α} analogue was done on 11th days after 1st one. Following 1st and 2nd injection the pregnancy rate was observed at 57.8% and 65.6%, respectively. The oestrus synchronization and pregnancy rate (39.0% and 65.6%) after double PGF_{2 α} analogue were significantly ($p < 0.05$) higher. The oestrus synchronization (66.6%) by Ovuprost® and pregnancy rate (61.4%) after artificial insemination was better than Prostenol® (50.0% and 60.0) and control (20% and 58.3%). It was revealed that Local \times Friesian crossbred cows responded better to the PGF_{2 α} analogue with a synchronization rate of 35% at day 1 and 42.3% at day 11 compared to Local \times Sahiwal crossbred cows with synchronization rates of 30% and 39.2%, respectively. Local cows however, consistently showed poor PGF_{2 α} analogue response with a synchronization rate 30% and 35.7%, respectively. The best reproductive performance was observed in cattle having an excellent body condition and being between 4 and 8 years. Worthwhile to note, well managed mini farms with cows having body weights between 250 and 350 kg showed a significantly higher ($p < 0.05$) sensitivity to prostaglandin. It may be concluded that double injection of prostaglandin reduced the calving interval and improves fertility status in cows at Rajshahi district of Bangladesh.

P 202 | Epididymis in the agouti (*Dasyprocta azarae*) raised in captivity – light microscope study

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The agouti is a wildlife rodent that has been raised in captivity because it can be used as an alternative source of animal protein. Thus, the knowledge of morphology of the reproductive organs is important for understanding and improving their reproductive efficiency. The aim of this study was to describe the epididymis in the Azara's agouti (*Dasyprocta azarae*), by light microscopy. Samples of epididymis were obtained from five adult animals during castration surgery performed at the Municipal Zoo of Catanduva, Brazil. Fragments of the epididymal regions (caput, corpus and cauda) were collected and destined to histological routine with HE and Masson's trichrome stains. The epithelium lining of epididymis was pseudostratified columnar with principal, basal, apical, clear, narrow and halo cells. The caput epididymis was divided into two different regions: initial segment and caput. The initial segment has a very wide lumen, a high epithelium. The caput epididymis, showed a lower epithelium than the initial segment, large amounts of spermatozoa in the lumen, and a cytoplasmic vacuolization. The cauda epididymis could be divided into two regions: a proximal region that showed ducts with appearance of pseudostratified epithelium and a distal region with ducts with appearance of cuboidal epithelium, perhaps because of its low height. Large amounts of spermatozoa are also present in the lumen of cauda epididymis. The pattern of the epithelium lining the duct of the agouti epididymis does not differ greatly from that reported in other domestic and wildlife mammals, such as dog and paca. These findings can cooperate with further investigations especially those related to rational exploration of these animals. (This study was supported by FAPESP, Grants 2015/23822-1.)

P 203 | Environmental temperature influences hair coat, heart rate and reproductive parameters in Shetland stallions

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We hypothesized that accommodation of stallions in a thermoneutral temperature in autumn and winter influences metabolism and hair coat and improves semen quality. Shetland stallions were kept outside (CON, n = 8) or in indoor stables with temperature >10°C (ST, n = 8) from October to March. Rectal temperature, heart rate, plasma testosterone, cortisol concentration as well as hair coat quality and length were determined at regular intervals. Semen was collected once weekly and sexual behavior and semen quality were analysed. Statistical analysis was performed by ANOVA using a general linear model for repeated measures with season as within and group as between subject factor. In autumn and winter, rectal temperature was higher in ST than in CON stallions (p < 0.05). The hair coat underwent seasonal changes (p < 0.001) and differed between groups (p < 0.05) with shorter guard hair, slower hair regrowth and earlier hair change in group ST. Season influenced heart rate (autumn > winter) but did not differ between groups. Testosterone and cortisol concentration and sexual behavior changed with season but were not affected by group.

Semen volume and total sperm count were influenced by season (p < 0.01) and increased more pronounced from winter to spring in CON than ST stallions (p < 0.05). In summary, environmental temperature affected body temperature and hair coat while effects on semen were minimal. Seasonal reproductive function in horses thus depends on day length but less on other environmental cues.

P 204 | Sperm fatty acid content changes with season in stallions with good and moderate semen quality after cryopreservation

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Spermatozoa contain polyunsaturated fatty acids (PUFA) which provide the sperm membrane with fluidity required for fertilization. Cryopreservation damages sperm membranes making them less functional after thawing. We have analysed the lipid composition of equine spermatozoa from January to June and compared between "good freezer" (GF, n = 6) and "moderate freezer" stallions (MF, n = 9). We hypothesized that seasonal and individual differences in the cryoresistance of spermatozoa reflect sperm lipid patterns. Semen was collected once weekly and two ejaculates per month were submitted to cryopreservation and analysis of sperm fatty acids, respectively. Differences over time and between groups were determined by repeated measures GLM-ANOVA with time as within and group as between subject factor. Content of saturated palmytic and stearic acid decreased from January to March (p < 0.001) while content of the PUFA docosapentaenoic (p < 0.001) and arachidonic acid (p < 0.05) and total PUFA (p < 0.001) increased. Docosapentaenoic acid was the predominant PUFA and the only one with a constantly higher content in GF than MF stallions (p < 0.05). In conclusion, the fatty acid composition of stallion spermatozoa undergoes seasonal changes and PUFA content increases from the non-breeding into the breeding season. We suggest that seasonal and individual differences in sperm membrane fatty acids in part explain changes in the resistance of equine spermatozoa to cryopreservation.

P 205 | Impact of heat stress on follicle size and estrus expression in dairy cows

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The objective of this study was to investigate the impact of heat stress on the expression of estrus signs and follicular diameter at the day of estrus in dairy cows under field conditions. Cows reported in estrus were examined by a veterinarian (n = 572 estrus events from 233 cows). Uterine contractility and the largest diameter of all ovarian structures were determined by transrectal palpation

and ultrasonography, respectively. The amount of estrus discharge, mounting traces and the color of the vaginal mucosa was determined in an external examination and scored on a 3-points scale. Blood samples were obtained for analysis of serum progesterone concentrations. Cows with a high uterine contractility and high amount of estrus discharge were 4.05 and 1.72 times more likely to have an estrus follicle (≥ 12 mm) than cows expressing a low amount of the estrus signs, respectively. The likelihood for a pink vaginal mucosa, clear stringy estrus discharge and mounting traces at the cows' back decreased continuously with increasing temperature-humidity index (THI) at the day of estrus. The likelihood for a serum progesterone concentration < 1 ng/ml at the day of estrus decreased continuously with increasing THI ≥ 74 . Follicular size decreased 0.1 mm with each incremental THI point at the day of estrus. The present study proves, that the amount of estrus discharge and the intensity of uterine contractility are useful clinical signs to detect cows with an estrus follicle under field conditions. Heat stress at the day of estrus significantly reduces the intensity of external signs of estrus; however, the intensity of uterine contractility is a useful clinical signs to detect cows with an estrus follicle under heat stress.

P 206 | Significance of physiological and technological factors in development of mastitis in lactating cows

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The aim of the researchers was to study the effect of pregnancy ($n = 178$, 1st experiment) and changes of optimal parameters of mechanical milking ($n = 320$, 2nd experiment) on the incidence of sub-clinical mastitis in dairy cows. Diagnosis of mastitis was realized with the use of diagnostic reagent Kenotest and by bacteriology. The 1st experiment stated that conception and pregnancy in cows were accompanied by a decrease in the milk ejection rate by 16–23.1% in comparison with non-pregnant animals and a 1.48 time increase in the risk to suffer from mastitis during observation period (120 days). We attribute this to the 4.6 times increase of progesterone production, that suppresses immune reactions to bacterial agents within the udder, suppressing also the processes of oxytocin receptor formation and hence milk ejection reflex. The 2nd experiment demonstrated that a two fold decrease of the normative vacuum reserve in the milking installation led to a 1.3 fold increase of the number of cows with teat-end hyperkeratosis and 3.1 times increase of mastitis during one month. Decreasing of the vacuum level below optimal by 9–12% (from 41–37 kPa to 36–34 kPa) in the teat chamber, increased the number of teat-end damages with hyperkeratosis by 2.8 times and the morbidity with mastitis – by 1.8 times. Milking of cows during one month with the threshold of automatic cluster/teat cup removal of 100 g/min in comparison with 200 g/min led to a 3.2 fold increase of teat-end hyperkeratosis and 3.1 times increase of mastitis. The data

obtained allow to conclude that maintenance of optimal mechanical milking parameters for pregnant cows and optimization of the technology of pregnant cows' udder preparation for milking are important for mastitis prophylaxis in lactating cows.

P 207 | Epigenetic modulation of ram sperm during cryopreservation

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Ram semen cryopreservation is a process which is widely used for long term storage of sperm. However, it can induce several detrimental damages to the sperm cells. These damages may involve cellular and epigenetic patterns of sperm. Epigenetic modulation of cryopreserved sperm may impact the outcome fertility potential of cryopreserved sperm. Therefore, the purpose of the present study was to consider several epigenetic indices in ram sperm before and post freezing. Motility, total DNA methylation and histone acetylation (H3K9) were measured in ram sperm before and post cryopreservation. Semen was collected from four mature rams, diluted with defined extender and cryopreserved according to our standard protocol. A significantly higher percentage of total motility and progressive motility were observed in the fresh group (85.1 ± 1.25 and 70.2 ± 1.8 , respectively) compared to the frozen group (52.6 ± 1.74 and 21.9 ± 2.1 , respectively). Furthermore, the percentage of sperm with methylated DNA was significantly increased in the frozen (62.2 ± 1.1) vs. the fresh group (35.1 ± 1.45). No significant differences were detected between the two groups with regard to histone acetylation (11.2 ± 0.25 vs. 40.3 ± 1.02). Due to the crucial impact of epigenetics on the reproductive outcome, further studies are necessary to determine the effect of cryopreservation on the epigenetic modulation of sperm cells.

P 208 | High caudal epidural anaesthesia and standing ketamine stun for transvaginal unilateral ovariectomy in cattle

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General anesthesia is rarely carried out in cattle. The most common procedures are undertaken using epidural anesthesia, paravertebral nerve or local infiltration blocks. High and low caudal epidural anesthesia is advocated in cattle for pain relief and muscle relaxation during obstetric manipulations and surgical procedures including uni- or bilateral ovariectomy. The aim of the study was to obtain the ovarian tissue while defined oestrus cycle time during transvaginal unilateral

ovariectomy under high caudal epidural anesthesia and standing ketamine stun. The 9 Holstein-Friesian type cows during induced oestrus cycle were enrolled into the study. The elective, transvaginal procedure under high epidural anesthesia (between S5 and Co1 vertebra) following pharmacological stun premedication with xylazine 5–30 mg/100 kg (Xylapan 20 mg/ml, Vetoquinol Biowet, Poland), ketamine 0.04 mg/kg (Bioketan 100 mg/ml, Vetoquinol Biowet, Poland), butorphanol 0.01 mg/kg (Butomidor 10 mg/ml, Richter Pharma AG, Austria) was performed. The ovaries were removed to obtain luteal tissue for further detailed examinations. The postoperative antibiotic therapy with procaine benzylpenicillin 1 ml/30 kg (Penillin 30%; 300 mg/ml; ScanVet Poland) in combination with postoperative analgesia flunixin 2 ml/45 kg (Flunimeg 50 mg/ml; Scanvet Poland) was continued for 3 days. The lidocaine deposition was successful into the sacrococcygeal space. The onset of paralysis of the tail occurs in 1 min. The block and ketamine stun last up to 2 h. No pelvic limb weakness and recumbency occurred. High caudal epidural anesthesia and standing ketamine stun provide great quality analgesia for transvaginal ovariectomy.

P 209 | Deviations in endometrial biopsies collected from mares

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The biopsy of the endometrium is considered to be the reference standard to diagnose endometriosis in mares. The aim of this study was to examine the relationship between deviations in endometrial biopsies in terms of cyclooxygenase-2 (COX-2) and fibronectin expression and polymorphonuclear cell (PMN) infiltration, as well as scoring degeneration in two endometrial biopsies collected from one mare. The research was conducted on 53 subfertile mares, Icelandic horses. All of the mares had no discharge and no intrauterine fluid, and they had been unsuccessfully inseminated during three oestrus cycles. We took two endometrial biopsies from the base of either uterine horn with Kevorkian biopsy forceps and fixed in 10% formalin. The biopsies were examined for the presence of PMNs infiltration within the luminal epithelium, stratum compactum and stratum spongiosum, as well as for the Kenney-Doing score. The biopsies were considered as evidence of acute endometritis if there were more than three PMNs per five fields of high magnification (x400). The biopsies were also stained for cyclooxygenase-2 (COX-2) and fibronectin expression. We found positive Spearman correlations between COX-2 expression in the surface ($R = 0.49$; 0.7) and glandular epithelium ($R = 0.2$; 0.21), as well as fibronectin expression in the stroma ($R = 0.41$; 0.55) and the Kenney and Doig (1986) grading score. There were observed an association between multiparous mares older than ten years and

endometrial biopsies with scores IIB /III in at least one biopsy sample and also a moderate to strong immunointensity for fibronectin. In conclusion, there was a relationship between the COX-2 and fibronectin expression and biopsy scores, according to Kenney and Doig (1986) grading system.

P 210 | Elastography of ovine fetal lung as a tool for gestational development evaluation

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The aim of this study was to evaluate the fetal lung stiffness by acoustic radiation force impulse (ARFI) elastography during gestation in ewes and correlate these analyses with fetal development age. Twenty-four healthy pregnant ewes were included in the study. The stiffness of fetal lungs was determined weekly, from the tenth week until parturition using ACUSON S2000® (Siemens®) ultrasound system, equipped with software for qualitative and quantitative elastographic-ARFI evaluation (Virtual Touch Tissue Quantification) and convex multi-frequential transducer. The stiffness characteristics assessed by grayscale elastogram were compared between the adjacent tissues qualitatively, and the fetal lung shear wave velocities (SWV m/s) compared between gestational weeks by mean of Friedman's test and correlated by regression models ($p < 0.05$). At elastogram analysis, the fetal lung was not deformable and stiffness characteristics showed light gray shades, indicating to be a soft tissue when compared to other organs (liver and kidneys). Lung SWV varied throughout the gestational period ($p < 0.001$) from 0.73 ± 0.11 m/s to 0.62 ± 0.12 m/s decreasing gradually from 16th to 21st gestational week and closely correlates with fetal age ($R^2 = 0.80$), indicating that pulmonary density undergoes modifications during the gestational weeks. In conclusion ARFI-elastography has shown to be an applicable and safe technique, enabled qualitative and quantitative tissues elastographic characterization and allowed detect pulmonary stiffness modifications related with fetal development and gestational age. We suggest that these results could be used as reference for pregnancy pathophysiological studies in ovine and extrapolated for gestational researches in other mammals' species.

P 211 | Effect of sample size on fish (*Prochilodus reticulatus*) sperm head morphometric measurements

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Computer-assisted sperm morphometry analysis has been previously standardized for fish semen; however there is little information in fish

(*Prochidolius reticulatus*). The aim of this study was to assess the effect of the number of cells analysed on fish sperm head morphometric measurements. Semen was collected from 7 fish by abdominal massage (in the region North of Santander, Colombia,) extended in DMSO (dimethylsulfoxide) diluent. All the samples showed physiological values concerning routine semen parameters (motility, morphology and sperm concentration). A final concentration of 450 million sperm/ml. Morphometric slides were stained with Diff-Quik and 300 sperm heads analysed using the Sperm Class Analyser (ISAS, Prosein, Spain). Subsets of 200, 150 and 100 sperm cells were randomly selected and mean measurements of length (L), width (W), area (A), perimeter (P), ellipticity (E), rugosity (R), regularity (RE) and elongation (EL) compared between groups by ANOVA. No significant differences ($p > 0.05$) for L (3.73^a vs. 3.74^a vs. 3.70^a), W (3.45^a vs. 3.45^a vs. 3.43^a), A (10.50^a vs. 10.56^a vs. 10.47^a), P (12.08^a vs. 12.11^a vs. 11.99^a), E (1.08^a vs. 1.08^a vs. 1.08^a), R (0.90^a vs. 0.90^a vs. 0.90^a), RE (0.96^a vs. 0.96^a vs. 0.96^a) and EL (0.42^a vs. 0.42^a vs. 0.41^a) were found when 200, 150 or 100 spermatozoa were analysed. Our results suggested that the analysis of 100 sperm heads was enough for the morphometric assessment of fish semen, reducing considerably the time to perform an accurate analysis. It is concluded that 100 sperm heads should be analysed to morphometrically characterise the whole sperm population of fish (*Prochidolius reticulatus*) semen samples.

P 212 | Effects of the prolonged culture conditions on the apoptosis resistance and developmental competence of bovine oocytes

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A delayed natural and artificial activation of matured mammalian oocytes results in a fall of their quality. The goal of the present work was to study effects of different conditions of the prolonged in vitro culture on the apoptosis resistance and developmental competence of bovine oocytes. Bovine cumulus-enclosed oocytes (CEOs) were matured for 16, 22 or 32 h in TCM 199 containing 10% fetal calf serum (FCS), 10 $\mu\text{g/ml}$ FSH, and 10 $\mu\text{g/ml}$ LH. All CEOs matured for 16 h were transferred to TCM 199 supplemented with 10% FCS and cultured for additional 16 h in the absence (Control) and in presence of 50 ng/ml prolactin (PRL) or 10 ng/ml growth hormone (GH). After culture, oocytes were used for apoptosis detection or activated by treatment with ionomycin and 6-dimethylaminopurine and then cultured up to the blastocyst stage. Apoptosis was detected in oocytes and blastocysts by the TUNEL method. An increase in the culture duration from 22 to 32 h led to a rise in the rate of apoptotic oocytes (from 9.4 ± 1.0 to $15.3 \pm 0.8\%$, $p < 0.01$), to a decline in the yield of blastocysts (from 20.6 ± 2.6 to $12.0 \pm 1.6\%$, $p < 0.05$), and to an increase in the rate of apoptotic nuclei in blastocysts (from 4.1 ± 2.3 to $12.6 \pm 2.0\%$, $p < 0.01$). As compared to the continuous 32-h culture, the transfer of CEOs to the medium containing PRL (but not to

the control medium or medium with GH) caused a decrease in the rate of oocyte apoptosis (to $7.1 \pm 1.9\%$, $p < 0.01$), an increase in the yield of blastocysts (to $20.8 \pm 1.7\%$, $p < 0.05$), and a reduction in the rate of blastocyst apoptosis (to $6.6 \pm 0.7\%$, $p < 0.01$). Thus, PRL is able to maintain the apoptosis resistance and competence for parthenogenetic development of bovine CEOs during their prolonged in vitro culture. (The research was supported by FASO Russia and RFBR (15-08-99473).)

P 213 | Postpartum hysteroscopic evaluation in Santa Ines sheep – preliminary results

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Postpartum fertility in sheep depends of physiological uterine involution. There is poor information available on the time between partus and cervix closure. Hysteroscopy allows direct visualization of the uterine structures and is an excellent method for uterine cavity evaluation. It is a very useful method for detection of abnormalities during the postpartum period. The aim of this study was to evaluate the cervical closure time by hysteroscopy in Santa Ines sheep. A rigid endoscope (5 mm Diameter) without angulation was used, and adequate visualization of the cervical canal and uterine cavity was done with the aid of a speculum and a 300W xenon light lamp. The real time video signal was transmitted to a TV monitor. Twenty animals were examined every 6 h after parturition until it was impossible to enter the cervical canal. Of 22 ewes evaluated, 20% had cervical closure between 6 and 12 h, 30% between 12 and 24 h, 25% between 24 and 30 h and 15% between 30 and 36 h. In 10%, the uterus could not be assessed due to anatomic features. One animal had a uterine prolapse on day 5 post partum. In conclusion, these results provide evidence that cervical closure occurred within in the first 36 h after parturition and hysteroscopy is an alternative technique for cervical and uterine evaluation in ewes.

P 214 | The content of collagen in the equine cervix during estrus cycle

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Proper cervical contractility and relaxation are major factors affecting mare's fertility and uterine defense mechanisms. Cervical disorders are predisposing factors for fluid accumulation and contamination of the uterus, might impair fertilization as well as influence pregnancy and parturition. Histologically, the cervix consists mostly of connective tissue and smooth muscle. In the cervical extracellular matrix, collagen is the most abundant structural protein. Collagen reorganization and decrease in its concentration have an influence on the process of cervical relaxation. The aim of this study was to assess the collagen content (CC) in the equine cervix in follicular and luteal phase. Fresh whole thickness samples of the cervix uteri were collected from 29 slaughtered mature mares with no signs of reproductive tract pathologies. The samples were stained with hematoxylin eosin (HE) and Masson's Trichrome Stain (MTS) and imaged using light microscopy and scanning cytometry for CC evaluation (mean±SEM, %). The tunica mucosa formed folds with connective tissue underneath, demonstrating significantly different ($p = 0.0063$) collagen content in follicular (57.09 ± 5.40) and luteal (34.33 ± 4.62) phase. The CC in tunica muscularis did not change during estrus cycle ($p > 0.05$) obtaining 58.84 ± 3.19 in follicular and 66.12 ± 3.30 in luteal phase respectively. CC was significantly higher ($p < 0.001$) in tunica muscularis in comparison to tunica mucosa during follicular phase; however, no differences were found in luteal phase ($p > 0.05$). In conclusion, the CC in the equine cervix undergoes hormonal regulation and differs significantly in tunica mucosa depending on the phase of the estrus cycle.

P 215 | Trichostatin A (TSA)-treated peripheral blood-derived fibroblast-like cells turn out to be a suitable source of genomic DNA donors for efficient production of nuclear-transferred pig embryos

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The current study was undertaken to assess the in vitro developmental outcome of porcine cloned embryos reconstructed with oocytes receiving the cell nuclei of adult peripheral blood-derived fibroblast-like cells (APB-FLCs) that had been epigenomically transformed by exposure to non-selective inhibitor of histone deacetylases, designated as TSA. Prior to their use for somatic cell nuclear transfer (SCNT), the adherent APB-FLC lines that had been established from primary cultures originating from the blood samples of a postnatal female piglet and were subsequently passaged twice or thrice underwent treatment with 50 nM TSA during 24-h serum starvation. Reconstruction of enucleated metaphase II-stage oocytes was achieved by their electrofusion with TSA-exposed APB-FLCs that was evoked by two successive DC pulses of 1.2 kV/cm for 60 μ s. The same DC pulses that triggered the fusion of ooplast-APB-FLC couplets were simultaneously applied to induce activation of reconstituted oocytes (clonal

cybrids). The frequencies of cleaved embryos (231/283; 81.6%^A), morulae (195/283; 68.9%^A) and blastocysts (108/283; 38.2%^A) developing from NT embryos that were reconstructed with TSA-treated APB-FLCs were significantly higher as compared to the TSA-untreated group (174/269; 64.7%^B, 132/269; 49.1%^B and 67/269; 24.9%^B, respectively) [A,B: $p < 0.001$; Chi-square test]. Collectively, the enhancements in both cleavage rate of porcine SCNT-derived embryos and their morula/blastocyst yields appear to result from increased functional abilities for proper onset and progression of epigenetic remodeling and reprogramming of TSA-exposed APB-FLC nuclei in a cytoplasm of clonal cybrids. (This work was supported by the National Centre for Research and Development (grant number INNOMED/1/17/NCBR/2014).)

P 216 | Effect of the bicarbonate concentration on the intracellular pH increase and protein kinase A activation in porcine in vitro sperm capacitation

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The transmembrane intake of HCO_3^- in the sperm has been associated with the increase of intracellular pH (pHi) and activation of PKA/cAMP pathway. This work evaluated the effect of the concentration of HCO_3^- on sperm pHi and capacitation through protein kinase A substrates phosphorylation (PKAs-P). For this purpose, samples ($n = 4$) of porcine epididymal spermatozoa were incubated for 60 min in a capacitating medium (TALP) with a fixed pH of 7.4 and containing 0, 5, 15 or 25 mM of HCO_3^- and in a non-capacitating medium as control (NCAP). The pHi was measured by spectrofluorometry at 1 and 60 min of incubation and PKAs-P was detected by western blot and semi-quantified at 60 min. Data (mean \pm SEM) were analyzed by one-way ANOVA and compared using the Tukey test. Differences were considered statistically significant at $p < 0.05$. The pHi results at 1 min of incubation showed no differences between groups ($p > 0.05$). The results after 60 min revealed that the HCO_3^- presence in the media was essential to elevate the pHi during sperm capacitation. However, no relation with the specific HCO_3^- concentration was found (NCAP: 6.65 ± 0.03^a , 0 mM: 6.77 ± 0.01^a , 5 mM: 6.93 ± 0.02^b , 15 mM: 7.00 ± 0.03^b and 25 mM: 7.06 ± 0.05^b). Moreover, the semi-quantification of relative amount of PKAs-P showed that using 15 or 25 mM in the capacitation media produced a higher PKAs-P (NCAP: 212.3 ± 36.0^a , 0 mM: 274.3 ± 28.1^a , 5 mM: 307.2 ± 22.2^a , 15 mM: 488.3 ± 22.3^b and 25 mM: 576.0 ± 10.2^b). In conclusion, 15 mM of HCO_3^- is an optimum concentration to elevate the pHi of spermatozoa and produce a high grade of capacitation in vitro. Nevertheless, further studies are needed to find out if this fact has a beneficial effect on in vitro fertilization in porcine species. (Supported by MINECO-FEDER AGL2015-66341-R.)

P 217 | Modulation of boar sperm capacitation and fertilizing ability in vitro by an ethanol extract of an oenological commercial oak-derived tannin

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Although excessive ROS levels induce sperm damage, sperm capacitation is an oxidative event that requires low and controlled amounts of ROS. The aim of this study was to evaluate the effect of an ethanol extract of a commercial oenological tannin (Tan'Activ R[®], T) with antioxidant activity (*Food Chemistry* 2017, 215:50) on in vitro sperm capacitation and fertilization (IVF). Boar sperm was capacitated for 1 h in presence of different concentrations of T (0, 1, 10, 100 µg/ml). Sperm viability (SYBR green/PI) and tyrosine-phosphorylation of sperm proteins (by immunofluorescence) were evaluated. For IVF, gamete co-incubation (1 h) was performed in presence of T (0, 1, 10, 100 µg/ml). After 19 h of oocyte culture in fresh IVF medium, the penetration rate was evaluated. Sperm viability was significantly ($p < 0.05$) higher compared with control in presence of T100 (67.0 ± 14.8 vs. 35.4 ± 10.8). Tyrosine-phosphorylation of sperm proteins was significantly influenced by T: T10 induced a decrease of the percentage of cells displaying A pattern (non capacitated cells) (51.4 ± 9.0 vs. 32.6 ± 10.7 , ctr vs. T10) and a parallel increase of B pattern (capacitated cells) (48.6 ± 9.0 vs. 67.0 ± 10.6 , ctr vs. T10) while an opposite effect of T100 was recorded (pattern A: 65.9 ± 29.8 ; pattern B: 33.3 ± 29.2). The penetration rate was significantly increased by T10 (69.0 ± 14.8 vs. 91.9 ± 4.0 , ctr vs. T10) while it was strongly inhibited by T100 (4.9 ± 8.3). These results demonstrate that T10, maintaining ROS at low levels, stimulates capacitation resulting in a higher fertilization rate while T100, excessively decreasing ROS levels, inhibits both capacitation and fertilizing ability and reduces the decline of viability that occurs during capacitation. (Work supported by "Fondazione del Monte di Bologna e Ravenna".)

P 218 | Molecular interactions between the porcine AhR and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor activated by environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is thought to be the most harmful dioxin exerting adverse effects on health and reproduction of domestic animals. It alters sexual behavior and causes decreased

spermatogenesis, diminished fertility, endometriosis, teratogenesis and abortion. TCDD influences also the metabolism and secretion of steroid hormones. Upon ligand binding, the TCDD/AhR complex translocates to the nucleus, dimerizes with AhR nuclear translocator and binds to a dioxin-responsive element present in promoter of target genes. In the present study, homology model of the porcine AhR-ligand binding domain (LBD) was constructed on the basis of crystalline structure of human HIF-2 α protein. The cavity volume for AhR-LBD was estimated at 804.6 Å³. Molecular docking of TCDD to the porcine AhR-LBD revealed high binding affinity between TCDD and AhR (-8.8 kcal/mol). TCDD was stabilized within the binding pocket by hydrophobic interactions with residues: H289, F293, P295, L306, L313, F322, F349, L351 and S363 of the AhR and a single hydrogen bond with Q381. Formation of the TCDD/AhR complex and TCDD-induced activation of AhR were confirmed with the use of electrophoretic mobility shift assay. It was found that TCDD bound to the AhR and activated the receptor. The current study provides a framework for examining the key molecular events involved in the ligand-dependent activation of the AhR. Better understanding of AhR function in the ovary is crucial to improve fertility and breeding of domestic animals. (This study was supported by grants No. 2012/05/B/NZ9/03333, N N303 815240 and 2016/21/B/NZ4/00202.)

P 219 | Bovine mastitis originating *S. aureus* has more virulence genes than coagulase-negative staphylococci

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Staphylococcus (S.) aureus may cause even severe bovine clinical mastitis but coagulase-negative staphylococci (CNS) mainly subclinical or mild clinical mastitis. Differences in virulence may exist between CNS species. The aims were to study the presence of putative virulence genes in *S. aureus* and three CNS species isolated in bovine mastitic milk samples, and to evaluate the potential association between bacterial genotype and type of mastitis (clinical vs. subclinical). Virulence gene orthologues were searched in 24 annotated whole genome sequences of *S. aureus* (4), *S. agnetis* (4), *S. chromogenes* (8), and *S. simulans* (8) isolates. Half of the isolates of each species originated from mild to moderate clinical, and the other half from subclinical bovine mastitis. The *S. aureus* isolates had much more virulence gene orthologues than the CNS isolates. One third of the virulence genes were detected only in *S. aureus*. Nearly 100 virulence genes were present in all *S. aureus* isolates, as contrast to about 40–50 present in each CNS isolate. *S. simulans* differed most from the other CNS: several of the virulence genes detected among CNS were harbored only by *S. simulans*, but it also lacked a number of genes present both in *S. agnetis* and *S. chromogenes*. Identical virulence genes were detected in isolates of

the same species both from clinical and subclinical mastitis. The virulence gene profiles or the cumulative number of different virulence genes were not associated with the type of mastitis, indicating that host derived factors such as the immune status play a pivotal role in the manifestation of mastitis.

P 220 | Evaluating the effectiveness of two mastitis vaccines in a dairy farm in Mashhad, Iran

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The objective of this study was to evaluate the efficiency of two commercial mastitis vaccines (Mastivac[®] and Startvac[®]) under field conditions. In total, 164 Holstein dairy cows were selected and divided into 3 groups. Three milk samples were collected from each cow at the day of drying-off, 3 days and 2 weeks after parturition. The first group (n = 55) received Mastivac[®] vaccine following the label regimen. The second group (n = 54) received Startvac[®] vaccine following the label regimen, and the third group (n = 55) was left unvaccinated as negative controls. The data were analyzed by using SPSS software (SPSS Inc., 22). Analysis of the mastitis prevalence after parturition in triple groups was performed using chi-square test. The comparison of the severity of infection and also the duration of treatment in all groups were analyzed by using Kruskal-Wallis test. The differences between groups with a p-value <0.05 were considered significant. In total, within one month after parturition, 70 cases of clinical mastitis occurred in the 3 study groups, but we detected no significant difference in the prevalence of clinical mastitis between any of the 3 groups. The average severity of clinical cases in three groups were 1.36, 1.24 and 1.45 for Mastivac[®], Startvac[®] and control group, respectively and not significant. Duration of treatment did not differ between groups and was 3.7, 3.29 and 3.6 days for Mastivac[®], Startvac[®] and control group, respectively. New infection rate after parturition was assessed by CMT and did not differ between groups. New infection rates were 38.5% for Mastivac[®], 26.5% for Startvac[®] and 34% for control group respectively. In general, we observed no significant difference in above-mentioned variables between any of the 3 groups.

P 221 | Use of dexamethasone as antenatal therapy affects placentome circulation in goats?

– Preliminary data

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The aim of this study was to evaluate and compare the spectral Doppler indices of arterial branches of placentome in pregnant goats submitted to different dexamethasone base protocols. Three protocols of dexamethasone (Dexacort, Hertape, Brazil) administrations i.m. were tested: G1 (n = 4) goats received a single 20 mg of drug at Day 139 of gestation; G2 (n = 5) were given 2 mg once a day between Day 133 and Day 136, 4 mg at Day 137 and at Day 138 and 20 mg at Day 139; and G3 (n = 5) were given 16 mg twice a day at Day 139 and Day 140. All animals were submitted elective cesarean section at Day 141 of pregnancy to obtain premature kids. Hemodynamic changes of two placentome were assessed by spectral Doppler ultrasonography (MyLabvet70[®], ESAOTE, Genova, Italy), connected to a 3–6 MHz convex multifrequency electronic transducer. Data were compared between groups (mean±SD, Tukey test, p < 0.05). There was no difference between the different treatments for flow velocity integral (6.78 ± 1.38 cm/s, p > 0.67), pulsatility index (1.61.69 ± 0.68 cm/s, p > 0.21), resistive index (0.60 ± 0.17 cm/s, p > 0.40); peak velocity (25.14 ± 3.41 cm/s, p > 0.55), end diastolic velocity (11.26 ± 1.93 cm/s, p > 0.86) and mean velocity (15.40 ± 1.69 cm/s, p > 0.36). In conclusion, the placentome circulation in peri-partum of goats is not affected by the different doses or corticosteroid administration time.

P 222 | Intracervical artificial insemination of Saanen goats in different breeding age with frozen-thawed semen

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Artificial insemination (AI) in goat is currently limited by the poor fertility obtained following non-surgical intracervical insemination of frozen-thawed semen. The objective of the present study was to determine the reproductive performance of Saanen goats in two different breeding ages in Turkey. The effects of synchronisation, inseminators and fertility rate were examined. Ninety nine (99) Doeling Saanen goats were used, divided in two groups: Group 1 = 49 doeling goats (7 month old) and Group 2 = 50 doeling goats (19 month old). Estrus was synchronized by impregnated intravaginal sponges (20 mg; FGA), and administration im of 125 mg of cloprostenol (PGF_{2α}) and PMSG (400 IU), at 48 h before sponges removal. Intracervical fixed time AI was carried out 43 h after removal of the FGA sponges during the non breeding season. Estrus symptoms of goats were detected by teaser goat. The occurrence of oestrus in Group 1 and Group 2 were 74%, 71.4%, respectively, and there were no significant differences between groups (p > 0.05). Sixty eight (68) goats were inseminated with frozen-thawed semen. The pregnancy rate was determined by transabdominal ultrasound scanning. Pregnancy rate were (Group 1 31.4%, Group 2 35.5%) not differ significantly between different breeding age nor between inseminators (p > 0.05). Fertility results were quite acceptable considering the various age groups and

inseminators. Intracervical insemination is a simple, less costly and time consuming technique compared to the others. As it is the final goal to establish a technique that could be applied similarly on a large scale by all farms, intracervical insemination must be considered as a method that simplifies the use of liquid buck semen in Turkey.

P 223 | Genetic transformation of chicken spermatogonia in vitro

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Spermatogonia are precursors of male germ cells. These cells are considered as promising target cells to deliver recombinant DNA for production of genetically modified animals. The present research aimed to study spermatogonia transformation efficiency in vitro using various gene delivery systems: lipophilic transfection, lentiviral infection and electroporation. Comparative analysis of the delivery efficiency of a gene has shown high performance modification of the target cells using the lentiviral infection and electroporation method. Efficiency of delivery of genetic constructs using the lipophilic transfection method was low and amounted to about 5%. Optimization by DNA and lipophilic agent quantities did not give an improved efficiency. Otherwise, optimization of the conditions of spermatogonia transformation using the lentiviral infection and electroporation allowed to obtain the sufficiently high transformation efficiency of the target cells – up to 65%. The high efficiency of transfection using the lentiviral delivery method was achieved at multiplicity of infection (MOI) = 1, using the lentiviral titre 1.7×10^7 TU/ml. The following electroporation parameters – voltage 350 V, pulse time 10 ms – were selected as optimal. The technology of transgenic animal production using donor spermatogonia is provided by injection of these cells into the testes of male-recipients. Due to the high quantity of the cells (2–3 million cells per testis) necessary for injection, the electroporation method can be recommended as the optimal method that allows for the simultaneous transfection of suspension cells in a high concentration. (Supported by RSF (16-16-10059).)

P 224 | Effect of different levels of nanoparticle of soybean lecithin on cryopreservation of goat spermatozoa

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The purpose of the current study was to evaluate the effects of nanoparticles from lecithin on goat sperm cryo-survival. The semen ejaculates were collected from four fertile goats using an artificial vagina and diluted with nine different extenders. Different concentrations of

soybean lecithin nanoparticles (NL, 1%, 2%, 3% and 4% w/v) were compared to different concentration of soybean lecithin microparticles (L, 1%, 2%, 3% and 4% w/v) and 15% (v/v) egg yolk extender (EY). In order to reduce the particle size in nano-lecithin-based extenders, the samples were sonicated for 30 min. Average diameter of particles size was 94.6 nm. Transmission electron microscopy (TEM) was used to examine the molecular structure. Sperm motility and motion parameters, the percent of normal sperm, plasma membrane integrity and activity, and apoptotic status were determined after freeze-thawing process. The results of this study showed that no significant difference was observed for sperm progressive motility, plasma membrane activity and integrity ($p < 0.05$) in 2% NL media (47.83%, 68.87% and 65.92%, respectively) and EY medium (47.16%, 64.06% and 62.16%, respectively). The results of apoptotic status showed that the highest ($p < 0.05$) percentage of total viability (81.73%) and the lowest percentage of dead sperm (18.26%) were achieved by inclusion of 2% nanoparticle of soybean lecithin into the semen extender. Sperm motion characteristics (VAP, VSL, VCL, ALH, STR and LIN) were improved in 2% nanoparticle of lecithin-based extender compared to EY- and lecithin-based extender. Results suggest that reducing particle size of lecithin in semen extender could improve sperm cryosurvival in goat.

P 225 | The effect of progesterone on the endometrial expression of PRa in the dairy cows

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Progesterone co-ordinate many reproductive events and are critical regulators of development, cell differentiation, morphogenesis and organ physiology. Progesterone autoregulate production of their own receptors (endometrial progesterone receptors-PR, isoforms A and B) and increase the rate of transcription in the response gene as an effect of hormone binding to target tissues. This study determined changes in the abundance of progesterone receptor isoform A (PRa) in bovine endometrium under the influence of exogenous progesterone. Uterine biopsy samples were collected over the anoestrus before intravaginal progesterone administration (progesterone insert, 1.55 g, 7 days) and after 7 days from 25 HF non pregnant lactating cows. The samples were fixed, immunofluorescent stained (IF) using primary (mouse anti-human progesterone receptor 1A6) and secondary (donkey anti-mouse AF 568) antibodies and quantified by scanning cytometry using SCAN^{AR} screening station. From corresponding samples full RNA was isolated, underwent reverse transcription reaction onto cDNA and multiplied using specific paired starters (PGR-a-GAPDH) and QuantStudio Real-Time PCR system. We found significantly lower ($p = 0.001$) mRNA expression (mean \pm SEM) before (0.745 ± 0.061) than after (0.759 ± 0.323) treatment. The PRa expression in tissue before treatment (2.82 ± 0.41) was also significantly lower ($p = 0.003$) than after (6.02 ± 0.81). No

significant correlations between expression of mRNA and PRa were found both before ($Sp=0.20$; $p = 0.34$) and after ($Sp = -0.11$; $p = 0.63$) treatment. Conclusively, a seven days progesterone administration affected progesterone endometrial receptors expression on both mRNA and tissue level. The results confirm the stimulatory effect of progesterone on progesterone receptor isoform A expression.

P 226 | Apoptotic-like changes in boar sperm – new ejaculate selection criteria for cryopreservation

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The aim of the study was to investigate the relationship between the presence of apoptotic-like changes (ALC) in fresh boar sperm and its influence on the effectiveness of cryopreservation. Thirty ejaculates collected from five Maxter boars were frozen in extender supplemented with 1.0 mM butylated hydroxytoluene as we previously described (Trzcińska et al. 2015). Only ejaculates with greater than 80% progressively motile (PM) sperm and 80% morphologically normal spermatozoa were used. The experimental material was divided into: group I (21 ejaculates with $\leq 10\%$ of ALC) and group II (9 ejaculates with $> 10\%$ of ALC) based on fluorescent methods for identification: membrane integrity (YO-PRO-1/PI) and caspase-3 activation (CASP-3) with phosphatidylserine (PS) translocation (Dual Apoptosis Assay Kit). Group I and group II with 3.6 ± 1.1 vs. $24.6 \pm 3.3\%$ of viable sperm with ALC (YO-PRO-1 + /PI-) and 4.4 ± 1.3 vs. $19.3 \pm 2.2\%$ of sperm with simultaneously CASP-3 activation and PS translocation (CASP-3 + /PS+) differed significantly ($p \leq 0.01$; Duncan test). After freeze-thawing percentage of PM sperm was significantly higher ($p \leq 0.05$) in group I (65.7 ± 8.4) compared with group II (49.8 ± 4.3). Moreover, the low level of sperm DNA fragmentation (TUNEL+: $2.1 \pm 1.1\%$) was observed in fresh and frozen-thawed semen. Moreover, the results showed that the suitability of boar semen for cryopreservation should not be solely verified based on assessment of sperm motility. In conclusion, the identification of apoptotic-like changes in sperm may be an additional criterion for selection of ejaculates for cryopreservation. (The research leading to these results received funding from the National Centre for Research and Development under project agreement No.297267/14/2016 (BIOSTRATEG 2).)

P 227 | Associations of cytological endometritis and ill health with fertility and culling risk in multiparous Holstein cows

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To study the effects of ill health (IH) and cytological endometritis (CE) on fertility and culling risk, the health states of 119 multiparous

Holstein cows from a single farm were recorded, and uterine cytology samples at 40 ± 2 days postpartum were collected. The cytological criterion was set at $> 8\%$ of polymorphonuclear neutrophils as the threshold indicator of CE. Milk samples were taken twice weekly for progesterone (P4) measurement by EIA. Resumption of ovarian cyclicity (ROC) was defined as the first measurement of P4 > 5 ng/ml. Cows were divided into four groups according to their health and CE status: 1) IH+ and CE+ (18.5%), 2) IH- and CE- (45.4%), 3) IH+ and CE- (24.4%), and 4) IH- and CE+ (11.8%). The IH- and CE- group was chosen as the reference group. Group differences were tested using Fisher's exact test. Medians were compared with the Mantel-Cox log-rank test. First AI pregnancy rate (PR), 180 days PR and culling rate in IH- and CE- cows were 57.7%, 81.5% and 7.4%, respectively. The CE+ status both in IH- and in IH+ cows was associated with significantly decreased first AI PR (OR = 0.22 and OR = 0.14, respectively), 180 days PR (OR = 0.23 and OR = 0.13, respectively), and significantly increased culling rate (OR = 5.00 and OR = 7.14), respectively. Median intervals to ROC were 33 days, 61 days, 34 days, and 39 days in the IH- and CE-, IH+ and CE+, IH- and CE+, IH- and CE+ groups, respectively. The IH+ and CE- status did not significantly affect 1st AI PR, 180 days PR or culling rate. Only the IH+ and CE+ status significantly prolonged the median time to ROC. The results suggest that the CE+ status in IH+ cows is an important risk factor associated with delayed ROC, decreased fertility and increased culling risk. (Supported by IUT8-1.)

P 228 | Administration of different eCG doses to induce estrus activity in mix-breed dairy goats at the end of the seasonal anestrus under subtropical conditions

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One of the strategies to induce estrus activity in anovulatory goats is the use of equine chorionic gonadotropin (eCG) in a dose of 200 IU. The aim of the present research was to evaluate if reduced doses of eCG are able to induce estrus activity in goats during the seasonal anestrus. A total of 29 mix-breed dairy goats under subtropical conditions (26° N) were randomly located in three experimental groups; goats were balanced for body weight and body condition score. During June (reproductive transition period), the hormonal treatment was administered to each female, the treatment consisted of 25 mg progesterone and, 24 h afterwards, an administration of eCG: 200 IU (T200; $n = 10$), 100 IU (T100; $n = 10$) and 50 IU (T50; $n = 9$); both, progesterone and eCG, administered intramuscularly. After eCG administration, estrus activity was evaluated, using 3 sexually active male goats, which were exposed to the females twice daily, for 7 consecutive days. Analysis of the percentage of females in estrus

considered a contingency chart with the Chi square statistic, while latency and estrus length considered an ANOVA; mean separation considered the Tukey test. No differences ($p > 0.05$) occurred for percentage and estrus length, having a 100% of females in estrus for T200 and T100 and 78% for T50 with an estrus length of 32.4 ± 5.4 ; 34.8 ± 4.2 and; 34.3 ± 9.2 h, respectively. No differences for estrus latency were observed between T200 and T100 ($p > 0.05$; 42 ± 3.7 ; 49 ± 2.8), but differed ($p < 0.01$) with respect to T50 (94.9 ± 10.0). Results of our study demonstrate that reduced doses of 100 and 50 IU of eCG can be efficiently used to induce estrus activity in mix-breed goats with a high percentage of incorporated dairy genes during the ending period of the seasonal anestrus under subtropical conditions.

P 229 | Isolation of male poultry spermatogonia

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Spermatogonia are the precursors of male germ cells. We have elaborated a technique to culture spermatogonia from roosters of different poultry species. High performance separation of spermatogenic cells occurred using the sequential enzymatic treatment of testis tissue with a 0.25% trypsin and 0.1% collagenase solution. Based on histological studies, it was found that the highest possible content of spermatogonia in the seminiferous tubules of the testes was observed at the age up to 5 weeks in chicken males, up to 3 weeks in quails and up to 6 weeks in ducks and guinea fowls. Spermatogenic cell cultures isolated from the testes at a given age was presented mainly by Sertoli cells and spermatogonia. Purification of spermatogonia from other cell types was performed by their separation due to adhesion. After 24 h of culturing spermatogenic cells, the supernatant with non-adhered cells represented mainly by spermatogonia was transferred to a new plate for further culturing. The maximum homogeneity of the cell population was registered while separating cells by three-fold transfer of the supernatant with 24-h interval: the number of spermatogonia in the suspension reached 85%. Moreover, the optimal feeder layer for the cultivation of spermatogonia were their own Sertoli cells. Spermatogonia colony formation was observed on days 3–5 of culturing, depending on the species and the growth medium. (Supported by RSF (16-16-04104).)

P 230 | Induction of bull semen capacitation and hyperactivation

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The aim of this work was to evaluate the possibility to induce Capacitation (C) and Hyperactivation (Hy) of bull sperm in vitro, by adding Heparin supplemented with Epinephrine/Penicillamine/Hipotaurine (HPE) and Procaine respectively, in order to assess whether the bull, time and treatment improve sperm motility parameters and highlight some typical motility characteristics by C.A.S.A. 75 frozen/thawed medium straws, from 5 Piedmontese bulls with proven fertility were submitted to the procedure of in vitro C (2 μ l/ml heparin and 20 μ l/ml of PHE) and Hy (procaine 5 mM). For each work session (25 total), 3 straws (total volume 1.5 ml) from the same ejaculate were thawed in a water bath (at 37°C for 1–2 min) and their content was gently stirred in order to obtain a uniform sample. Variation in the percentage of progressive sperm, LIN, STR, VSL, VAP and beat cross frequency (BCF) were evaluated after thawing (sample 0), after dilution with extender (s1), after C (s2) and after Hy (s3) and after combined treatments (C+Hy) (s4) and then after separation by swim-up in C and Hy sample on the upper (s5, s6) and on the bottom fractions (s7, s8). All evaluations were performed by C.A.S.A., immediately after thawing (T0) and then after 15', 30' and 60' incubation (T1, T2, T3) in the above mentioned solutions. Statistical analysis has shown the successful C, with increase of VSL ($p = 0.00002$) and BCF ($p < 0.05$) in 1, 2, 5 and 7 samples and significant rates (0.00017 to 0.001 and VSL for BCF) is the time relationship for treatment. Statistical analysis has shown the successful Hy with increased VCL ($p = 0.0001$) in 3, 4, 6 and 8 and meaningful treatments (0.0009) relationship/time ratio. It seems possible to induce Hy with procaine, irrespective of C, in bull frozen/thawed semen.

P 231 | Recovery of spermatogenesis in male chickens by transplantation of donor spermatogonia

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Spermatogonia are undifferentiated male germ cells that can be used as a tool for obtaining transgenic and chimeric poultry. The aim of our research was to examine the effectiveness of spermatogenesis recovery in male chickens by transplantation of donor spermatogonia. Shutdown of spermatogenesis in recipients was performed by introducing a solution of busulfan into the adults testes. In 3 weeks after busulfan treatment, donor spermatogonia isolated from the testes of 4-week-old chicken were injected into the testes of 6-month-old recipients ($n = 4$) at concentration of 2 million cells per testis. Optimal concentration of the donor cells and chicken age for obtaining spermatogonia culture were determined experimentally. The effectiveness of conducted manipulations on transplantation of donor spermatogonia was studied using Hoechst 33342 and a comparative microsatellite analysis of DNA extracted from the recipients (blood, sperm) and donors (muscle tissue) cells. Histological studies revealed that an effective concentration of busulfan for removal of own spermatogenic

cells was 80 mg per kg of body weight (Mann-Whitney, $p < 0.01$). After the introduction of donor spermatogonia labeled with Hoechst 33342 into testes of recipients, the presence of fluorescing cells in the seminiferous tubules was observed. The presence of sperm cells in the ejaculate of experimental males was found in 2.3 ± 0.4 months after the introduction of the donor cells. Identical microsatellite profiles were confirmed for recipient sperm DNA and donor muscle tissue DNA, while for recipient blood DNA and donor muscle tissue DNA different profiles were observed. (Supported by RSF, 16-16-10059.)

P 232 | Influence of the progesterone device on the corpus luteum function in prepubertal Nellore heifers

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The aim was to evaluate previous exposure to progesterone (P4) on the function of the corpus luteum (CL) in prepubertal Nellore heifers. Fifty seven females (17–18 months old, BW 289.6 ± 32.3 kg, BCS 5.7 ± 0.6) were randomly divided into two groups: GP4 + GnRH ($n = 29$), which received intravaginal P4 device (CIDR[®]) of 3rd use for 10 days and 48 h after CIDR removal was injected 0.02 mg of busserelin acetate (BA); and GGnRH ($n = 28$), which received only BA. The luteal dynamics were monitored starting 96 h after BA injection, with B-mode and color Doppler sonographic evaluations (US) until detecting the decrease of the luteal blood flow. Blood samples were collected at each US for P4 concentrations. Duration of the luteal phase (DLF) was determined starting 24 h after BA injection until the last day P4 concentrations were >1 ng/ml. Three animals from each group did not ovulate after the treatments. Three CL functions were established: (1) normal duration (ND) – luteal phase ≥ 16 days; (2) premature regression (PR) – luteal phase $<$ than 16 days and; (3) afunctional (AF) – P4 concentration at all evaluations <1 ng/ml. Significant difference were observed for the DLF between groups ($p < 0.0001$) and CL functions ($p < 0.0001$). In GP4 + GnRH were observed 81% (21/26) of CL with ND and 19% (5/26) with PR; while in GGnRH, 4% (1/25) were ND, 44% (11/25) PR and 52% (13/25) AF. This study confirmed the importance of previous exposure to P4 in prepubertal heifers in the quality of the subsequent CL formed. (Financial support: FAPESP (2015/13079-0).)

P 233 | Effect of fractionated seminal plasma on post-thaw boar semen quality

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This study investigated the effects of fractionated seminal plasma (SP) on quality characteristics of boar spermatozoa following

freezing-thawing. Boar sperm-rich fractions (SRFs) were split into three parts and each portion was incubated with chromatographically fractionated SP comprising proteins less than 40 kDa (SP 1) and more than 40 kDa (SP 2), and with whole SP (wSP) for 1 h. Following incubation, the samples were diluted with BTS extender, stored for another 1 h at 17 degrees C and were frozen. No marked differences ($p > 0.05$) in the sperm quality characteristics were observed among the treatment groups in the pre-freeze semen. SP source had a significant effect ($p < 0.05$) on post-thaw semen quality. Total motility of frozen-thawed spermatozoa of SP 1, SP 2 and wSP varied significantly ($p < 0.05$) among the boars and averaged $48.8 \pm 3.6\%$, $48.5 \pm 4.1\%$ and $37.7 \pm 4.6\%$, respectively. Samples supplemented with either SP 1 ($42.5 \pm 3.9\%$) or SP 2 ($40.2 \pm 3.5\%$) exhibited higher ($p < 0.05$) proportions of viable frozen-thawed spermatozoa (YO-PRO1-/PI-) compared with those of the wSP ($29.3 \pm 2.4\%$). Post-thaw sperm mitochondrial function (JC-1/PI) and acrosome integrity (FITC-PNA/PI) differed among the treatment groups. It was found that post-thaw sperm acrosome integrity averaged $42.9 \pm 3.2\%$, $47.0 \pm 2.7\%$ and $29.8 \pm 1.9\%$ for samples supplemented with SP 1, SP 2 and wSP, respectively. The results of this study indicated that pre-cooling of boar semen in fractionated SP, prior to freezing, rendered the spermatozoa less susceptible to cryo-induced damage. (Supported by a NCN project in Poland (2016/21/N/NZ9/02289).)

P 234 | Unilateral cryptorchidism in the dog: the case report

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Cryptorchidism is one of the most common genital defects in dogs. This report describes a case of cryptorchidism that resulted in hind limb lameness and the feminizing syndrome. The 13 years old, male cocker spaniel, weighing 14.7 kg was presented with a history of right hind limb lameness, bilateral skin alopecia and weight loss. An orthopaedic and radiographic examination did not reveal any alteration in the limb. Alopecia was mild, non-pruritic, situated symmetrically on the the ventral abdomen and flanks. The prepuce appeared pendulous. The abdomen was sensitive to palpation. There was no prostatic alteration which is very common in hyper-oestrogenism. Physical examination and ultrasonography revealed a soft tissue mass $15 \times 27 \times 10$ cm within the caudoventral right abdomen. A complete blood count, serum biochemistry panel, and urinalysis were unremarkable. Sertoli cell tumor was suspected in the retained testis, thus an exploratory midline laparotomy was carried out to locate and remove right testis. During the surgery also left testis was removed from scrotum. Neoplastic testis was weighing 1.56 kg. Post-operative treatment included anti-inflammatory drugs for 3 days and antibiotics for 7 days. A few days after an orchiectomy, the lameness was completely resolved. After 1 month, the clinical symptoms significantly improved. Complete remission of all symptoms occurred within 3 months after

orchietomy. Histopathological examination confirmed the presence of Sertoli cell tumors, that were primarily produced the feminizing syndrome. The size of tumor was additionally responsible for lameness, which is not typical in such cases.

P 235 | High incidence of morula and blastocyst formation among nuclear-transferred pig embryos results from epigenetic modulation of peripheral blood-derived fibroblast-like cells

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The current research was aimed to explore the influence of epigenetically transformed or non-transformed adult peripheral blood-derived fibroblast-like cells (APB-FLCs) that provided a source of nuclear donor cells (NDCs) on the extracorporeal development of porcine cloned embryos. Clonal APB-FLC lines were epigenetically modified by exposure to 50 nM trichostatin A (TSA) during 24-h contact inhibition that took place before use of NDCs for somatic cell cloning. Ex vivo-matured oocytes that had been enucleated underwent insertion of TSA-treated APB-FLCs (Group I) or TSA-untreated APB-FLCs (Group II) into their perivitelline spaces. The ooplasts were then electrofused with NDCs and simultaneously activated by applying two consecutive DC pulses of 120 V/mm for 60 μ s. In Groups I and II, 229/257 (89.1%)^a and 205/234 (87.6%)^a oocytes were successfully fused/activated and intended to be cultured, respectively (a, a: $p \geq 0.05$; Chi-square test). Groups I and II yielded proportions of 192/229 (83.8%)^A and 132/205 (64.4%)^B for cleaved embryos, respectively (A, B: $p < 0.001$). The proportions of cloned embryos that reached the morula and blastocyst stages were 168/229 (73.4%)^A and 89/229 (38.9%)^C or 107/205 (52.2%)^B and 56/205 (27.3%)^D in Groups I or II, respectively (A, B: $p < 0.001$; C, D: $p < 0.01$). Cumulatively, the competences of the cell nuclei that had been inherited from TSA-exposed APB-FLCs to support both cleavage divisions and development of nuclear-transferred pig embryos to morula and blastocyst stages were remarkably higher than the competences of those that had been inherited from TSA-unexposed APB-FLCs. (Presented work was financially supported by the National Centre for Research and Development (grant number BIOSTRATEG2/297267/14/NCBR/2016).)

P 236 | Oxidative stress in sperm causes developmental and epigenetic defects during bovine early embryonic development

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Cryopreservation is the standard technique for semen in assisted reproductive techniques. Freezing and thawing might induce oxidative stress, thereby leading to DNA damage. To determine the impact of oxidative stress in sperm during early development, we monitored embryos obtained through in vitro fertilization with H₂O₂ treated sperm. Although the fertilization rate was not affected, we found that oxidative stress in sperm induced an arrest at 2–4 cell stage (Control: 25.33 \pm 4.3, n = 127 oocytes; H₂O₂: 49.33 \pm 0.67%, n = 141 oocytes; P: 0.0054) and a drastic reduction in blastocyst formation (Control: 40 \pm 4.3%, n = 161 oocytes; H₂O₂: 9 \pm 2.7%, n = 180 oocytes; P: 0.0009). Upon fertilization, the paternal genome (pPN) undergoes DNA demethylation. Immunofluorescence (IF) analysis in zygotes and LC-MS in 2-cell stage embryos revealed that the loss of DNA methylation (5 mC) is impaired in embryos generated with H₂O₂-treated sperm (mean 5 mC intensity; control: 3.75 \pm 0.46, n = 24 zygotes; H₂O₂: 5.32 \pm 0.538, n = 26 zygotes; P: 0.0335). IF analysis indicated an accumulation of a key base excision repair (BER) component at the pPN (mean XRCC1 intensity; control: 1.37 \pm 0.07, n = 120 zygotes, H₂O₂: 1.764 \pm 0.07, n = 85 zygotes, p-value: 0.0123). The data suggested that oxidative stress in sperm induces an arrest in early embryonic development due to the impairment of DNA demethylation, a process linked to DNA repair pathways.

P 237 | Effects of inclusion of mucolytic agent to treatment of clinical endometritis in dairy cows

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The study was designed to evaluate the effects of inclusion of a mucolytic agent to treat clinical endometritis in dairy cows and post treatment reproductive status. Sixteen Montbéliard cows between 60 and 70 days after calving with clinical signs of endometritis after unsuccessful intrauterine antibiotic treatment were submitted to a transrectal ultrasonography and ovarian status was determined. The cows were randomly divided in two groups I (no mucolytic; n = 8) and II (with mucolytic; n = 8). According to the ovarian status hormonal therapy for estrus induction was done and estrus detection time was accepted as Day 0 of the treatment. Group I received intrauterine Jodofarm[®] aerosolum spumescens (40 g) on Day 2 and Day 9 and 0.2 g pilocarpine hydrochloride as 5% solution on Day 3 and Day 5. Group II received intrauterine infusion of 80 ml mucolytic (1.5% solution of N-acetylcysteine in saline) on Day 0 and the same treatment as in group I. Clinical treatment rate (percentage of cows with clear uterine discharge) was accounted during the first post treatment estrus and animals with no clinical signs of endometritis were artificially inseminated (AI). Pregnant animals after insemination during the first, the second post treatment estrus and total number of pregnancies were also determined. The results were statistically analysed. The clinical treatment rate and the pregnancies after the first AI were higher in group II than group I (87.5% and 75% vs. 50% and 25%). Statistically

($p < 0.05$) higher total number of pregnancies was observed in group II than group I (100% vs. 50%). In conclusion, supplementation with a mucolytic agent to treat clinical endometritis in dairy cows provided better clinical treatment rate and improved the reproductive performance.

P 238 | The effect of Sat-Som preparation on functional activity of sex glands of male and female animals

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The aim of the research was to study the use of a somatostatin-containing peptide for the normalization of the functional activity of sex glands in begetters with low indices of sperm production and in cows with postpartum ovarian inactivity. The preparation is registered in Russia as a drug for the regulation of metabolic (anabolic) processes in animals. The mechanism of its action is based on the induction of anti-somatic antibody production in animals' organisms, the decrease of somatostatin content and increase of endogenous somatotrophic and follicle-stimulating hormones. The trial included 10 Holstein bulls, 6 Large White boars and 48 Simmental cows. The preparation was subcutaneously injected in a dose of 5 mg of protein per 100 kg of body weight two times with an interval of 14 days. The volume of ejaculate obtained increased by 26.3% in comparison with the initial level, the number of sperm doses – by 25.3% in 45 days after the 2nd injection of the preparation. The average volume of ejaculate in bulls increased by 12% in 60 days, by 28.7% in 90 days, the number of obtained sperm doses – by 31.2%. Testosterone blood concentration increased by 41% in comparison with the initial level. The injection of the preparation provided the recovery of sexual cyclicality and fertilization of 81.8% of animals during 1.5 month in comparison with 38.5% in the control group. Therefore, the nonhormonal preparation Sat-Som can be used in practice for controlling reproductive function of animals, although more studies are necessary to determine the underlying mechanism of action.

P 239 | Associations between farrowing duration, parity and follicular development at the subsequent oestrus in sows

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The study examined the effects of duration of farrowing on preovulatory follicle development of oestrous sows during subsequent insemination. A total of 30 crossbred sows were allocated into two groups

based upon duration of farrowing: 1) SHORT ($n = 14$): between 103 and 192 (average 159 ± 29 min), and 2) LONG ($n = 16$): between 326 and 878 (average 533 ± 190 min). The sows included in the study were also divided into two age classifications: 1) YOUNG ($n = 14$): parity between 1 and 3 (average 2.5 ± 0.8), and 2) OLD ($n = 16$): parity between 4 and 11 (average 6.4 ± 2.3). After a four week lactation period, the sows were confined in stalls. Transrectal ultrasound examinations of the both ovaries were performed at 09:00 and 15:00, starting from three days after weaning until ovulation. The follicle size (diameter in mm) represented means of the five largest follicles of each ovary. Farrowing duration and parity of the sow did not affect the days for weaning-to-oestrus interval and when the five largest follicles reached their maximum size between three days after weaning and ovulation. Follicle diameters of the sows in LONG group tended to be larger than in SHORT group on day four after weaning ($p = 0.06$), while not differed on the other days. The sows in OLD group tended to have larger follicles on day five after weaning, or had larger when the follicles reached their maximum size after weaning until ovulation compared to the sows in YOUNG group ($p = 0.07$, $p = 0.02$). The results indicate that prolonged farrowing and higher parity might be associated with larger size of preovulatory follicles of the oestrous sows. However, further studies are needed to demonstrate causal relationships between farrowing duration, parity and follicular development in the sows.

P 240 | Proliferative potential of transplanted and endogenous cells after MSC transplantation into porcine cervix

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Mesenchymal stem cells (MSC) during in vitro expansion undergo a progressive loss of proliferative potential which reduces the efficacy of the treatment. The aim was to detect the proliferation of MSC after transplantation into the cervix of Polish Landrace sows ($n = 9$). MSC were autotransplanted after 3 weeks in vitro culturing with PKH26 and DID markers. Twenty eight days after transplantation, pigs were neutered, cervixes were collected, fixed and immunofluorescence (IF) labeled with anti-Ki67 antibodies. Then MSC and Ki67 were imaged using confocal and scanning microscopy. Living MSC were found in all transplantation places and where accounted (mean \pm SEM) for PKH26 22.19 ± 3.33 and DID 15.87 ± 5.62 of the cells with no significant differences between both marked-types. Cells positive for PKH26 or DID showed Ki67 staining in nuclei confirming

that PKH26/DID-labeled MSC underwent *in vivo* multiplication in transplantation places. The Ki67 expression was significantly higher in PKH26 (22.28 ± 3.13) and DID (28.87 ± 9.19) positive MSC with respect to controls (13.16 ± 1.94), but with no significant differences between both marked-types of MSC. Ki67 expression was significantly higher in PKH 26 (16.01 ± 2.75)/DID (8.64 ± 2.40) negative cells close to MSC (transplantation places) with respect to control (no transplantation places). MSC survived after transplantation into the muscle layer of the porcine cervix and demonstrated a significantly higher proliferative potential than smooth muscle cells. Endogenous cells (PKH 26/DID negative) in the vicinity of the MSC demonstrated a significantly higher proliferative potential than distant endogenous cells. Transplanted MSC probably stimulate the endogenous cells of the porcine cervical muscle layer via paracrine signaling.

P 241 | Effectiveness of three estrus synchronization protocols in Polish native cattle breeds

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The aim of this study was to compare the effectiveness of different estrus synchronization protocols in Polish Red and Lowland Black-and-White cattle. The study included cows ($n = 80$) from a farm in north-eastern Poland. The animals of the control group A ($n = 23$) were inseminated (AI) during spontaneous estrus within 60 days after parturition (p.p.). Cows in group B ($n = 21$) expressed no signs of estrus by day 50 p.p. After detecting follicles with a diameter of at least 8 mm on ultrasound examination, they were injected with GnRH (buserelin acetate). Seven days later PGF_{2 α} (cloprostenol) was given followed by GnRH another two days later. Eighteen hours after the second GnRH injection AI was performed (Ovsynch). In group C ($n = 19$) PGF_{2 α} was administered twice 11 days apart, after detecting a corpus luteum. Estrus occurred between 2 and 4 days after injection and AI was performed 12 h after the onset of estrus. In group D cows ($n = 17$) a PRID was inserted for 7 days. PGF_{2 α} was administered the day before the device was removed. AI followed 12 h after signs of estrus appeared. All cows were examined for pregnancy between 40 and 50 days after AI. The protocols were evaluated based on the pregnancy outcomes. In group A pregnancy was confirmed in 52.17% of the cows, in group B in 42.85%, in group C in 36.84%, and in group D in 23.52% of the cows. Although there was no statistical difference between the groups ($p > 0.05$), the Ovsynch protocol tended to produce better results than the two PGF_{2 α} injections 11 days apart and the PRID protocol. The results of groups B and C were satisfactory. Results in group D suggested that the PRID protocol was less effective in these breeds, although studies using larger groups are required.

P 242 | Oviductal fluid from different oestrous cycle stages modulate capacitation in boar

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The secretions of oviduct epithelial cells differ with the stage of the oestrous cycle, therefore the effect on sperm function may vary by time of oestrus. Thus, in this work we evaluated if porcine oviductal fluids (POF) from different oestrous cycles stages have any influence in protein kinase A substrates phosphorylation (pPKAs) and tyrosine phosphorylation (pY) in boar. For this purpose, sperm were incubated for 3 h in capacitation medium (TALP) with presence or absence (control) of POF and evaluate phosphorylation by western blot. The immunoblotting analysis of pPKAs and pY indicated that sperm incubated with periovulatory POF decreases significantly the phosphorylation of these residues during capacitation ($p < 0.05$). Addition of periovulatory fluid maintained sperm viability without induction of capacitation. These findings indicate that the particular fluids might delay sperm capacitation, perhaps because of inhibitory proteins. However, POF of the late luteal phase increases pPKAs ($p < 0.05$) and pY was detected as a 38 kDa band, similar to that observed in control. In conclusion, the evidence indicates that POF from different oestrous cycle stages modulates capacitation. (Supported by MINECO-FEDER AGL2015-66341-R.)

P 243 | Effect of social status upon estrus activity in anovulatory goats treated with intramuscular progesterone plus equine chorionic gonadotropin

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To determine if social status affects estrus response in anovulatory goats submitted to a hormonal treatment based on progesterone (P4) plus equine chorionic gonadotropin (eCG), Saanen goats were used. Social status of each female was determined by a behavioral study that consisted in registering agonistic interactions between goats at the feeding periods during 7 consecutive days. With the registration of agonistic interactions, as well as their consequences (win or lose the encounter), the success rate was calculated (SR), which was classified in 3 social statuses: 0 to 0.33 low social status (LST); 0.34 to 0.66 medium social status (MST) and; 0.67 to 1 high social status (HST). According to their behavior, 18, 28 and 14

females were grouped on each social status, respectively. During March, the hormonal treatment (25 mg P4 and, 24 h later, 350 IU eCG, both intramuscularly) was administered. For the percentage of females in estrus, a contingency chart with the Chi square statistic was used, similarly, for estrus latency and length, ANOVA was used. No differences ($p > 0.05$) were observed for estrus latency between groups (LST 57.6 ± 4.66 , MST 69.9 ± 4.32 and HST 68.35 ± 3.15 h) nor for estrus length (31.2 ± 5.12 , 26.4 ± 2.55 and 30.26 ± 3.19 h).

However, percentage of females in estrus was higher ($p < 0.01$) in the HST-goats (96%) regarding to the other two social statuses, with no differences ($p > 0.05$) between the last (LST 56% and MST 76%). Results of our study demonstrate that social status of Saanen goats influences the main variable of estrus activity (percentage of females in estrus), being goats of high social status the ones that achieved a higher success in this variable compared to female goats of lower social statuses.