ABSTRACT

POSTER PRESENTATIONS

P 1 | The presence of light-induced sexually active rams prevents the seasonal inhibition of LH in OVX-E2 ewes

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We determined whether the presence of sexually active rams prevents seasonal decrease of LH plasma concentrations in ovariectomized ewes bearing a subcutaneous implant containing estradiol 17-β (OVX-E2). Control rams were kept under natural photoperiodic variations (n = 6); light-treated rams were rendered sexually active by exposure to 2 months of artificial long days (16 h light/8 h dark) in two sub-groups from Nov 1st (SAR1, n = 3) or Dec 1st (SAR2, n = 3). The first group of ewes (SAR; n = 10) was kept with control rams from Oct 1st to Feb 15th, with SAR1 from Feb 16th to Mar 31st, and with SAR2 from Apr 1st to May 31st. All rams displayed intense sexual behavior. The second group of ewes remained with control rams throughout the study (C; n = 10), and the third group was isolated from rams throughout the experiment (ISO; n = 10). Blood samples were collected weekly from Nov to May and plasma LH concentrations were analyzed and compared by ANOVA and t-test. Plasma LH concentrations were high and did not differ between groups during the breeding season (Nov-Feb; SAR: 2.00 ± 0.34; C: 1.88 ± 0.16; ISO: 1.67 ± 0.51 ng/ml). In contrast, from Mar to May, LH plasma concentration decreased to very low levels in the C and ISO groups (1.30 ± 0.20 and 0.48 ± 0.04 ng/ml, respectively) but were maintained at the same level as during the breeding season in the SAR group (2.30 ± 0.17 ng/ml; $p < 0.001$). In conclusion, the permanent presence of the sexually active rams prevents the seasonal decrease of plasma LH concentration, probably by preventing the seasonal negative feedback of estradiol on LH secretion.

P 2 | Unilateral perineal herniation of a mineralized paraprostatic cyst in a dog, a case report

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Perineal hernia (PH) almost exclusively affects male dogs (intact or castrated). Factors that may predispose to PH include tenesmus, anatomic variation of the pelvic diaphragm and hormonal influence. However, there is a little information about mineralized paraprostatic cyst (MPC) as an underlying cause of PH. A 10 years-old intact male Teckel was referred with a history of tenesmus, dysuria and a mass in the perineal area (PA). Physical examination revealed a unilateral right perineal swelling. Haematological and biochemical analyses showed polycythemia and hyperglobulinemia. Urinalysis revealed haematuria, pyuria and presence of bacteria. Abdominal and PA ultrasound examination showed an enlarged heterogeneous prostate (2.7 × 4 cm) with intraprostatic cysts/abscesses and a mineralized cyst structure with anechoic liquid into PH. A presumptive diagnosis of benign prostatic hyperplasia with intraprostatic and MPC was made. Computed Tomography revealed the same findings. The MPC was drained by perineal access and a perineal herniorrhaphy was performed. Then resection and omentalization of PC by laparotomy and conventional orchiectomy were performed. Marbofloxacin (oral dose of 2 mg/kg once daily) during ten days was administered. The dog recovered uneventfully. Histopathologic study confirmed the diagnosis of MPC with chronic multifocal osseous metaplasia. Uni or bilateral PH can be developed secondary to a prostatomegaly. However, although an inguinal herniation of a MPC has been reported, to the author's knowledge this is the first case of a perineal herniated MPC in the dog. (Head et al 2002, J Am Vet Med Assoc 221:533–5; Vititoe et al 2017, Can Vet J 58:1309–12).

P 3 | Ultrasonic characteristics of uterus and ovaries during estrus and their relationship with pregnancy rate in dairy cows

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The purpose of the present study was the evaluation of characteristics of the reproductive tract using ultrasonography. The focus was laid on follicle size (Mokhtari et al. 2016, Theriogenology 85:747–753) and possible accumulation of fluids in the uterine lumen during estrus, and the effect of these findings on pregnancy rate in dairy cows. The study was conducted on 486 lactating Holstein cows detected in estrous in a large commercial dairy herd in Shiraz, Iran. Transrectal ultrasound was performed at the time of artificial insemination. Reproductive tract characteristics included follicle diameters, presence of corpus luteum in ovaries, thickness, folding and edema of the uterus and intrauterine fluid visualized and scored by ultrasonography. Cows were followed after insemination and their pregnancy rate was determined. The

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effects of ultrasonographic findings were investigated on pregnancy rates. The data were analyzed using logistic regression analyses. Results indicated that pregnancy rate was significantly higher in cows with a follicle size >14 mm (38.8%) compared with ≤14 mm (27.3%) after adjusting for parity of animals, days in milk and mean daily milk production (OR = 1.84, p = 0.005). No association between pregnancy rate and other ultrasound characteristics of the reproductive tract during estrus was observed in this study (p > 0.05). In conclusion, follicle size is positively associated with pregnancy rate of dairy cows in estrus. However, other ultrasonographic findings of the uterus including intrauterine fluid did not show any association with pregnancy rates.

P 4 | Assessing the most effective way for overnight cooling of epididymal dog sperm prior to freezing stored in situ or in extender**

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The cauda epididymis is a good sperm source in case of an unexpected decease or castration. We aimed to elucidate if canine epididymal sperm is better preserved at 4°C within the epididymis or in an extender. Testicles were retrieved after neutering and one epididymis was dissected and cooled for 24 h and the other was flushed, sperm were extended in CaniPlus Chill® at 100 × 10^6 sperm/ml and preserved at 4°C. After 24 h of cooling, the other epididymis was flushed and processed as the former. Samples when then centrifuged, and the obtained pellet was resuspended at 100 × 10^6 sperm/ml in CaniPlus Freeze® medium. Sperm were packed in 0.5 ml straws, cooled at 4°C (1 h) exposed to LN2 vapors for 20 min and plunged in liquid LN_2. Sperm quality was evaluated after thawing (37°C for 1 min). Total motility (TM) was estimated with a CASA system, while viability, mitochondrial membrane potential (MMP) and ROS production were assessed by flow cytometry using SYBR-14/PI, JC-1 and MitoSOX respectively. Results are expressed as mean ± SEM in % comparing epididymal cooling vs. extended sperm; a paired t-test was run to compare treatments. Significant differences were found for viability (52.2 ± 5.0 vs. 42.2 ± 5.7) and high MMP (51.0 ± 4.6 vs. 41.8 ± 5.4; p < 0.05) but not for TM (31.4 ± 5.2 vs. 28.8 ± 8.7) and sperm producing ROS (80.6 ± 6.2 vs. 81.6 ± 2.1). Our study demonstrates that epididymal sperm should be cooled within the epididymis before cryopreservation in order to maintain their fertilization potential.

P 5 | Dosage of iron oxide nanoparticles in selection of Angora buck semen before freezing: preliminary results**

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Nanotechnology has allowed sperm selection in human, bull and boars but its effect on freezability is not yet reported. In our study we aimed to use magnetic iron oxide nanoparticles (np) coated with annexin-V, silica magnetite and pisum sativum agglutinin obtained from Clemente Associates on goat sperm and analysed the effects on sperm parameters after thawing. We used different concentrations of nanoparticles to select Angora buck sperm before freezing and determined the concentration having the best effect. Angora bucks (n = 3) were selected after andrological examination. A total of 9 semen samples were collected and extended with Tris-based extender. Samples were then divided into 3 groups; (1) control (2) 1 μg/ml and (3) 10 μg/ml np selection. Samples in control group were directly placed in 0.25 French straws, equilibrated for 1.5 h, frozen and stored in liquid nitrogen. In groups 2 and 3, samples were treated with 1 or 10 μg/ml of nanoparticles, then processed and stored as the samples in control group. After thawing, the samples were analysed using SCA, CASA. The np treated groups were significantly different from the control group in VCL and VAP (p < 0.05). The median values were: VSL (μm/s) 13.5 ± 2.51, 21.9 ± 3.97, 21.2 ± 3.74; VCL (μm/s) 36.2 ± 3.09, 45.1 ± 6.35, 54.7 ± 5.17; VAP (μm/s) 20.4 ± 2.88, 31.8 ± 6.58, 40.9 ± 4.74, respectively. Median values of TM (%) and PM (%) were: 63.4 ± 4.04, 64.6 ± 5.47, 83.8 ± 6.14; 3.2 ± 1.35, 7.7 ± 8.29, 10.5 ± 2.35, respectively. Although no difference was observed in TM and PM, the median values of these 2 parameters reveal the requirement of further detailed studies. Our preliminary results indicate that 10 μg/ml np may be the better option for selection of buck sperm. To obtain more thorough data on this topic, further analyses are needed.

P 6 | Are FOXL2 mutations involved in mare granulosa cell tumors (GCT)

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Pathogenesis of granulosa cell tumor (GCT), the most common ovarian tumor, is unknown in mares, while in human ovaries a point mutation in the coding region of the forkhead transcription factor FOXL2 (C402G) leading to altered amino-acid sequence (C134W), is linked to adult GCT. Therefore, our study aimed to determine 1) the GCT case prevalence in UK equine practice; 2) FOXL2 localization in GCT samples from 5 mares compared with medium and large antral follicles from 21 control mares; and 3) whether

the mutation identified in human GCT also existed in equine GCT. Data mining of consultation records from 26,019 mares using SimStat v2.6 /WordStat v7.0 (Provalis Research) revealed 812 GCT cases (3%), but with only 11 cases true GCT positive (0.04%). Nuclear expression of FOXL2 was seen in granulosa (GC) and theca interna cells of all control follicle walls (FW), and in GC of cysts and solid areas of GCT. Following DNA extraction from formalin-fixed GCT (10 samples from 8 mares) and control FW (10 follicles from 5 mares), PCR amplification of the region spanning the FOXL2 C→G mutation was carried out before submitting the products for sequencing using an Illumina NextSeq 500. The mutation was detected rarely and thus attributed to sequencing error. However, a second, proximal C→T mutation was identified in 25% of all reads (mean ± SEM: GCT 27.1 ± 4.7, controls 23.5 ± 2.2, $p = 0.5$), yet not predicted to change the protein. In conclusion, we identified a very low prevalence of confirmed GCT in UK practices. While we could not link the specific human SNP to equine GCT in our samples, we did identify a further frequent mutation in the small region amplified. Therefore, further study of the equine FOXL2 gene may identify other mutations with functional significance in GCT formation

P 7 | Bacterial load and sperm quality during storage of cooled stallion semen**

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Both bacterial contaminants in semen and antibiotics (AB) in semen extenders may be detrimental to sperm quality. We therefore determined the effects of bacteria and AB on sperm quality during cooled storage. Semen from six pony stallions (n = 18 ejaculates), extended in EquiPlus extender with or without AB, was processed by Single Layer Centrifugation (SLC). Pellets were resuspended in the appropriate extender and aliquots of uncentrifuged and SLC samples were sent for culture and bacterial identification by MALDI-TOF mass spectrometry. Sperm quality (motility, viability, chromatin integrity, mitochondrial membrane potential and reactive oxygen species production) was evaluated after 96 h. Pearson correlations were analysed between bacterial load (BL) at 0 h and sperm quality at 96 h. The BL ranged from 1.1×10^5 (cfu/mL) to 4.2×10^6 (cfu/mL) and 5.1×10^5 (cfu/mL) to 1.4×10^7 (cfu/mL) in controls with and without AB respectively, and from 1.1×10^4 (cfu/mL) to 7.4×10^5 (cfu/mL) and 2.1×10^4 (cfu/mL) to 3×10^6 (cfu/mL) for SLC-selected samples with and without AB, respectively. Negative correlations were seen between BL and total motility ($r = -0.258$), live hydrogen peroxide negative spermatozoa ($r = -0.322$), live superoxide (LS) negative spermatozoa ($r = -0.29$), LS positive spermatozoa ($r = -0.25$) ($p < 0.05$ for all). A positive correlation was seen between BL and chromatin damage ($r = 0.275$, $p < 0.05$) and a trend towards significance between BL and membrane integrity ($r = -0.22$, $p < 0.068$). In conclusion, bacterial contamination of stallion semen has a negative effect on some aspects of sperm quality. Preparing the semen by SLC reduced the bacterial contamination considerably.

P 8 | A comparative analysis on post-thawed quality of ram sperm stored in three different freezing media

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The aim of this study was to evaluate the effect of three different cryoprotectants: soybean lecithin (SL), butylated hydroxytoluene (BHT) and powdered egg yolk (PEY) used for ram semen cryopreservation. Briefly, ejaculates from eight sexually matured males were collected by artificial vagina twice a week (two ejaculates/male/day). Fresh ejaculates were immediately pooled, centrifuged twice at 600 g for 10 min and diluted in three different Tris-based media containing 15% PEY, 1% SL or 0.6 mM BHT. Thereafter, all diluted samples were refrigerated for 4 h at 5°C before freezing in liquid nitrogen. After post-thawing, sperm quality was determined by flow cytometer via a quadruple staining technique with the following fluorescence probes: SYBR-14 and Propidium Iodide for plasma membrane integrity (viability), Phycoerythrin-Peanut Agglutinin (PE-PNA) for acrosome integrity and Mitotracker deep red for mitochondrial activity. All data were analyzed (mean ± SE, n = 6) using the Statistical Analysis System software JMP (SAS version 10). Results showed significant differences ($p \leq 0.05$) among the extenders, with SL having higher values on sperm viability (59.49 ± 2.05^a) than PEY (36.11 ± 2.13^b) and BHT (22.81 ± 0.66^c) and lower total acrosome damage (22.6 ± 1.73^b) compared to BHT (39.52 ± 2.29^a) or PEY (45.65 ± 1.45^a). However, viable sperm with mitochondria activity was significantly lower in SL (1.5 ± 0.82^c) compared to BHT (21.79 ± 2.66^b) or PEY (35.05 ± 2.25^a). In conclusion, SL extender had a negative effect on mitochondrial functionality. (Supported by INIA (RZP2014-00001-00-00).)

P 9 | Seminal plasma does not upregulate the anti-inflammatory IL-10 gene in the oviductal sperm reservoir of pigs

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Boars deliver fractionated ejaculates containing both spermatozoa and seminal plasma (SP). SP is rich in exosomes, proteins, miRNAs

and cytokines, all able to signal functional modifications of the female immune system before fertilization. We studied the effect of sperm-free SP deposition (AI) on the gene expression of the anti-inflammatory cytokine IL-10 at the functional peri-ovulatory oviductal sperm reservoir (UTJ). UTJ-samples were surgically removed from 20 sows 24 h after AI with either the whole ejaculate (SP-Ejac, n = 4) or solely from the sperm-peak fraction (SP-P1, n = 4). Mating and P1-insemination (P1-AI) served as a positive controls (n = 8) and AI of BTS was used as negative control (n = 4). Global transcript analysis was done using microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix). The data were normalized (Robust Multiarray Average) and analysed with the Transcriptome Analysis Console (RMA-method, −1 > fold changes >1, $p < 0.05$) and biological processes, particularly immune process-related, were identified by using PHANTER. As expected, IL-10 gene expression in the UTJ was up-regulated in the positive controls (sperm-bearing mating and P1-AI) but remained statistically unaffected by the infusion of sperm-free SP. The findings highlight the relevance of the in vivo colonization of the UTJ for the tolerance of a restricted sperm number ensuring fertilization. (Supported by FORSS (745971) and The Swedish Research Council FORMAS (2017-00946), Stockholm, Sweden.)

P 10 | A new device for intrafollicular oocyte transfer in cattle**

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The aim of the study was to develop and verify a new device for intrafollicular oocyte transfer (IFOT) in dairy cattle. Three instruments for IFOT were developed: aspirator, applicator and injector. An aspirator is a micromanipulator for oocyte aspiration into an applicator. An applicator is composed by an insulin syringe, adapter and disposable needle (25G, 0.55 × 40 mm). A shortened semen straw was inserted into the stainless steel adapter to eliminate any dead space. The injector is an instrument that allows the attachment of the applicator and movement of the syringe piston by the pulling rod. The content of the applicator is injected into the preovulatory follicle under sonographic control. The function of the instruments used for IFOT was firstly verified under laboratory conditions. Oocytes derived from abattoir ovaries were aspirated into the instruments. Then the content of instruments was injected to the Petri dishes to find out if all oocytes left the needle and straw. Recovery rates in vitro ranged from 89.4% to 97.5% according to the study conditions. Synchronized Holstein heifers were used for in vivo test. Intrafollicular injection of saline (n = 9) was performed to find out whether ovulation was affected by the injection. Then, IFOTs of PBS with 20 oocytes (n = 19) were performed into the preovulatory follicles and followed by 7 days old embryos collection. Total ovulation rates were 85.7% (24/28). Total recovery rates (oocytes+embryos) were 26.1%, embryo recovery rates were 12.3%. The new instrument allows to perform IFOT by one person, however the method needs further investigation.

P 11 | A preliminary study of instrumental alternatives on the ram sperm cryopreservation process under field conditions

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The sperm freezing in nitrogen vapours is a commonly used methodology (affordable and simple) but little studied. The aim of this study was to assess the safety limits from the distance between the sperm straws and the liquid nitrogen, assessing the sperm function after thawing. Three freezing heights were assessed (1, 2.5 and 5 cm). Ten ejaculates from 5 mature Assaf breed males were used in this work. Each ejaculate was diluted 1:1 (TesTris-Fructose-Egg yolk-Glycerol) and cooled to 5°C. Once at this temperature, the samples were diluted down to a final concentration of 100×10^6 sperm/ml. Then the samples were packed in 0.25 ml French straws and frozen in nitrogen vapours following the 3 different heights previously described. After thawing, the viability with intact acrosome (VIA) and mitochondrial activity (MA) were assessed by flow cytometry. After thawing, 1 cm showed the worst results, being significant lower ($p < 0.05$), not only in VIA (41.8 ± 1.7%) but also in MA (28 ± 1.5%) compared with 2.5 cm (VIA: 48 ± 1.6%; MA: 33.6 ± 1.5%) and 5 cm (VIA: 44.6 ± 1.7%; MA: 33.3 ± 1.3%). This data suggests that 1 cm could be a very aggressive freezing ramp, finding the safety height in 2.5 cm. Further investigation should be carried out in order to assess whether this lower viability and mitochondrial activity after thawing in those samples (1 cm) can cause a lower fertility rate respect the other ones (2.5 and 5 cm). (This work was supported by (AGL2017-83098-R).)

P 12 | Significance of dairy cow γδT lymphocytes in regulatory mechanisms

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Regulatory lymphocytes (Treg) are a phenotypically heterogenic group of cells responsible for controlling the functions of the immune system. Treg $CD4^+$ $CD25^{high}$ $Foxp3^+$ lymphocytes are characterised by the ability to inhibit the immune response and they take part in controlling the inflammatory response in infectious diseases in humans and rodents. In cows, the regulatory function of these cells is debatable. Bovine lymphocytes produce genus specific surface particles BoWC1 – BoWC15. These lymphocytes can be activated by the TCR and WC1 receptors. This does not preclude the

possibility that in cows the $CD4^+$ and $CD8^+$ lymphocytes may possess regulatory properties. It has been proven however, that the Tγδ ($WC1.1^+$, $WC1.2^+$) lymphocytes as well as $CD14^+$ monocytes may perform regulatory functions. A study was carried out on 30 Polish Holstein-Friesian cows. Animals were characterized by elevated somatic cell count (>400 k/ml) and negative results of bacteriological milk examinations. The control group consisted of 10 cows, which in two subsequent tests were characterized by lks <100 k/ml and also with negative results of bacteriological milk examinations. At the same time blood samples for lymphocyte immunophenotype evaluation were taken. The $CD4^+$, $CD8^+$, $WC1^+$ Tγδ, CD335, $CD14^+$ and T $CD4^+$ $CD25^{high}$ (FACSVerse/ BD- Biosciences- US) subpopulations were evaluated. Full recognition and understanding of action mechanisms of those cells in cows in significant way explains the immune system activity control mechanisms, whereas a pharmacological or immunological modification of their function may, in the future, serve as a form of immunotherapy.

P 13 | Effect of humic substances on the quality of ram sperm**

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The aim of the study was to research the influence of the feed additive "Humapol", which includes humic substances, on the quality of ram sperm. Therefore, four groups of rams were formed: two experimental groups – rams of Romanov breed (GR1, n = 5) and rams of catadin breed (GC1, n = 5), which were fed with "Humapol" in a dose of 10 g/head three times a week for 60 days; and two control groups (GR2, GC2, n 1,2 = 5), which received the basic ration. Objective was to assess the density and activity of sperm, the concentration, intensity of breathing of sperm, based on the rate of reduction of methylene blue and determined the percentage of alive spermatozoa. As a result of the studies, we found that in the GC1 the activity of spermatozoa was on average 3.85% higher than in the GC2 group and averaged 9 points. The intensity of the oxidation-reduction processes in the sperm of the rams of the GC1 (8.1 min) was 29% higher than in the GC2 (11.5 min), which is confirmed by a higher concentration of sperm. In the GC1, the concentration was 3.87 billion/ml, and in the GC2 2.87 billion/ml. The number of live spermatozoa in the GC1 was also higher than in the GC2 and was 92%. In the GR1, the activity of spermatozoa was 8.6 ± 0.55 points. The intensity of breathing in this group was higher by 42%, which is explained by a higher concentration and a high percentage of live spermatozoa, which amounted to 3.68 ± 0.14 billion/ml and 92.34 ± 3.12%, respectively. Thus, during our studies it was found that feeding the feed additive "Humapol" in a dose of 10 g/head, three times a week for 60 days has a positive effect on the quality of sperm of rams.

P 14 | Ultramicroscopic finding of ovine spermatozoa after cryopreservation

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In the current study, ultramicroscopic morphological lesions have been analysed in ovine spermatozoa subjected to different cryopreservation protocols, concentrations of sucrose and maintenance temperatures before vitrification. For this purpose, the ejaculates were diluted in Tris-based extender and 4 aliquots were prepared (300×10^6 spz/ml). The mentioned aliquots were evaluated a) after the semen collection, b) after conventional freezing, c) after vitrification of samples maintained at room temperature (22°C), and d) after vitrification of samples maintained at 5°C. Samples were diluted (1:2) in Tris-based extender + 2% bovine serum albumin (control group) or with the same components supplemented with sucrose (0.2 M, 0.3 M and 0.4 M, final concentrations). After thawing, morphological changes of spermatozoa were evaluated and described by scanning electron microscopy and transmission electron microscopy. In addition, we also assessed the dimensions of area, length and width of spermatozoa head. Unidirectional variance analysis (ANOVA) and Duncan Post Hoc test was used as statistical method. The results point out that the cryopreservation processes decrease the dimensions of area, length and width of spermatozoa head. The maintenance of spermatozoa at 5°C prior to vitrification and the use of 0.4 M sucrose pointed out lower dimensions of area, length and width than the rest of the groups. It could be hypothesized that greater intracellular fluid loss during vitrification could prevent damages in the spermatozoon throughout the reduced ice crystals formation. This is the first ultramicroscopic study in ovine vitrified spermatozoa, and further studies are needed in order to improve sperm quality.

P 15 | Effect of time of year over the seminal characteristics in quarter mile horses

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The aim of this study was to evaluate the effect of seasonality over seminal characteristics of male horses. The study included sexually experienced Quarter Horse stallions (n = 9; 7 ± 0.8 years) with a body condition score (BCS) of 3 ± 0.7 (scale 1-5) and live weight (LW) of 500 ± 0.9 kg, with good physical and health condition and similar management and housing conditions. The study was conducted in 2015, throughout two seasons: winter (January-February)

and summer (June-July). The offered diet consisted of dry alfalfa hay and a commercial concentrate (CP=12%) with free access to water, minerals and shades. In order to extract the samples, a Colorado artificial vagina model was used. Response variables included sperm motility (semen was assessed initially for wave motion with a score ranging from 0 (nonmotile) to 5 (highly motile)), spermatic concentration, scrotal circumference and ejaculate latency. The data was analyzed through the general linear models with repetitions across time (SYSTAT program). The response variables were higher ($p < 0.05$) in summer than winter, with respective values of: (i) sperm motility (3.3 ± 0.3 vs. 1.0 ± 0.57 units), (ii) sperm concentration (146.66 ± 26.03 vs. 66.43 ± 16.13 mill/ml), 3) scrotal circumference (42.00 ± 0.57 vs. 39.17 ± 0.72 cm). The response was higher ($p < 0.05$) in winter than summer for: 4) ejaculate latency (183.0 ± 16.1 s vs. 130.3 ± 26.4 s). The obtained results demonstrate seasonal long days effect upon semen quality characteristics, with improved seminal parameters during summer; such information should be of practical importance in equine reproductive practices.

P 16 | Effect of L-2-Thiohistidine on goat semen cryopreservation: preliminary results

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The aim of the study was to investigate the effect of L-2-Thiohistidine (L2TH) on goat semen cryopreservation. Briefly, goat ejaculates were collected and seminal plasma had been separated by centrifugation. Then, pellets were diluted with skim milk based extender (skim milk containing 10% egg yolk and 5% glycerol) with 0 (control), 0.25, 0.5, 1, 2.5 or 5 mM L2TH. Diluted semen was loaded to 0.25 ml straws. Straws were equilibrated (5°C/2 h) and frozen (−120°C/15 min in liquid nitrogen_LN vapor) and stored in LN. Total of 12 replicates had been performed throughout the study. However, only straws from 3 replicates were thawed (37°C/1 min) and post-thawing sperm parameters (motility, live sperm, membrane integrity, acrosome integrity and abnormal sperm percentages) were obtained to date. Motility percentages was higher in 5 mM L-2-TH (45 ± 8.1%) and lowest in control (35 ± 4.0%). Highest acrosomal integrities were determined in 0.5 and 1 mM L2TH (79.3 ± 4.5 & 79.4 ± 4.7%, respectively), while 2.5 mM L2TH (65.2 ± 8.1%) had lowest acrosomal integrity. Highest abnormality was determined in 2.5 mM L2TH (38.3 ± 6.7%) and lowest abnormalities were determined for 0.25 and 1 mM L2TH (22.3 ± 5.8 & 23.6 ± 4.6%, respectively). Highest membrane integrity percentage was determined in 5 mM L2TH (60.4 ± 0.9%) while lowest percentage was determined in 0.25 mM L2TH (51.5 ± 1.0%). It was concluded that different doses of L2TH may beneficially affect freezability of goat semen. Nevertheless, all replications results must be considered and future studies should be carried out in order to determine the exact effect of L2TH usage on cryopreservation of goat semen. (This research has been supported by Kafkas University Scientific Research Projects Coordination Unit. Project Number: TS-51, Year: 2017.)

P 17 | Maternal nutritional restriction modulates placental VEGF inmunolocalization and fetoplacental development in the rabbit

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Maternal nutritional disorders during pregnancy can modify placental vascularization and therefore, fetal development. The aim of this work was to assess the effect of a feed restriction of 60% of the nutritional requirements during pregnancy on fetoplacental development, placental efficiency (fetal/placental weight) and expression of vascular endothelial growth factor (VEGF) in the rabbit. Multiparous pregnant rabbits were fed ad libitum (C; n = 17) or food restricted (R021, n = 25) from day (D) 0 to D21. On D28, 11 dams were euthanized to study fetoplacental features and the rest (n = 31) were used to assess perinatal survival and birth weight. Immunohistochemical studies of paraffined placentae (ABC method) was performed for VEGF. No differences were found in the number of viable fetuses between C and R021 groups (11.6 ± 2.9 vs. 12.0 ± 2.0). However, fetuses of C group showed higher size (thoracic diameter: 20.5 ± 2.8 vs. 19.1 ± 1.8 mm, occipital-nasal length: 29.0 ± 1.4 vs. 27.9 ± 1.4 mm); total weight (39.2 ± 7.3 vs. 34.7 ± 5–9 g), and separated head and body weights (9.1 ± 1.5 vs. 8.1 ± 1.1 and 29.3 ± 6.0 vs. 25.6 ± 4.8 g, respectively) than R021 ($p < 0.05$). Placental efficiency was lower in R021 than in C group ($p < 0.05$). VEGF was mainly immunolocalized in endothelial cells in labyrinth zone in both groups being slightly intensive in C group. Mean number of born alive per doe (10.4 ± 3.0 vs. 12.3 ± 3.1) and their weight (59.1 ± 9.8 vs. 56.4 ± 6.8 g) were similar between groups. In conclusion, maternal feed restriction seems to modulate VEGF expression, placental efficiency and fetal development in the rabbit. These effects were not reflected in a low body weight at parturition. (Funds by AGL2015-65572-C2.)

P 18 | Administration of GnRH on day 23 post AI enhances plasma progesterone, embryonic survival, and herd fertility in lactating Nili-Ravi buffaloes

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The objective of the present study was to determine if administration of GnRH at Day 23 post AI enhances plasma progesterone

(P4), embryonic survival, and herd fertility in Nili-Ravi buffaloes (n = 129). Lactating, cyclic buffaloes with mixed parity were synchronized using CIDR-GnRH protocol and timed artificial insemination (TAI) was performed (Day 0). Buffaloes that did not ovulate (n = 18) or returned to estrus (n = 9) were excluded from the study. At Day 23, the treatment group received GnRH (n = 53) while control group was administered normal saline (n = 49). Plasma P4 was quantified on Days 23 and 30 posts TAI using radioimmunoassay. Serial ultrasonography was conducted in each buffalo to monitor the pregnancy rate and embryonic and fetal losses at Days 30, 45, and 60 post TAI, respectively. Results revealed that plasma P4 (means ± S.E.M) was higher ($p < 0.05$) at Day 30 (8.9 ± 0.4 ng/ml) than Day 23 (5.0 ± 0.5 ng/ml) in GnRH-treated buffaloes. It was not different ($p > 0.05$) in the control group (4.8 ± 0.3 vs. 4.9 ± 0.2 ng/ml). Likewise, the mean plasma P4 level in pregnant buffaloes was higher ($p < 0.05$) at Day 30 in GnRH-treated (10.4 ± 0.2 ng/ml) as compared to control group (6.7 ± 0.1 ng/ml). The pregnancy rate at Day 30 was higher ($p < 0.05$) in GnRH-treated as compared to control buffaloes (75% vs. 53%). The embryonic losses at Day 45 were lower ($p < 0.05$) in GnRH-treated than control buffaloes (13% vs. 33%). However, fetal losses at Day 60 post TAI did not differ ($p > 0.05$) between both groups (6% vs. 11%). Therefore, it is concluded that administration of GnRH at Day 23 not only increases plasma P4 but also enhances the herd fertility by reducing embryonic losses in lactating Nili-Ravi buffaloes.

P 19 | Expression profile of Toll-like receptor 7 in the ovine corpus luteum during prostaglandin $F_{2\alpha}$-induced luteolysis

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The aim of this study was to elucidate the expression profile of Toll-like receptor 7 (TLR7) in the ovine corpus luteum (CL) during prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteolysis. For this purpose, $PGF_{2\alpha}$ were injected to ewes on day 12 of the estrous cycle and CLs were collected at 1 h (PG1 h; n = 4), 4 h (PG4 h; n = 4), and 16 h (PG16 h, n = 4) after injection. For control groups, the CLs were collected from cyclic ewes on days 12 (C12, mature CL, n = 4) and 16 (C16, regressed CL, n = 4). Quantitative polymerase chain reaction (qPCR) was used to evaluate the expression profile of TLR 7 while in situ hybridization and immunohistochemistry were used to define the spatial localization of TLR7 mRNA and protein in the CL. Expression of TLR7 mRNA was significantly increased at both PG16 h and regressed groups (C16, $p < 0.05$). Although, in situ hybridization failed to detect TLR7 mRNA expression at C12, prominent staining for TLR7 mRNA was detected both endothelial and luteal cells at PG16 h. Similarly TLR7 protein was particularly localized in endothelial cells on C12, but prominent signals corresponding to TLR7 was detected in luteal cells at PG16 h. The results suggest an involvement of TLR7 in the luteolytic mechanism in ovine CL, as indicated by differential expression level of TLR7 during $PGF_{2\alpha}$-induced luteolysis. Moreover, the present study indicates that TLR7 is more important during the structural stage of luteolysis.

P 20 | Relationship between biochemical parameters of equine semen plasma and motility before and after freezing

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The aim of the research was to study relationship between biochemical parameters of equine semen plasma and motility before and after freezing. Ejaculates (n) from 21 stallions were collected during the breeding season (February-May). Each ejaculate was divided into two parts: one part was frozen following a freezing protocol of Russian Institute for Horse Breeding, and the other part was centrifuged at 600 g for 15 min to get seminal plasma free from spermatozoa (SPF). The concentrations of total protein, albumin, glucose, urea, total calcium, phosphorus were determined in SPF by analyzer "ChemWell 2902V". Motilities were measured by eyes. Data was processed by cluster analysis (K-means). As a result, the data was divided into 3 clusters. There was the lowest motility both before freezing (less than 40%) and after freezing (less than 20%) in cluster one (C1) (n = 9).The motility before (higher than 50%) and after freezing (higher than 35%) did not differ much in the second (C2) (n = 4) and third (C3)(n = 8) clusters. There was a high glucose (mean ± SD) 6.7 ± 6.8 mM in C1 vs. 1.0 ± 1.04 and 2.1 ± 1.5 mM in C2 and C3, respectively. C2 was characterized by a higher protein, urea and calcium, and C3- by larger phosphorus. High glucose in semen plasma and low motility may be due to low mitochondrial activity, as glucose is a substrate for oxidative phosphorylation. (Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01109 (collection and evaluation of sperm, biochemical parameters), the Federal Agency for Scientific Organizations (FASO Russia), development program of Bioresource collections "Cryobank of genetic recourses All-Russian Research Institute for Horse Breeding" and project No. AAAA-A18-118021990006-9 (cluster analysis).)

P 21 | Impact of the use of large-scale embryo transfer programs in the increase of inbreeding and relativeness in the Argentinean Polo horse**

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The Argentine Polo horse is outstanding among equine breeds of Argentina. Its phenotype was strongly selected for sport for decades which resulted in an excellent recognition of this breed worldwide. The breeding program largely depends on the use of embryo transfer and associated technologies, with almost 70% of registered offspring resulting from this technique in recent years. The objective of this study was to compare the genetic variability of individuals produced by embryo transfer or natural mating, using the pedigree data. The information was obtained from horses registered by the studbook of Argentinean polo horses in 2010–2015. The population was divided into two groups of 10259 and 6341 individuals, produced by either ET or NM. The analysis included the estimation of different population parameters such as generation interval, inbreeding coefficient (F), average relatedness (AR) and effective number of founders (Fe) and ancestors (Fa) in both groups. The generation interval was lower in the ET group compared to NM (8.8 vs. 9.4 years, respectively). The values of F and AR were 22% and 84% higher in the ET group ($p < 0.001$), while Fe was 38 and 377, and Fa 39 and 337 for TE and NM, respectively. The parameters indicate that the TE group shows a progressive reduction in variability, attributable to the generalized use of embryo transfer. Assisted reproductive technologies can enhance genetic improvement by increasing the contribution to the gene pool of superior animals and by shortening the generation interval. However, lack of breeding programs as in the study population, can result in increased degrees of inbreeding which may finally lead to genetic impairments.

P 22 | Free-radical oxidation evaluation in Saanen goats

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The aim of this research was to study free-radical oxidation and antioxidant system markers in pregnant Saanen goats. The research was conducted in North-Western region of Russian Federation and in biochemistry and physiology department laboratory of FGBOU VO "SPbSAVM" on Saanen goats. The experimental group included 30 pregnant Saanen goats, 1–4 years old, picked using matched pairs method; control group included 30 non-pregnant Saanen goats, same age. The blood samples were taken 5 times, each month during pregnancy. The levels of lipid peroxygenation markers (MDA, diketone and conjugated dienes) and SOD and catalase activity were assessed by standard methods. During pregnancy the lipid peroxygenation markers were: in the 2nd month MDA 2.15 ± 0.2 mM ($p < 0.05$); in the 3rd month diketone 0.075 ± 0.005 U ($p < 0.05$); in the 4th month diketone 0.1 ± 0.005 U ($p < 0.05$); in the 5th month: MDA 4.5 ± 0.15 mM ($p < 0.05$), conjugated dienes 0.24 ± 0.01 U ($p < 0.05$). During the first 4 months of pregnancy compensatory stage of oxidative stress was also shown by increased catalase activity from 1.45 ± 0.05 U to 3.5 ± 0.035 U ($p < 0.05$), SOD activity increased from 14.8 ± 1.89 U/min to 30.5 ± 3.0 U/min ($p < 0.05$). The 5th month of pregnancy was marked by lipid peroxygenation markers continuously increasing and a reduction of the activity of oxidation preventive enzymes. The catalase activity had a downward trend (to 3.11 ± 0.025 U), SOD activity reduced to 21.5 ± 2.11 U/min ($p < 0.05$), this indicated the decompensation stage of oxidative stress. Our study results describe the free-radical oxidation activation during pregnancy in Saanen goats. During the last month of pregnancy the oxidative stress was decompensated, which should be taken into consideration and be reduced.

P 23 | Giant vaginal cyst in female sphinx cat – a case report

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A 3.5-year-old regularly cycling female Sphinx cat after multiple matings in previous heats has never been pregnant. The cat was presented to the clinic in good clinical condition. CBC revealed slight leucocytosis (19.600 G/l). Hormone levels were: estradiol – 87 pg/ml and progesterone – 14.2 ng/ml. The ultrasound showed large (27 × 63 mm), thick-walled (2–3 mm) cyst deriving from vagina and cystic endometrial hyperplasia (CEH) with lack of intrauterine fluid. An ultrasound guided needle aspiration of the fluid was performed. Ten ml of dense purulent fluid was removed. Bacteriological culture revealed no aerobic nor anaerobic growth. Because of the high breeding value of the cat, a decision of conservative treatment was made. Aglepristone was given on day 1, 2, 7, 14 in dose of 15 mg/kg and tolfenamic acid (4 mg/kg) for 5 consecutive days. Unfortunately, the patient showed no improvement. A decision of surgical treatment was made. The procedure revealed the presence of a subserous cyst with local adhesions to the urinary bladder and the left ureter, scar tissue in the uterine cervix, significant hyperplasia in both uterine horns, well-visible ipsilateral oviducts with a diameter of 2–3 mm and ovaries with the presence of single corpora lutea and multiple small follicles. Because of the severity of the pathologies an ovariohysterectomy was performed. Histopathological investigation showed chronic hyperplastic inflammation of tubal mucous membrane, CEH and adhesions in the cervical lumen. The source of infertility in this queen was not clear, however, the type of changes suggests a hormonal background while the described cyst seems to be a congenital defect.

P 24 | Pregnancy-Specific Protein B (PSPB) concentration in different breeds of pregnant ewes

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Early pregnancy diagnosis has economical benefits in sheep production. Detection of the ovine PSPB (oPSPB) concentration from blood 35 days after AI is possible by ELISA test. Earlier studies indicated that fetal numbers could correlate to serum oPSPB concentration. The aim of our study was to detect differences in oPSPB concentrations in different sheep breeds commonly delivering single or twin lambs. BioPRYN® ELISA assay kit was used for detection of pregnancy in experimental animals. Sixty-four ewes of three breeds (British Milksheep, n = 15; Lacaune, n = 29; and Transylvanian Racka, n = 20), each from different farms in Hungary were included in the study. British Milksheep (BM) and Lacaune (L) ewes were artificially inseminated (AI). The Transylvanian Racka (TR) flock was natural mated over a six-week period. Thirty-five days after AI (or at the end of the mating period), ultrasound pregnancy check was used for confirming pregnancy. All pregnant ewes were bled and serum samples were assayed by the BioPRYN test. Twin lambing rate was over 70% in BM and L ewes, but no twin delivery was recorded in TR ewes. No significant differences of oPSPB concentrations were found among the 3 breeds (BM: 3.23 ± 0.19 ng/ml, L: 3.09 ± 0.8 ng/ml and TR: 3.17 ± 0.27 ng/ml). Detection of serum oPSPB by ELISA technology is an accurate method for ovine pregnancy testing (Karen et al. 2001, Acta Vet Brno, 70:115–126). Although twin lambing rate was highly different, just some non-significant breed differences were detectable in serum oPSPB concentrations in pregnant ewes. (This research was supported by FM (theme code: TNATEJ).)

P 25 | The expression of hsp70 on mRNA and protein levels in cattle embryos co-culture with BOECs and culture in KSOMaa at elevated temperature after activation of embryonic genome

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Heat shock protein 70 (HSP70) is one of the proteins responsible for the protection of embryos from the effects of heat stress. The objective of this study was to determine the influence of elevated temperatures (40.5°C and 41°C) on hsp70 gene expression at both mRNA as well as protein levels in embryos co-cultured with bovine oviduct epithelial cells (BOECs) and cultured in synthetic medium (KSOMaa) from activation of the embryonic genome. Zygotes obtained in vitro were co-cultured with bovine oviduct epithelial cells (BOECs) to the 8-cell stage (72 h post fertilization) at control temperature (38.5°C) and from 8-cell to blastocyst stage (168 h post fertilization) both at control (38.5°C) and elevated temperatures (40.5°C and 41°C). After 168 h, cattle embryos co-cultured with BOECs and embryos cultured in KSOMaa at control (38.5°C) and experimental temperatures (40.5°C and 41°C) were used for analysis of hsp70 mRNA level by RT-PCR, and protein levels by Western Blot, methods. At the control temperature of 38.5°C, the level of hsp70 gene expression at both mRNA and protein levels was significantly higher than at elevated temperatures (40.5°C and 41°C) independent of system culture ($p < 0.001$). However, at the control temperature of 38.5°C and elevated temperatures (40.5°C, 41°C), the level of hsp70 gene expression at both mRNA and protein levels was significantly higher in embryos co-cultured with BOECs, compared to embryos cultured in KSOMaa ($p < 0.001$). In conclusion, elevated temperature has a negative influence on hsp70 gene expression in cattle embryos after activation of embryonic genome. However, BOECs stimulate embryos to switch on their defence mechanisms to a significantly higher level than in embryos cultured in KSOMaa. (Financed by COST 453/N-COST/2009/0)

P 26 | Repeated low doses of buserelin in anovulatory anestrus cows develop ovarian follicles and can be used as treatment method

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The aim of the study was to evaluate follicular development and fertility after treatment of anovulatory anestrus dairy cows using repeated low doses of GnRH. There were 46 healthy cows (36 in treatment and 10 in control groups) included into the study, 50–60 days after parturition without previously observed heat with follicles ≤5 mm and without corpus luteum. They were examined by ultrasound to control uteri and the ovaries within standard herd health visits. Next they received 0.0042 mg (1 ml) of buserelin (Receptal, MSD, Poland) i.m. for 5 days 24 h apart. Cows from control received no treatment. From that point on all cows were examined every week to control follicular development. Cows with follicles larger than 10 mm, and having visible heat followed by insemination were diagnosed as cured. The average number of follicles on both ovaries when at least one follicle ≥10 mm was found, reached 5.7 mm (2–10) and the average interval from the 1st day of treatment to insemination was 26.87 ± 18.3 days and to pregnancy was 72.8 ± 8.4 days. Eleven (33.3%) cows conceived after the first insemination after treatment, next 11 (33.3%) needed at least two inseminations for pregnancy and 11 (33.3%) were not pregnant. Control cows had an average of 4.2 mm follicles (2–8) and the average interval from examination to

insemination was 38.3 ± 31.4 days. We conclude that repeated low doses of buserelin treatment of anovulatory anestrus cows improve follicular development and allow to successfully inseminate cows, however this treatment is time consuming.

P 27 | Adipolin, a novel adipokine expressed in ovarian pig follicles

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In human and rodents, Adipolin, also called CTRP12 (C1q/TNF-related protein-12), is a novel adipokine, mainly expressed in adipose tissue and involved in insulin sensitivity. Several novel adipokines including adiponectin and chemerin have been recently showed to be expressed in porcine ovarian cells and to be involved in the regulation of ovarian physiology. However, adipolin expression and its role in gonads have never been investigated. The aim of the present study was to assess adipolin expression in visceral adipose tissue and also ovarian follicles. We collected large (LF), medium (MF) and small follicles (SF) from two pig breeds with different fattening and prolificacy: Large White (LW) and Meishan (MS), (LW>MS). By RT-PCR, we showed a higher adipolin expression in MS visceral adipose tissue as compared to LW ($p < 0.05$). Interestingly, we observed that the adipolin mRNA expression was dependent on the size of the follicles (LF>MF>SF) in prepubertal and mature LW animals. Furthermore, in MF, adipolin expression was higher in MS as compared to LW ($p < 0.05$). By immunohistochemistry, we located adipolin mainly in granulosa and theca cells from follicles of LW. By ELISA assay, we measured plasma and follicular fluid adipolin concentration in both breeds. Moreover, we used recombinant adipolin protein to investigate the in vitro effect of adipolin on proliferation, signalling and steroidogenesis in LW granulosa cells. Taken together, we showed that adipolin mRNA is expressed in porcine ovarian follicle and its expression is dependent on the size of follicles suggesting a role in folliculogenesis. In addition, its higher expression in adipose tissue and MF of MS suggest that this novel adipokine could be involved in the metabolism and reproduction interactions.

P 28 | The transforming growth-factor (TGF)-β1, -β2 and-β3 is synthetized by most of the boar internal genital organs

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Boar seminal plasma is rich in cytokines, including transforming growth-factor (TGF)-β1, -β2 and -β3, whose concentrations differ among ejaculate fractions, suggesting different sites/levels of expression along the boar's genital tract. Accordingly, this study evaluates their localization using immunohistochemistry in samples from the testis, epididymis (caput, corpus and cauda segments) and accessory sex glands of 6 healthy and fertile boars. The three TGF-β isoforms were immunohistochemically localized using rabbit polyclonal primary antibodies (ab25121, ab113670, resp ab227711, Abcam, Cambridge, UK). TGF-β1, TGF-β2 and TGF-β3 were expressed throughout the genital organs, without obvious differences among boars. The three cytokines were localised most specifically in the Leydig cells, the principal epithelial cells and the surrounding smooth muscle of the entire epididymis and the accessory sexual glands (strong cytoplasmic location in both prostate and seminal vesicle for TGF-β1 and TGF-β2, but weakest for TGF-β3 and an interstitial staining in the bulbourethral glands). In conclusion, the expression of TGF-β1, TGF-β2 and TGF-β3 supports the concentration differences seen between boar ejaculate fractions. (Supported by MINECO & FEDER EU-Funds (AGL2015-69738-R) Madrid, Spain; Seneca Foundation, Murcia, Spain (19892/GERM/15); FORSS (grant 745971) and The Swedish Research Council FORMAS (grant 2017-00946), Stockholm, Sweden.).

P 29 | Expression of connexin 43 during testicular regression after exposure to short photoperiod in the Syrian hamster

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Connexin 43 is a protein that forms part of the GAP junctions. Some studies have shown that regardless of its ability to form GAP junctions, connexin is also able to control cell proliferation. In the testis, not only Sertoli cells establish direct communications with germ cells through GAP junctions but Leydig cells also establish them in the testicular interstitium. However, there is controversy about the changes related to cellular communications during testicular regression. Therefore the aim are of this work was to perform an immunohistochemical and quantitative study of connexion 43 expression in the testis of Syrian hamster during testicular regression. For this, a total of 17 Syrian hamsters were divided into four groups: Control, Middle Regression, Strong Regression and Total Regression groups. The presence of connexin 43 was detected immunohistochemically. The quantitative study was made in testis by western-blot technique. During regression connexin 43 positivity was observed in the cytoplasm and the plasmatic membrane of Leydig cells and in the junctions between Sertoli

cells and spermatogonia and Sertoli cells and spermatocytes. The expression of connexin 43 in testis was higher at the end of the regression, and the number of GAP junctions in the Leydig cells decreased as the regression progressed. In conclusion, it does not seem that the higher expression of connexin 43 is related to the testicular interstitium so it will probably be related to the seminiferous tubule. The decrease GAP junctions in Leydig cells during regression may be related to the decreased steroidogenic activity. (Funded by GERM 19892/15 from Fundación Séneca CARM.)

P 30 | Effect of astaxanthin on frozen-thawed boar semen – a preliminary study

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Astaxanthin (ASX) is a xanthophyll carotenoid with strong antioxidant capacity. The aim of the study was to test whether overnight preincubation at 17°C of boar semen with ASX prior to freezing (Trial I) or ASX supplementation only at thawing (Trial II) may exert a protective effect against injury related to freezing and thawing. Entire ejaculates from 4 boars were collected, pooled, extended 1:1 (BTS; v:v) and split in 4 groups [Control (1): no treatment; low ASX (2): semen with ASX at 0.5 μM; medium ASX (3): semen with ASX at 5 μM; and high ASX (4): semen with ASX at 15 μM] for cryopreservation procedure. Sperm quality and functionality were evaluated for all groups (3 replicates) prior to freezing (Trial I) and after thawing (30 and 150 min; 37°C; trial II). Sperm motility was evaluated by a CASA system, while sperm viability and acrosome integrity (PI, FITC/PNA), ROS production (DHE, CM- H2DCFDA, MitoSox Red), lipid peroxidation (C11-BODIPY581/891) and apoptosis (Annexin-V) were analyzed by flow cytometry. In trial I, no effect of ASX on any of the evaluated parameters was observed. In trial II, a positive effect of ASX on apoptosis was found in group 2 (low ASX), demonstrating better ($p = 0.023$) values for apoptosis 30 min post-thawing compared to group 1 (control) (3.2 ± 1.91 vs. 6.8 ± 3.3 for group 2 and 1, respectively). In conclusion, although this study is still in progress, the results showed a mild beneficial effect of ASX on cryopreserved boar semen after its addition at thawing. (Funded by SENECA foundation, Spain (19892/GERM/15), the Erasmus+ Students Mobility Programme and the State Scholarships Foundation (IKY), Greece.)

P 31 | The post-thawing quality of INRA180 ram sperm held 4 h at 15°C prior to cryopreservation

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Semen storage requires that animals of interest are within reasonable proximity of laboratories. However, in many cases, those animals are located far from lab facilities. Therefore, we aimed to study the effect of holding semen at 15°C for 4 h post dilution before freezing on post-thaw quality of INRA180 ram sperm. Semen samples were collected using an artificial vagina from two groups of animals. Group 1: The rams were kept 170 km far from the lab (G1) and group 2: The rams were kept next to the lab (G2). Immediately after collection, semen samples were evaluated for volume, sperm concentration, mass (MM) and individual (IM) motility. The semen was extended in egg yolk and soy lecithin-based extenders to 0.2×10^9 spermatozoa/ml. Before freezing, the samples from G1 were transported in a temperature-regulated cooler box at 15°C to the laboratory (within 4 h) then cooled to 5°C, while those from G2 were directly cooled to 5°C during 2 h (G2). Total (TM) and progressive (PM) motility, and curvilinear velocity (VCL) were analyzed using the CASA system. Sperm viability was determined using the eosin-nigrosin staining. The data were statistically analyzed using JMP SAS 11.0.0. No significant difference was recorded between both ram groups, in terms of sperm volume (1.54 ± 0.04 ml), concentration (4.14 ± 0.11 (10^9 spz/ml)), MM (4.51 ± 0.06) and IM (92.47 ± 0.62%). After thawing, G2 recorded a higher TM (65.13 ± 1.69 vs. 58.34 ± 1.64%) and viability (70.89 ± 2.34 vs. 53.51 ± 1.78) compared to G1 ($p < 0.05$) while no significant difference was highlighted in VCL (91.39 ± 1.79 μm/s) and PM (26.33 ± 0.91%) between the two ram groups. Sperm transport during 4 h at 15°C prior to cryopreservation decreased the ram post-thaw semen quality. However, the quality was still satisfying in both treatments.

P 32 | Ovulatory response to GnRH following luteolysis by $PGF_{2\alpha}$ in Norwegian Red heifers

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Synchronization protocols are commonly applied for fixed timed AI in dairy cattle. In this study, the objective was to assess the time of ovulation after GnRH treatment in Norwegian Red (NR) heifers. Transrectal ultrasonography was used to detect ovulation in 32

heifers following synchronization of oestrus with $PGF_{2\alpha}$. Heifers were recruited from four dairy herds in eastern Norway. Each animal was treated with a luteolytic dose of $PGF_{2\alpha}$ twice, (Estrumat vet., 2 ml (i.m.)), defining time of first treatment as day 0 and second as day 11. Presence of corpus luteum was confirmed on day 11. GnRH treatment was given on day 13 (Receptal vet., 2.5 ml (i.m.)), directly followed by ultrasound examination (T0). Ultrasound was repeated 9 h later and from then on, every third hour until ovulation. For each ultrasound examination, the diameter of the largest follicle was recorded. Persistence of follicles was defined as time from T0 until these follicles were no longer detectable by ultrasound. Mean time from T0 to ovulation was 26.5 h (SD = 3.4). Altogether more than 90% of the heifers ovulated within a limited time interval of 9 h, only 3 animals did not ovulate. These results obtained in Norwegian Red heifers are comparable to those previously reported for most other dairy breeds.

P 33 | Antimicrobial resistance of isolates of microorganisms identified in the milk of cows with subclinical mastitis in the Ural region of Russia

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During 2017 n = 375 milk samples of dairy cows with subclinical mastitis from 18 farms of the Ural Region were examined in order to identify the main pathogenic agents and their antimicrobial resistance. Microbiological research on the milk using real time PCR using the device Rotor Gene-3000 (Australia), with the complex of reagents of the IDS company (Russia) was done, to identify DNA from biological material and determine antimicrobial resistance of microorganisms. The identified microorganisms included: *Staphylococcus spp.* –77.3%, *E. coli* – 37.3%, *S. aureus* – 38.7%, *S. agalactiae* – 14.7%. Associations of microorganisms included: *S. aureus*, *Staphylococcus spp.* – 17.3%; *Staphylococcus spp.*, *E. coli* – 21.3%; *S. agalactiae*, *E. coli* – 2.7%; *St. aureus*, *Staphylococcus spp.*, *E. coli* – 8.0%; *S. aureus*, *Staphylococcus spp.*, *S. agalactiae* – 2.7%; *S. aureus*, *Staphylococcus spp.*, *S. agalactiae*, *E. coli* – 1.3%. The gene of CTX-M that determines resistance of Enterobacteriaceae to Cephalosporin of the 1st generation was found in 3.6% of samples with *E. coli*. The gene of blaDHA that determines resistance of Enterobacteriaceae to penicillin and cephalosporin of the 3rd and 4th generations was found in 4.6% samples with *E. coli*.. 3.6% of the samples with *E. coli* had two mutational genes CTX-M and blaDHA. The gene MecA that determines resistance of *S. aureus* to Cephalosporin of the 2nd generation was found in 10.6% samples. Resistance of *Staphylococcus spp.* and *Streptococcus spp.* to macrolides of the 1st generation (the gene of ErmB) was identified in 45.3% samples. In conclusion, the research done proved the wide spread of antimicrobial resistance of pathogenic agents causing subclinical mastitis in dairy cows.

P 34 | Cases of male sterility of interspecific hybrids between *Phodopus campbelli* and *P. sungorus* (Rodentia, Cricetidae)**

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Sterility or reduced fertility is often observed in interspecies hybrids. Using histological approach and immunolocalization of key meiotic proteins at pachytene we analyzed gametogenesis and meiotic chromosome behavior in two closely related species of dwarf hamsters, *P. campbelli* (PCA), *P. sungorus* (PSU) and their F1 hybrids. Female hybrids showed normal fertility. Male hybrids were completely sterile. They showed multiple aberrations in the morphology of the seminiferous tubules and their contents. The ratio of pachytene spermatocytes to spermatogonia in the hybrids was two times higher than in the parental species. At the same time, we observed a severe shortage of early and middle spermatids. This meiotic arrest and massive germ cell death at the end of meiotic prophase was probably caused by asynapsis between the heterochromatic Xp and Yq, which was observed in most pachytene spermatocytes of F1 (77.9 ± 2.8% cells compared to 8.6 ± 2.3% in PCA and 11.0 ± 2.3% in PSU). In pachytene oocytes of F1, PCA and PSU females we observed a high incidence of centromere misalignment at the XX bivalent and completely suppressed recombination in heterochromatic Xp, where the pseudoautosomal region is located. We propose that this recombination pattern speeds up divergence of the X- and Y-linked pseudoautosomal regions and results in their incompatibility and asynapsis in the male hybrids. (This work was supported by the Federal Agency of Scientific Organisations via the Institute of Cytology and Genetics (Grant # 0324-2018-0019) and by Russian Foundation for Basic Research (Grant # 17-29-08019).)

P 35 | Biopsy of mammary gland in sows: A tool for studying colostrum production

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Mortality and health of newborn piglets are related to colostrum intake which is mainly limited by the colostrum yield of the sow and highly variable between animals. There is a need for studies focusing on factors affecting colostrum yield. One method that could be used is next-generation sequencing of mammary gland tissue which would allow to gain an understanding about the difference between mammary glands that produce a low and high amount of colostrum. The purpose of this study was to test different biopsy needles, evaluate the amount and composition of obtained tissue, and observe whether sows develop complications such as hematoma or abscess. The most suitable needle was an automatic one with a diameter of 14 gauge, a

length of 10 cm and a penetration depth of 22 mm (Monopty, Bard Finland Oy, Finland). Biopsies were taken from eight sows three days before expected farrowing from the lateral-caudal part of three different mammary glands. Before the biopsy, glands were disinfected three times with a povidone-iodine solution (7.5% Betadine, Leiras OY, Finland). During the biopsy, food was provided to the sows. The needle was inserted through the skin, about one centimeter into the tissue and triggered. After the biopsy, the tissue was placed in formalin and later transferred to paraffin for histological examination. The size of obtained tissue was about 30 mm^2 and the composition homogenous. Sows showed no reaction during the biopsy. Minor local bleeding occurred but no pathological changes were observed afterwards. The experiment shows that biopsy of the mammary gland is feasible and does not affect the health and welfare of the animal and therefore can be used as a tool for studying colostrum yield in sows.

P 36 | Conceptus products differentially regulate expression and activity of peroxisome proliferator-activated receptor (PPAR) isoforms in luminal epithelial and stromal cells of the porcine endometrium

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The present studies aimed to analyze the effect of conceptus products on mRNA and protein expression and DNA binding activity of peroxisome proliferator-activated receptor (PPAR) isoforms (α, β/δ, and γ) in cultured porcine endometrial cells. Conceptuses were collected from Day 12 pregnant gilts (n = 6) and incubated in Medium 199 supplemented with 1% steroids-free newborn calf serum for 24 h. Then, medium was collected and used as conceptus-exposed medium (CEM). Luminal epithelial (LE) and stromal (ST) cells of the endometrium were enzymatically isolated from Day 10–12 cyclic gilts (n = 6) and treated with CEM for 24 h. PPARs mRNA and protein expression was analyzed with Real-time PCR and Western blot, respectively. DNA binding activity of each isoform was determined using ELISA. Paired t-test was conducted for data analyses. Conceptus products stimulated PPARα protein level ($p < 0.05$) in LE cells. In ST cells, both mRNA and protein expression of PPARα increased in response to CEM ($p < 0.05$). Moreover, the addition of CEM to ST cells resulted in greater PPARβ/δ transcript abundance compared to control value ($p < 0.05$). PPARγ mRNA expression decreased after CEM treatment in LE and ST cells. Conceptus products stimulated DNA binding activity of PPARα in studied cells ($p < 0.05$). To conclude, conceptuses differentially regulate PPARs expression and activity in endometrial cells; which in turn, may affect uterine preparation for implantation in the pig. (Supported by NSC grant 2013/11/B/NZ9/00806)

P 37 | Electron paramagnetic resonance (EPR) spectroscopy to study the fluidity of equine sperm membrane

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Background: The sperm quality might be affected during freezing leading to the loss of motility and to the decrease of the cell viability. Ochsendorf et al. studied the fluidity of sperm plasma membranes in fertile and infertile men by EPR spectroscopy. We have previously shown that the use of INRA 96 extender enriched with hydroxypropyl beta cyclodextrin-cholesterol complex (CDC) and 1% glycerol (INRA96-CDC-G) compared to INRA-freeze (IF) medium, significantly improved post thawing semen quality. Objective: this study aimed: i) to describe an EPR spectroscopy process to study the membrane fluidity of equine spermatozoa (SPZ) using 5-DSA and 16-DSA as probes on 5 stallions having a good fertility and a good semen quality post-thawing ii) to compare the values obtained with two different extenders used in our Center. Mat & Met: Frozen semen in IF or INRA96-CDC/G was thawed at 37°C, centrifuged (600 × g, 10 min, 37°C) and SPZ were washed once with HBSS+glucose (HBSS/g) (pH 7.4). After washing, the resulting pellets were resuspended in HBSS/g to obtain 5×10^7 cells in 95 µl + 5 µl of the probe (5-DSA or 16-DSA, 0.5 mM). Cells were transferred in the capillary, sealed and placed in EPR tube for analysis. Results & Conclusion: Our results showed good EPR characteristic signals for each probe. Both conservation media (IF and INRA96-CDC-G) were not different: 5-dsa probe IF (2A// = 58.9 ± 1.8G; I-hf = 4.6 ± 1.7) and INRA-CDC-G (2A// = 55.6 ± 4.8G; I-hf = 5.9 ± 1.3), likewise, for 16-dsa (IF: 24.37 ± 5.82 G and INRA-CDC-G: 26.87 ± 3.17 G). Results are means+/-SD (n = 5). Altogether, our results indicate that EPR technique can be applied to evaluate the membrane fluidity of equine sperm cells and suggest that INRA96-CDC-G does not modify the membrane fluidity compared to IF medium.

P 38 | The relation between PGE2 synthesis and expression profile of the molecular markers of implantation ability and developmental competence in bovine blastocysts derived from good and poor quality oocyte

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Prostaglandin E2 (PGE2) plays an important role in early embryo development and the establishment of pregnancy in cows. The expression of numerous genes in early stage embryos is directly associated with their developmental competence. The aim of the study was to determine possible correlation between mRNA expression of factors involved in PGE2 synthesis and mRNA expression of developmentally important genes in the bovine blastocysts. The expression of enzymes involved in PGE2 synthesis (PTGS2, mPGES-1, mPGES-2, cPGES) and embryo quality markers (PLAC8, IGF1R, IGF2R, OCT4, SOX2) in in vitro produced blastocysts derived from early and late cleaved embryos (separated at 30 and 36 hpi, respectively) were examined by Real-time PCR (n = 5 per each group). Embryo quality of the early, developed, expanded and hatched blastocysts from each group was assessed according to International Embryo Transfer Society. Data were analyzed using Pearson correlation (GraphPad PRISM 6.0). There were dynamic changes of mRNA abundance for all analyzed enzymes and embryo quality markers in the early and late cleaved embryos at blastocyst stage. There were several positive correlations between mRNA expression of PTGS2, mPGES-1, mPGES-2, cPGES and mRNA expression of PLAC8, IGF1R, IGF2R, OCT4, SOX2 in type A and B of early, developed and expanded blastocysts and type A of hatched blastocysts derived from early and late cleaved embryos. Summarizing, the conducted research accounts for differential correlation between mRNA expression of enzymes involved in PGE2 synthesis and expression of markers of implantation ability and developmental competence in the bovine blastocysts, produced in vitro from the two diverse types of oocytes. (Supported by Polish National Science Centre: 2014/13/N/NZ9/03924).

P 39 | Effect of Ficoll 70 on vitrification of donkey embryos: preliminary results

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The inclusion of a macromolecule in a vitrification solution (VS) could increase the survival of cryopreserved embryos. Ficoll 70 has been previously used with this aim for the cryopreservation of embryos in horses and mice, but not in donkeys. This study evaluates the in vitro viability of donkey embryos vitrified in two different vs. (supplemented or not with Ficoll 70). Day 6 and 7 embryos were measured and morphologically evaluated. Only grade 1 or 2 morula, early blastocyst and blastocyst stage were vitrified-warmed using the cryotop technique. Embryos were randomly distributed into two groups: (i) VS1 (n = 5): vitrified using non-supplemented vs. (15% ethylene glycol + 15% dimethyl sulfoxide + 0.5 M sucrose in TCM-199), and (ii) VS2 (n = 5): vitrified using vs. supplemented with 18% of Ficoll 70. After 24 h of warming, the embryos were measured and evaluated for their morphology, development and viability (Propidium Iodide-Hoechst 33342 dyes). Data were evaluated by the chi-square test and ANOVA. Post-warming survival was equal (60%; $p > 0.05$) for both treatments. Similarly, no differences ($p > 0.05$) were observed between groups for diameter (250.0 ± 31.6 vs. 240.0 ± 18.7 μm) and grade (2.4 ± 0.7 vs. 2.6 ± 0.6). However, percentage of viable cells was higher ($p < 0.05$) in VS1 than in VS2 (98.8% vs. 95.6%). In conclusion, the addition of Ficoll to vs. containing ethylene glycol and dimethyl sulfoxide did not improve both morphology and viability of donkey embryos. (Supported by Grant AGL2013-42726-R).

P 40 | Cryo-scanning and conventional electron microscopy of Iberian ibex (*Capra pyrenaica*) sperm cryopreserved using slow and ultra-rapid cooling protocols

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To improve the sperm cryopreservation techniques it is required to understand how the processes provoke cellular damages during the freezing-thawing process. Sperm damages are caused by the extra/intracellular formation of ice. It has been suggested that ultra-fast cooling of small volume samples raises the viscosity of the milieu, avoiding ice formation during cooling. Due to the practicality of this cryopreservation technique, its use is recommended for in situ application in wild ungulates. Several authors indicate that both the intracellular milieu and the extracellular environment of the sperm cells must become vitrified to use the term 'vitrification'. The aims of this work were: 1) to evaluate the extracellular state in sperm samples of Iberian ibex processed by two cryopreservation methods (ultra-rapid and slow) to determine if during ultra-rapid freezing a extracellular vitrified state is acquired; 2) to compare cellular damages of spermatozoa between both methods using scanning and transmission electron microscopy. Sperm samples (N = 4) were obtained by transrectal ultrasound guided massage method (TUMASG) in anesthetized ibexes, and cryopreserved using both techniques. Vitrified state was not found after ultra-rapid cooling rate. Crystal dimensions and morphology were smaller and more stretchmarked after ultra-rapid freezing. Damages in plasmalemma and mitochondria seem more marked after ultra-rapid cooling. This study increases the knowledge about sperm damages after a cryopreservation process, necessary for improve the freezing techniques in this species. (Supported by MINECO grant AGL2014-52081-R.).

P 41 | Use of hCG on the induction of accessory corpora lutea in Morada Nova ewes

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The objective of this study was to evaluate if the use of hCG seven days after the synchronized estrus will induce the formation of accessory corpora lutea in Morada Nova ewes. For this, 115 multiparous Morada Nova ewes were used (mean weight of 36.6 kg and body condition score of 3.13, scale from 1 to 5). Estrus was synchronized with intravaginal sponge impregnated in medroxyprogesterone acetate (60 mg, Progespon®, Zoetis, USA) for six days and eCG (200 UI, i.m. Novormon®, Zoetis, USA) plus $PGF_{2\alpha}$ analog (0.0375 mg, D-Cloprostenol, i.m., Vetglan®, Hertape Calier, Spain), both administered 24 h before the sponge removal. Seven days after the synchronized estrus, hCG (300 IU, i.m., Vetecor®, Hertape Calier, Spain; n = 57) or physiological solution (1 ml, i.m., 0.9% NaCl, Eurofarma Lab SA, Brazil; n = 58), were injected. B-mode ultrasound examinations of the ovaries were performed on Day 7 (corresponding to the day of hCG or physiological solution administrations), and six days later (Day 13), in order to quantify the corpora lutea present. Data were analyzed by ANOVA with Turkey's post hoc test (mean ± SEM; $p < 0.05$) using SAS software. The number of corpora lutea on Day 7 was similar ($p > 0.05$) between the hCG and control groups (1.58 ± 0.09 vs. 1.57 ± 0.08), respectively. However, on Day 13, the number of corpora lutea was higher ($p < 0.05$) in the hCG group (2.65 ± 0.13) than in the control group (1.69 ± 0.07). In conclusion, the use of 300 IU of hCG seven days after synchronized estrus in Morada Nova ewes is efficient in inducing the formation of accessory corpora lutea, as demonstrated by the increase in the number of corpora lutea. (Financial support: CNPq and EMBRAPA (process n° 02.13.06.026.00.02).).

P 42 | A single injection of triptorelin or of buserelin acetate in saline solutions induce ovulation in mares as a single injection of hCG

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In the 1980's, it was concluded that a single injection of GnRH or its agonists was not able to induce ovulation of preovulatory follicles in mares. Subcutaneous implants releasing deslorelin during 48 h and injection of deslorelin in long acting base are commercially available and are widely used around the world to induce ovulation in mares. Recent studies showed that a single subcutaneous injection of large dose (6 or 3 mg) of buserelin acetate in saline solution is able to induce ovulation. To inject this dose, it is necessary to use a human drug (Suprefact®), but its production is definitively stopped. The aim of this study was to test another analog of GnRH, triptorelin. A total of 737 oestrus of donor and recipient mares were checked. A control group included 114 oestrus without treatment (spontaneous ovulations). In 5 other groups mares in oestrus having a growing follicle with a diameter of 35 mm were injected either intravenously with hCG (1,500 iu) (Gh n = 145), subcutaneously with triptorelin (0.1 mg) (Gt n = 96) or with buserelin acetate at 3 different doses 1 mg (Gb1 n = 67), 2 mg (Gb2 n = 141) or 3 mg (Gb3 n = 174). Size of follicle before ovulation was significantly higher ($p < 0.01$) in the control group than in the 5 treated groups, showing that all treatments induced ovulation. Rates of ovulation occurring during 48 h after injection (Gh 95%, Gb1 97%, Gb2 97%, Gb3 93% and Gt 95%) and between 24 and 48 h after injection (Gh 87%, Gb1 87%, Gb2 83%, Gb3 85% and Gt 85%) were not significantly different among the 5 treated groups. From a practical point of view triptorelin commercially available on human drug (Decapeptyl®) can be use in place of Suprefact® with same efficacy to induce ovulation.

P 43 | The effect of Hydrostatic Pressure Treatment (HHP) on quality of poor boar ejaculates after cryopreservation

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The aim of this study was to evaluate the effect of HHP treatment (Applied Cell Technology, Hungary) of poor quality of fresh boar ejaculates, in which progressive motility (PM%) were 55.3 ± 3.8%. The sperm-rich ejaculates fractions collected from 5 boars (n = 20) were used in the experiment. Before freezing ejaculates were split: I (control without HHP treatment); II, III and IV were treated with 30 MPa, 35 MPa and 40 MPa for 1.5 h at 21°C, respectively. Cryopreservation procedure was carried out as previously described (Trzcińska et al. 2015, Theriogenology 83:307-13). The quality of cryopreserved semen were verified by PM % (CASA); viable sperm with intact acrosome (PNA-/PI-) and live sperm without translocation of phosphatidylserine (AnV-/PI-) analyzed by flow cytometer. Data were analyzed by Duncan's test ($p \leq 0.05$). The results showed all treated groups (II, III, IV) differ significantly with control (47.6 ± 3.5; 50.8 ± 1.7; 46.9 ± 2.6 vs. 35.2 ± 2.1) in post-thaw motility and in % of PNA-/PI- sperm (43.2 ± 2.6; 48.3 ± 4.1; 42.5 ± 3.2 vs. 34.1 ± 3.5). No differences in % of AnV-/PI- sperm in experimental groups were observed. The highest % of AnV-/PI- freeze-thawed sperm (48.2 ± 1.7) was noticed in group treated with 35 MPa before cryopreservation. Study demonstrates that using HHP treatment before cryopreservation of poor quality boar semen increased cryotolerance of sperm during freezing and provided high sperm viability after thawing. (Financial support: BIOSTRATEG 2 No. 297267/14/2016.)

P 44 | Effect of seminal plasma and two types of freezing extenders on characteristics of frozen-thawed stallion epididymal spermatozoa

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The aim of this study was to investigate the viability and plasma membrane integrity of epididymal sperm after addition of seminal plasma (SP) to samples frozen in two different extenders. SP was collected after centrifugation of ejaculates from different stallions with known good motility after thawing. Epididymes were collected from 5 healthy stallions during routine castration. To collect the epididymal sperm, the ductus epididymes were filled with air, similar to the retrograde flush. Then the samples were diluted with freezing extenders (L-EDTA or INRA82) and filled into 0.25 ml straws. After that, the samples were frozen and stored in liquid nitrogen. The straws were thawed in a water bath (37°C, 30 s). After thawing, the SP was added to samples in a ratio 1:1. The viability and plasma membrane integrity were evaluated immediately after thawing (T0) and after 30 min incubation at 37°C (T30) by t-test ($p < 0.05$). Differences were observed ($p < 0.05$) in viability and plasma membrane integrity between two freezing extenders (in T0 viability in L-EDTA on average 47.4 ± 1.2%, in INRA82 38.3 ± 0.7%; plasma membrane in L-EDTA 35.4 ± 1.8%, in INRA82 15.8 ± 1.6%). Viability was not affected ($p > 0.05$) by addition of SP after thawing. Plasma membrane integrity decreased ($p > 0.05$) in INRA82 extender after addition of SP. We noticed differences between individual stallions in response to chosen extenders as well as to addition of SP to frozen-thawed epididymal semen. Incubation time did not influence evaluated parameters. In this experiment, L-EDTA was a better choice for cryopreservation of epididymal sperm. Effect of SP addition after thawing was not manifested.

P 45 | Mitochondrial activity in boar spermatozoa: some insights

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Mitochondria activity is of utmost importance in sperm cells as they control energy and ROS production for optimal sperm function. The aims of this work were to investigate the main mitochondrial functional patterns and their implication on sperm viability and motility. Sperm were collected from three fertile boars; analyses were performed on three ejaculates from each boar ($n = 9$). Cells were diluted in Androhep® at 30×10^6 spz/ml and treated with: Rotenone 5 μM (ROT) complex I inhibitor, Dimethylmalonate 10 mM (DMM), complex II inhibitor, CCCP 5 μM uncoupling agent, 2-deoxy-glucose 10 mM (2DG) glucose agonist and 2 μl DMSO, (CTR) vehicle. Spermatozoa were incubated for 1 h at 37°C, then motility (total, TM and progressive, PM) was analysed with the Image J BGM plug-in. Mitochondrial activity was analysed by JC1/SYBR/PI (200 cells were counted under epifluorescence microscope). Sperm motility was negatively affected by CCCP and ROT treatments with respect to CTR (TM 13.4 ± 6.3% and 19.8 ± 8.8% vs. 60.5 ± 8.8%; CTR; PM 4.1 ± 4.1%; 5.7 ± 4.7%; 28.9 ± 6.6%, respectively vs. CTR). No difference in sperm viability (ranging around 70%) was registered, while a strong inhibition of mitochondrial activity was shown after CCCP treatment: 100% spermatozoa with low $\Delta\psi$ (mitochondrial membrane potential). Mitochondrial respiration likely switches from the first to the second phosphorylation site during substrate oxidation, even if preferentially exploits NADH oxidation by complex I. In addition, both sperm cell motility and $\Delta\psi$ do not rely on anaerobic glycolysis in presence of 2-DG inhibitor. In conclusion, boar sperm motility seems to depend on mitochondrial ATP, while cell viability is maintained even under conditions of impaired mitochondrial function.

P 46 | Evaluation of a novel portable device for motility assessment in stallion semen

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In horse breeding, quality assessment of semen before insemination is often requested. Non-laboratory-based techniques for objective analysis of sperm motility are thus of increasing interest. The aim of this study was evaluating a portable method of semen analysis (Ongo sperm test, Microfluidlabs, H-Budapest), and comparison with computer assisted semen analysis (CASA, SpermVision, Minitube, D-Tiefenbach). Semen was collected from 10 stallions and diluted in EquiPlus extender (Minitube) to 100, 50 and 25 mio sperm/ml. Aliquots were analyzed for total motility (TM) and progressive motility (PM) with both systems, and the 25 mio/ml dilution was further analyzed for intra-assay variation (5 measurements × 10 stallions). Agreement between methods was evaluated by correlation analysis and Bland-Altman plot. Intra-assay variation of Ongo was 2.9 ± 1.4% for TM and 2.3 ± 1.0% for PM. Pearson's coefficient of correlation was $r = 0.79$, 0.88 and 0.83 for 100, 50 and 25 mio/ml for TM, and $r = 0.87$, 0.89 and 0.87 for PM, respectively (all $p < 0.001$). At the 100 and 25 mio/ml dilutions, the difference between the two systems deviated significantly from 0, while no such bias existed at the 50 mio/ml dilution (TM Ongo 85.0%, CASA 82.3%; PM Ongo 64.1%, CASA 66.1%). The 95% confidence interval was 19.9, 18.9 and 19.2% ± mean for TM and 20.7, 17.4 and 20.3% ± mean for PM at 100, 50 and 25 mio/ml, respectively. In conclusion, Ongo sperm test sperm motility data were strongly correlated with data obtained by CASA. In addition, at a concentration of 50 mio/ml values measured with both systems are close to identical. At this concentration, which is

recommended in equine AI, Ongo and CASA system can be used interchangeably.

P 47 | Effectiveness of plant-based additives application for reproductive indicators of breeding bulls

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The use of feed additives based on natural, environmentally friendly plant material is a growing topic. The new additive "Verva"- is a mixture of triterpenic sodium salts acids, derived from the fir tree wooden green shown to Increase the productivity of farm animals and birds and resilience to diseases (Mata-Campuzano et al., 2015). We report here the influence of "Verva" supplement on reproductive potential of stud bulls (n = 14), sperm quality and biochemical parameters (30 blood samples). Following treatment, changes in blood content (increase in Ca from 2.23 ± 0.08 mM to 2.33 ± 0.05 mM ($p < 0.01$); reduction of creatine kinase level from 365.28 ± 134.44 un/l to 243.75 ± 79.47 un/l ($p < 0.05$); decrease in GLDH from 49.56 ± 11.35 un/l to 31.80 ± 8.19 un/l ($p < 0.01$); decrease in aspartate aminotransferase concentration from 94.57 ± 14.33 un/l to 75.00 ± 10.31 un/l ($p < 0.05$) and decrease in cortisol from 114.87 ± 33.93 to 56.64 ± 6.99 nM ($p < 0.05$) were observed. Under supplementation, the volume of the ejaculate increased from 4.42 ± 0.65 ml to 4.72 ± 0.66 ml ($p < 0.05$) and concentration of spermatozoa increased from 1046.00 ± 154.79 mln/ml to 1115.00 ± 85.88 mln/m ($p < 0.05$). As a result of the experiment, there was an increase in the frozen doses of sperm obtained from bulls from 933.00 ± 356.72 to 981.25 ± 254.87 pcs ($p < 0.05$). Thus, the use of the feed additive "Verva" was economically beneficial and a promising way to improve the reproductive performance of breeding bulls, as well as the normalization of animals biochemical processes, which may lead to an improvement of the productive life of males. So, the use of the feed additive "Verva" positively influenced biochemical indicators of blood of bulls and their reproductive characteristics.

P 48 | Histological characterization of testis in Miranda Donkeys from juvenile to adult

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The development of testis from juvenile to adult is marked by structural changes in the gonads. The aim of the present research is to characterize the testicular histology and the various steps of spermatogenesis in Miranda donkeys (*Equus asinus*) during their growth and maturation. Testes (n = 6) were obtained from orchiectomy by of animals with 4 months, 12 months and 4 years. Testes were measured, weighed (mean: 10 g (SD = 1.25); 48 g (SD = 0.32); 193 g (SD = 6.48)) and the volume calculated (mean: 12 cm^3 (SD = 3.01); 42 cm^3 (SD = 1.02); 175 cm^3 (SD = 5)) for the three ages, respectively. Material was taken by systematic random sampling, immediately fixed with formaldehyde 10%. From each animal a fragment of the right testicle was cut and processed according to the routine histological technique for inclusion in paraffin. Sections with 3 µm were stained with H&E and a comparative morphological analysis was performed. In juvenile, the seminiferous tubules are narrow with the lining epithelium filled only with spermatogonia A and Sertoli cells. The adjacent connective loose tissue is highly developed with scarce disperse Leydig cells. In pre-pubertal, the seminiferous tubules are broad and covered with spermatogonia A and B, leptotene to pachytene spermatocytes I, II and Sertoli cells. In some portions seminiferous tubules possess only spermatogonia A and Sertoli cells. The adjacent connective tissue is scarce. In adult, the seminiferous tubules are broad with the lumen filled by germ cells in all consecutive steps of spermiogenesis and residual cytoplasmic bodies. Myoid cells and Leydig cells with lipofuscin inclusions are present in all testes. These results suggest that in Equus asinus testis, the cell types, its distribution and proportion are not random associated and depend on age.

P 49 | Effect of clinical mastitis from calving to end of the voluntary waiting period on reproductive performance in Holstein cows

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The aim of this study was to investigate the effects of occurrence of clinical mastitis (CM) from calving to end of the voluntary waiting period (VWP) on reproductive performance in high producing lactating dairy cows. Retrospective records of Holstein cows (n = 550) in a commercial dairy farm in İzmir, Turkey from December 2014 to December 2015, were used. Data obtained during routine health/reproductive visits by herd veterinarians/technicians were initially recorded on herd management software and then evaluated. CM was diagnosed with the presence of visible changes as abnormal milk or signs of inflammation of one or more quarters. Reproductive variables included in the study were calving to first service interval (CFSI, day), calving to conception interval (CCI, day), services per conception (SC). All cows were inseminated by the same technician. Pregnancies were diagnosed on 30 days after AI by using transrectal

ultrasonography. CFSI (83.68 ± 3.18 vs. 74.57 ± 1.08; p < 0.05), CCI (168.48 ± 8.59 vs. 132.86 ± 3.44; p < 0.001) and SC (3.16 ± 0.21 vs. 2.42 ± 0.09; p < 0.001) were higher in cows detected with CM compared to the healthy ones. The negative effects of mastitis on reproduction were observed regardless of calving season, the time of occurrence of CM and the lactation number (p > 0.05). In conclusion, results suggested that cows suffering from an episode of CM between calving and the end of the VWP had extended CFSI, CCI and SC without any interaction with the calving season, the timing of CM and parity.

P 50 | Recombinant Heat Shock Proteins (HSPs) do not improve the quality of cooled porcine seminal doses during summer

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The quality of semen doses is influenced by several factors including the environment. Several studies have demonstrated a negative impact of high temperatures on the quality of boar ejaculates. The objective of the present work was to improve the quality of cooled porcine seminal doses during summer season (June-July) by adding different recombinant Heat Shock Proteins (HSPs). Commercial doses (n = 6) were supplemented with 1 µg/ml of recombinant HSP60, 70 or 90 and were maintained at 17°C. After 96 h, total motility (TM) and progressive motility (PM) were evaluated using a CASA system and sperm viability (SYBR+/PI-) and membrane lipid organization (M540 + /Yopro-1-) were studied by flow cytometry. A one-way ANOVA was used to compare pairs of values. In our study none of the sperm parameters studied were affected by addition of the recombinant HSPs compared with control; p>0.05. Viability (75.3 ± 2.3 vs. 76.8 ± 1.4 vs. 74.2 ± 1.5 vs. 76.8 ± 2.2; % ± SEM, control vs. HSP60, 70 and 90). TM (65.9 ± 9.3 vs. 66.7 ± 12.6 vs. 62.7 ± 13.4 vs. 51.7 ± 14.2; control vs. HSP60, 70 and 90). PM (21.6 ± 4.0 vs. 27.0 ± 8.2 vs. 28.3 ± 9.4 vs. 20.0 ± 5.9; control vs. HSP60, 70 and 90). M540 + /Yopro-1- (15.4 ± 2.7 vs. 19.8 ± 5.1 vs. 20.0 ± 4.3 vs. 16.1 ± 2.7; control vs. HSP60, 70 and 90). More research is necessary to determine whether other concentrations of recombinant HSPs would improve the quality of cooled porcine seminal doses during summer or if the recombinant HSPs are involved in other fertilization-related events in boar spermatozoa. (Funded by AGL2015-73249-JIN (AEI/FEDER/UE) from the Spanish Ministry of Economy, Industry and Competitiveness.).

P 51 | Identification of intracellular nitric oxide synthase isoforms in ram sperm**

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Nitric oxide (NO) plays a fundamental role in sperm functionality. NO is synthesized from L-arginine by nitric oxide synthase (NOS). Three isoforms of NOS have been identified and partially characterized in somatic cells: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Neuronal and endothelial NOS are constitutive isoforms, regulated by calcium and calmodulin, whereas the iNOS is inducible by cytokines and lipopolysaccharide (LPS). All isoforms have also been detected in human, murine, bovine and porcine spermatozoa and an increment in NOS activity has been reported during in vitro capacitation. The aim of this work was to determine the presence and localization of the NOS isoforms in ram spermatozoa and analyze possible changes during in vitro sperm capacitation. Semen collected from nine healthy Rasa Aragonesa rams (2–6 years old) was selected by swim-up, and spermatozoa were incubated without (control) or with cAMP-elevating agents (cocktail) to induce in vitro capacitation. Western blot analysis using anti-nNOS, anti-iNOS and anti-eNOS antibodies (Abcam), revealed bands compatible with nNOS (~120 kDa) and eNOS (~100 kDa), but no bands associated with iNOS were detected. Indirect immunofluorescence assays evidenced the three isoforms in the post-acrosomal region of all the cells, along with immunoreactivity in the apical edge for some of them. Moreover, eNOS showed additional reactivity in the neck of all spermatozoa. Changes in the proportion of these labeling patterns were observed when spermatozoa were subjected to in vitro capacitation. In conclusion, NOS isoforms are present in ram spermatozoa, which suggest a possible NO role in sperm capacitation in this specie. (Grants: AGL-2017-83799-R, DGA 2016-A26, BES-2015-072034.).

P 52 | SlimCASA software to evaluate bovine sperm motility: preliminary results

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The objective of this study was to develop a software for the analysis of bovine semen from microscope images. The purpose of this software was to standardize sperm motility assessment performed by veterinarians during field andrological examinations, reducing the cost of acquiring specific equipment. There were three steps concerning the development of the software solution: 1) definition of the necessary equipment to guarantee the quality of the images of animal ejaculate; 2) development of the proposed application; 3)

validation, comparing to the results obtained by an existing hardware solution. The software was developed based on the cellular analysis functions of the existing open source solution ImageJ. 100 commercial bovine semen samples were removed from the bottle with liquid nitrogen and placed in a water bath at 37°C for 20 s to thaw. With a micropipette, a 10 µl droplet was added onto a slide and covered with cover slip for determination of motility. Simultaneously a video of 30 s was recorded for further analysis by the software SlimCASA, while the technician analyzed the motility under microscopy. Another drop was added to the Hamilton Thorne equipment for computerized analysis. Data on the motility sperm analyses were submitted to Analysis of Variance (SAS, 2013). The averages were compared by the Tukey test at a significance level of 0.05. In the validation test, the mean results obtained were 64.51% ± 18.43[a] for the subjective analysis of the technician, 64.69% ± 19.34[a] for the CASA and 64.47% ± 9.15[a] for the software SlimCASA. The developed software presented good performance in the test. It is concluded that the free software will be an option to standardize the quality of analysis of sperm motility for bovine semen.

P 53 | Platelet factor 4 sustains in vitro production of porcine blastocysts in a chemically defined medium

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Establishing an optimal in vitro culture (IVC) system is a major goal of pig embryo production. Most culture media contain bovine serum albumin (BSA); however, the development of chemically defined media may improve results reproducibility and reduce sanitary risks. Our aim was to establish a chemically defined IVC system, replacing BSA by the platelet factor 4 (PF4), which increases viability and differentiation of hematopoietic stem cells. Matured oocytes (N = 1723; 4 replicates) were incubated for 5 h with 1000 thawed spermatozoa per oocyte and cultured for 7 days in a sequential system. The basic IVC medium was NCSU23 supplemented with 0.3-mM pyruvate and 4.5-mM lactate the first 2 days, or 5.5 mM glucose for a further 5 days. Presumptive zygotes were divided in 3 groups and cultured in media supplemented with: 0.4 mg/ml BSA (control); 0.3 mg/ml polyvinyl alcohol (PVA group) or 0.3 mg/ml PVA and 100 ng/ml PF4 (PF4 group). Data were expressed as mean ± SD. The efficiency of the in vitro fertilization was 38.1%. No differences among groups were found in cleavage rate at Day 2 (range: 57.8 ± 6.6–60.6 ± 8.2%). However, the blastocyst yield related to cleaved embryos at Day 7 was lower ($p < 0.01$) in the PVA group (5.9 ± 5.3%) than in control (55.1 ± 17.5%) and PF4 (53.6 ± 15.1%) groups. The efficiency of blastocyst formation was similar for control (33.5 ± 11.2%) and PF4 (31.6 ± 11.2%) groups, while PVA group showed the lowest ($p < 0.01$) efficiency (3.8 ± 4.0%). In conclusion, PF4 successfully replaced BSA and sustained porcine blastocyst production in chemically defined conditions. (Supported by MINECO-FEDER (AGL2015-69735-R and BES-2016-077869) and SENECA (19892/GERM/15).).

P 54 | Melatonin effect on sperm capacitation in sheep breeds under equatorial climates**

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Exogenous melatonin can modulate ram sperm functionality in seasonal breeds. However, there is no information on the melatonin effect on sperm capacitation in ram spermatozoa from sheep breeds located in equatorial climates (12D:12N). To elucidate this question, semen from twelve rams (4 Colombian Creole, 4 Romney Marsh, and 4 Hampshire) was collected during the rainy (March-May) and dry (June-August) seasons in Colombia (4° 42' N & 74° 12' O). Spermatozoa were selected by swim-up and incubated for 3 h at 39°C and 5% CO_2 in a capacitation medium (TALP supplemented with db-cAMP, caffeine, and theophylline plus Ca^{2+} and $NaHCO_3$), with 1 µM or 100 pM melatonin or without hormone (control). The capacitation status (chlortetracycline staining, CTC) and protein tyrosine phosphorylation (Western-blot) were evaluated by X2 and two-way ANOVA, respectively. The percentage of non-capacitated spermatozoa increased in the melatonin-treated samples ($p < 0.05$) compared to the control group in both seasons and for the three breeds. Curiously, melatonin did not reduce the capacitation-related protein tyrosine phosphorylation in high molecular weight sperm proteins in any season or breed, although incubation with 1 µM or 100 pM melatonin increased protein tyrosine phosphorylation in low molecular weight proteins in the Creole spermatozoa during the rainy season ($p < 0.05$) when compared with the control group. We did not find any difference in low molecular weight bands in the other two breeds or during the dry season, but we found differences between seasons in Creole and Romney Marsh breeds ($p < 0.05$). In conclusion, melatonin can prevent sperm capacitation in ram breeds located in equatorial climate with no photoperiodic changes. (Grants: 110157635854-Col576-2012, AGL2014-57863-R).

P 55 | Evaluation of sperm fertilising ability of porcine spermatozoa after voltage-dependent anion channel 2 (VDAC2)-blocking

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Sperm capacitation is a process which comprises changes in intracellular concentrations of calcium requiring the transport of this cation through ion channels located at plasma and mitochondrial membrane (Adeoya-Osiguwa and Fraser 2003, Mol Reprod Dev 65:228–236). Given that VDAC2 is implicated in in vitro capacitation in boar sperm (Martínez-Abad 2017, Reprod Dom Anim 52, Suppl 4:65–68), the aim of this study was to determine whether VDAC2 inhibition affects in vitro sperm fertilising ability. With this purpose, boar spermatozoa were selected with a double density gradient and incubated for 1 h with or without the presence of two VDAC2-inhibitors: erastin at 10 μM (E10) and olesoxime at 100 μM (O100). Subsequently, spermatozoa were cocultured for 1 h with in vitro matured oocytes and penetration rate and number of sperm per oocyte were evaluated at 18 h post-fertilisation. Exposure of spermatozoa to erastin did not affect sperm fertilising ability (sperm penetration rate; control: 76.49 ± 9.82 vs. E10: 89.42 ± 7.68 or the number of penetrated sperm per oocyte; control: 3.93 ± 0.22 vs. E10: 4.21 ± 0.25). Similarly, the addition of olesoxime did not show significant differences in the number of penetrated oocytes (control: 56.33 ± 20.69 vs. O100: 55.40 ± 18.93) and the spermatozoa able to fuse with the oocyte (control: 4.63 ± 3.41 vs. O100: 3.91 ± 2.70). Our results indicate that, in these conditions, VDAC-2 blocking does not affect sperm fertilising ability, although VDAC2 is involved in boar sperm capacitation. In conclusion, erastin and olesoxime may be considered useful for blocking capacitation without affecting the sperm ability to fertilise.

P 56 | Presence and function of kisspeptin/kisspeptin receptor system in corpora lutea of pseudopregnant rabbits (*Oryctolagus cuniculus*): in vitro studies

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The hypothalamic neuropeptide kisspeptin (KiSS) and its cognate receptors (KiSSR) have crucial function in mammalian reproduction, regulating GnRH production and release. The KiSS/KiSSR gene and protein are also expressed in several reproductive organs including the ovary. In the present study, we examined the expression of the KiSS/KiSSR system and its functional role in corpora lutea (CL) of pseudopregnant rabbits. CL were collected at early- (day 4), mid- (day 9), and late- (day 13) stages of pseudopregnancy following GnRH injection (day 0). KiSS immunoreactivity was localized in the nucleus and cytoplasm of all luteal cells; the density of immune reactive cells decreased from early- to late luteal stage. Immunosignals for KiSSR were detected in the cytoplasm of luteal cells only at early and mid-stage of pseudopregnancy, but not at late stage; the density of immune reactive cells decreased from early to mid-stage. In CL cultured in vitro, the agonist KiSS-10 increased progesterone and decreased prostaglandins $F_{2\alpha}$ and E2 secretion at early- and mid-stages of pseudopregnancy, whereas the antagonist KiSS-234 counteracted the effects of KiSS-10. The present study indicates that KiSS/KiSSR system is present in rabbit CL and that it affects the luteal endocrine activity with a luteotropic effect.

P 57 | Study of coconut water as chilled stallion sperm extender**

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Currently, there are numerous natural and commercial extenders to increase the equine sperm lifespan. However, more affordable and efficient alternatives are being studied. The aim of this study was to evaluate the effect of coconut water in combination to dimethylformamide (DMF) as chilled stallion sperm extender. Semen samples were obtained from the epididymis of 12 stallions. Sperm samples were centrifuged (1000 g/5 min) and resuspended in 4 experimental extenders: INRA 96® (control medium), coconut water (312 ± 3 mOsm/L), INRA 96® with 5% DMF, coconut water with 5% DMF. Motility parameters, osmotic response of the plasma membrane, viability and acrosome integrity were analyzed at 0 h, 24 h and 96 h after storage at 4°C. Data were analyzed by GLM test. Coconut water with or without DMF did not improve sperm quality parameters compared to INRA® at 0 h and 24 h. However, the experimental extender with DMF compared to the control medium significantly improved ($p < 0.05$) sperm motility parameters (56% vs. 41.36%) and acrosome integrity (89.63% vs. 85.38%) after 96 h of storage. In conclusion, coconut water with or without DMF is a suitable chill extender for epididymal equine sperm (Supported by DGA and Fondo Social Europeo (IA2).).

P 58 | Urethral obstruction caused by lithiasis in an Anglo-Nubian buck: a case report**

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Stranguria and dysuria in bucks and rams are very often associated to urolithiasis caused by a high grain and low roughage diets. High

levels of phosphorus and magnesium and low level of calcium from these diets increase the risk of phosphate urolith formation. In our work, a case is described of a 2 years old, 56 kg bodyweight, Anglo-Nubian intact buck referred to the Clinical Hospital of Faculty of Veterinary Medicine from Bucharest, Romania, with a 3 days history of stranguria and hematuria. At the moment of referral, the male showed dysuria, a slightly increased heart and respiratory rate, pallor, decreased rectal temperature and appetite, and no glans penis abnormalities. Ultrasonography exam revealed a distended bladder and a hyperechoic structure in the perineal part of the urethra represented by uroliths. As emergency, a perineal ureterostomy was performed in the ischiopubian region by longitudinal section of bulbospongiosus muscle and urethra. An intermittent suture between uretra and skin was held for 20 days. Although the male was intact we preferred to do urethrostomy instead of a tube cystotomy, because the urethra was obstructed and the owner wanted to keep the buck as teaser. For this clinical case, our aim was to solve the urethral obstruction which has happened after healing.

P 59 | Reproductive response to different doses of progesterone plus ECG or male effect in anestrous Dorper sheep

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The aim of this study was to compare a dose of progesterone plus eCG or progesterone and the "male effect" for ovulation induction in anestrous ewes. In March (26° North L), multiparous Dorper ewes (n = 20; 24–60 month, live weight (LW) = 43.7 ± 1.4 kg, body condition score (BCS) = 2.5 in a 0–5 scale (0 = very thin and 5 = very fat), were divided into two homogeneous groups regarding LW and BCS. The first group of females (n = 10) was fitted with an intravaginal sponge impregnated with 20 mg of P4 (cronolone) for 6 days and, upon removal, 300 IU of eCG were applied (eCG20). The second group (n = 10) received the same progesterone treatment and was then exposed to two sexually active Dorper males (day 0) "male effect" (EM20). The sheep were observed every 12 h (0800 and 1800 h) to determine the percentage of estrus, the percentage of ovulation and corpora lutea number, determined by ultrasonography 10 day after day 0. To determine the association between the categorical variables estrus and ovulation, a Chi-square test was performed; number of corpora lutea was evaluated through ANOVA with the SPSS statistical program. Regarding the estrus percentage, no difference occurred between treatments (eCG20 0% and EM 0%; $p > 0.05$). The ovulation percentage favored to the eCG20 treatment (60% vs. 30%; $p < 0.05$). No differences ($p > 0.05$) for number of corpora lutea was observed; eCG20 = 1.7 ± 0.16 and EM20 = 1.0 ± 0. Based on the results, a significant increase in the ovarian activity was observed in those ewes treated with 20 mg of P4 combined with eCG, compared to the use of the "male effect" with P4 in Dorper ewes in northern Mexico.

P 60 | Passive transfer of immunity: evaluation of mare colostrum quality and immunoglobulin G concentration in the new-born foal

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Ingestion of high quality colostrum at birth is a determinant factor influencing a foal's health. As no antibodies pass through the mare's diffuse epitheliochorial placenta, failure of passive transfer (FPT) is an important cause of morbidity and mortality during the first month of foals' life. FPT is defined as serum immunoglobulin G (IgG) concentration <400 mg/dl at 24 h of age. Mare's breed, age, nutrition and vaccination protocol have been described as having influence on colostrum quality, however, previous results reveal inconsistencies. The aim of this study was to evaluate the influence of different factors related to the mare on colostrum quality (IgG concentration) and its relationship with foal's IgG serum levels between 12 and 24 h of life. In this study, 131 mares of different breeds (Lusitano, French Trotter, Warmblood and Arabian/Anglo-Arabian) and ages (4–10 years old) were monitored during four breeding seasons (2014–2017). All mares were from the same stud farm and had the same pre-partum management, within each year. Colostrum quality was evaluated using a Brix refractometer (RHB-32®) and IgG levels were accessed through a commercial kit (DVM Rapid Test II®). Considering the breed, Arabian/Anglo-Arabian mares showed the lowest Brix % ($p < 0.01$). Age, parity and foaling season had no influence on colostrum quality ($p > 0.05$). However, a progressive increase of colostrum quality was observed until 2016 ($p < 0.05$) which could be ascribed to an improvement of stud farm practices such as nutrition and vaccination protocols. In the present study, a positive correlation between colostrum density and IgG foals' serum concentrations was also detected ($r = 0.335$; $p < 0.001$) supporting the importance of good quality colostrum for passive transfer of immunity.

P 61 | Effect of different commercial extenders on cooled-stored stallion sperm

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The aim of this study was to assess a new extender for stallion sperm cooling, Hippex (Barex Biochemical, De Hoek, the Netherlands), in comparison to other commercial extenders (INRA96, IMV-Technologies, and BotuSemen, Botupharma). 10 samples were collected from 5 stallions (2 ejaculates from each animal), extended in each diluent to a final concentration of 25×10^6 sperm/ml and cooled-stored for 24 h in a styrofoam box. At 0 and 24 h, total (TM,%) and progressive sperm motility (PM,%) were analysed using the Sperm Class Analyzer (Microptic S.L.). Plasma membrane integrity (PMI,%) was assessed using Vital test® (Halotech SL) and sperm DNA fragmentation (SDF,%) was evaluated using the Halomax® Kit (Halotech SL). Results were compared between treatments by ANOVA and expressed as mean ±SEM. SDF at 0 h was lower with INRA96 (I) than using Hippex (H) and BotuSemen (B) ($I = 5.0 \pm 0.5^b$; $H = 7.0 \pm 0.4^a$; $B = 8.2 \pm 0.5^a$). No significant differences at 0 h were found for TM ($I = 94.2 \pm 0.9^a$; $H = 89.4 \pm 2.6^a$; $B = 87.2 \pm 2.6^a$), PM ($I = 70.8 \pm 2.6^a$; $H = 63.0 \pm 2.8^a$; $B = 63.0 \pm 3.7^a$) and PMI ($I = 91.0 \pm 1.1^a$; $H = 90.3 \pm 0.7^a$; $B = 88.6 \pm 3.3^a$). After cooled-stored for 24 h, SDF values were significantly lower for INRA96 (I) and Hippex (H) in comparison to BotuSemen (B) ($I = 10 \pm 0.4^b$; $H = 8.6 \pm 0.9^b$; $B = 13.6 \pm 0.7^a$), respectively. No significant differences were found for TM ($I = 76.4 \pm 2.2^a$; $H = 77.2 \pm 3.3^a$; $B = 72.4 \pm 1.2^a$), PM ($I = 55.4 \pm 4.0^a$; $H = 53.8 \pm 4.3^a$; $B = 54.2 \pm 1.4^a$) or PMI ($I = 86.7 \pm 06^a$; $H = 87.0 \pm 1.4^a$; $B = 86.4 \pm 1.5^a$) among extenders. In conclusion, stallion sperm can be cooled using Hippex as well as INRA96 or BotuSemen, particularly preserving a low DNA fragmentation index up to 24 h. (This study was supported by project AGL2013-42726-R.).

P 62 | Ovicoll™ colloid is useful for selecting high quality spermatozoa from small volumes of cryopreserved ram semen

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Ovicoll™ is a colloid (glycidoxypropyltrimethoxysilane-coated silica) for single layer centrifugation used for sperm selection in several species, but little tested in sheep or cryopreserved semen. We have assayed different protocols for selecting good quality spermatozoa from low volumes of thawed semen, combining colloid formulations, OS (Small, 1 ml) and OL (Large, 2 and 4 ml), RCF (300 and 600 × g; 20 min) and sample volumes (150 and 300 µl). Doses from 3 g (10^8 ml^{-1}) were thawed, pooled and centrifuged (3 replicates). Pellets were resuspended in TALP and analyzed by flow cytometry (viability, apoptosis -% within viable- and mitochondrial status). Data (shown as mean±SEM of %) were analyzed by linear mixed-effects models. Thawed samples yielded poor post-thawing quality (30.7 ± 2.2, 21.4% ± 5.4, 17.7 ± 2.5, respectively). Ovicoll™ increased quality of spermatozoa ($p < 0.001$), with small differences between protocols for viability (54.5 ± 1.9) and mitochondrial status (35.8 ± 1.7). The presence of apoptotic spermatozoa was lower for OL (9.9 ± 1.7 vs. 32.3 ± 2.8, $p < 0.001$), but the proportion of cells with active mitochondria within non-apoptotic spermatozoa was higher for OS (92.5 ± 3.5 vs. 72.8 ± 2.2, $p < 0.001$). Overall, centrifuging 300 µl of sample at 300 × g was the best option, although only OL-4 ml showed $p < 0.05$ for sperm viability in this protocol, with the rest of comparisons being $p > 0.05$. In conclusion, Ovicoll is effective for improving post-thawed ram samples. OS and OL select different sperm populations, and protocols could be adapted to different practical conditions or other requirements (e.g., cell recovery), given the small differences detected. (We thank Ovigen for providing the semen samples and Jane M. Morrell (Uppsala, Sweden) for Ovicoll™ colloid.).

P 63 | The effect of neurokinin A and B on the expression of PRL, D2R and TRHR genes in porcine anterior pituitary cells during the estrous cycle

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Neurokinins are considered to be regulatory factors of reproductive functions and their involvement in the modulation of prolactin secretion is suggested in many species, but in pigs this issue has not yet been elucidated. The current study evaluated the impact of neurokinin A and B on the expression of genes coding for prolactin (PRL), dopamine D2 receptor (D2R) and thyrotropin-releasing hormone receptor (TRHR) in porcine anterior pituitary cells in vitro. Cells isolated from anterior pituitaries of cross-bred gilts (Large White × Polish Landrace) on days 8–10, 15–16 and 18–20 (n = 3 × 5) of the estrous cycle were incubated with NKA and NKB (at doses 10^{-7}, 10^{-8}, 10^{-9} M). The expression level of PRL, D2R and TRHR genes was analyzed using Real-Time PCR and followed by relative quantification ΔΔCt method. Statistical analysis was performed using one-way ANOVA and Fisher's LSD post-hoc test. Neurokinin A elevated ($p < 0.05$) the expression of PRL, D2R and TRHR genes on days 8–10 of the estrous cycle (from control value 0.97 to 2.34, from 0.63 to 2.6 and from 0.78 to 1.9, respectively), TRHR on days 15–16 (from 0.88 to 1.58) and PRL on days 18–20 (from 0.79 to 1.54). In the presence of neurokinin B, the expression of D2R and TRHR genes was increased ($p < 0.05$) on days 8–10 (from 0.63 to 2.79 and from 0.78 to 2.43, respectively), as well as PRL and TRHR on days 18–20 of the estrous cycle (from 0.79 to 1.63 and from 1.17 to 2.16, respectively). The obtained results indicate that neurokinins may exert their effect at the pituitary level through altering the gene expression for prolactin and receptors for hypothalamic factors i.e. dopamine and TRH, involved in the regulation of PRL secretion.

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P 64 | Histopathological studies of mammary gland in dairy cows from Mexico

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Bovine mastitis is one of the most frequent pathologies in dairy cows; histopathological diagnostic allows to accurately establish the udder health status. The aim of this study was to perform the histopathological diagnosis of mammary glands (MG) of cows with or without macroscopic lesions of mastitis. MG samples, one per each quarter, were collected by necropsy during a 6-month period from cows housed in a dairy region of central Mexico. 40 Holstein Friesian cows were classified in 2 groups: i) healthy (H), 30 cows without clinical mastitis lesions, and ii) clinical mastitis (CM), 10 cows showing clinical mastitis lesions. Samples of all MG were fixed in 10% neutral buffered formalin, paraffin-embedded and cut in 5 μm thick sections. The slides were stained with Haematoxylin/Eosin protocol; data were analyzed using descriptive statistics. 10 CM (100%), and 25 H cows (83.3%) showed mastitis in at least one gland. A total of 28 glands (quarters) from CM, and 73 from H were diagnosed with one mastitis form (CM: 64.3% chronic lymphocytic, 32.2% acute purulent, and 3.5% chronic proliferative. H: 54.8% chronic lymphocytic, 24.7% acute purulent, and 20.5% chronic proliferative). Mastitis distribution into group CM was: 14.3% focal (F), 53.5% multifocal (MF), and 32.2% diffuse (D); into group H was: 1.4% F, 82.2% MF, and 16.4% D. Regarding severity, in CM was 32.2% mild (MI), 57.1% moderate (MO), and 10.7% severe (S); in H was 52% MI, 43.9% MO, and 4.1% S. Based on the histopathological findings, we can deduce that in both groups predominate chronic mastitis; a significant number of the "healthy" cases showed subclinical mastitis. This results suggest early diagnostic and appropriate treatment lack in those herds. (Supported by Leipzig University, UNAM PAPIIT IA2049 and PIAPI1).

P 65 | Nutritional supplementation plus testosterone treatment increases libido and scrotal circumference in goat males in resting season

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The aim of this study was to compare the libido and scrotal circumference of grazing bucks induced exogenously to sexual activity and submitted or not to nutritional supplementation in sexual resting season. This study was carried out in northern Mexico (26°N, 104°W), using 12 adult male goats in extensive grazing, divided into 2 groups (n = 6 each; body condition score 2.6 ± 0.57). Group NSGT was fed for 81 days (4th January to 25th March 2017) only with native vegetation, while SGT was fed with native vegetation plus 400 g of commercial concentrate (16% CP) and alfalfa (18% CP). Both groups received 25 mg of testosterone propionate, via IM each third day for 3 weeks (days 56–79). Body condition score (scale 1–4) and scrotal circumference (cm) were evaluated weekly, this data were analyzed with an ANOVA test, in SAS 2002, through an experimental design of random blocks. On March 24th and 25th, a sexual behavior test was performed. Each male was evaluated individually quantifying sexual behaviors (search and consummation). These data were compared using a Chi-squared test (MYSTAT 12). There were differences between groups ($p < 0.001$) in: body condition score (SGT: 3.67 ± 0.63 years NSGT: 2.38 ± 0.44)), scrotal circumference ($p < 0.001$; SGT: 30.00 ± 2.97, NSGT: 26.00 ± 3.59). Sexual search behavior was lower ($p < 0.001$) in NSGT compared with SGT (196 vs. 2051), likewise, the consummation behavior ($p < 0.001$; 7 vs. 142). We conclude that a nutritional supplementation in the resting season increases the body condition score, scrotal circumference and libido, which can be an important factor to induce female goats to ovulate through the male effect.

P 66 | Quantitative ultrasound attributes of ovine fetal lung development during ewe pregnancy

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Intrauterine diagnosis of fetal maturation and development are essential to increase fetal survival and, in humans, is assessed by means of tissue echotexture during ultrasonographic examination. The aim of this study was to examine ovine fetal lungs in order to establish a correlation between the changes in the composition of this organ associated with its maturation and the ultrasonographic characteristics of the images. Twenty-four pregnant ewes were included in the study. Ultrasonographic assessments were performed in B-mode, from the 9th gestational week (GW) until parturition. The lungs were located to evaluate the echogenicity and echotexture. All images were obtained at constant settings of the ultrasound scanner for overall gain, near and far gains and focal points. For quantitative evaluation of the ultrasonographic characteristics, a computerized image analysis was performed using a commercial software program (Image ProPlus®). The software assigns values from 0 (black color) to 255 (white color) and provides an indicator of tissue echogenicity. Mean numerical pixel values (NPVs), pixel heterogeneity (standard

deviation of NPVs), and minimum and maximum pixel values were measured by selecting five circular regions of interest in the lung. Pulmonary NPVmean, NPVmin and NPVmax decreased gradually through GW and mean NPV values ranged from 131.24 ± 22.44 to 64.98 ± 17.53 in the first and last evaluated weeks, respectively. It was concluded that the lung pixel analysis of ovine fetuses proved to be safe and practicable, obtaining satisfactory correlation results between changes in organ echogenicity and its development throughout pregnancy.

P 67 | Combined addition of superoxide dismutase, catalase and glutathione peroxidase improves cooled storage of equine spermatozoa

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Stallion spermatozoa experience oxidative stress during cooled storage, impairing subsequent sperm function. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are the main enzymatic components of the endogenous antioxidant defense of equine seminal plasma, and counteract reactive oxygen species. However, semen dilution reduces endogenous antioxidant levels. The aim of this study was to evaluate the effect on stallion semen quality during 72 h storage at 5°C of supplementing the semen extender with SOD, CAT and GPX. Ejaculates from seven stallions were split in two and diluted in INRA96 without (control) or with addition of 15 IU/ml each of SOD, CAT, and GPX. Semen analysis was performed within 3 h after semen collection and every 24 h during chilled storage. Viability, motility, DNA fragmentation, and relative levels of activated caspase-3 were evaluated. Differences between control and treated samples were evident after 48 h and 72 h. Antioxidant supplementation inhibited the increase of activated caspase 3 as indicated by Western Blot after 72 h ($p < 0.05$). Concomitantly, viable i.e. eosin negative sperm (69 ± 10% vs. 64 ± 11%), total motility (51 ± 20% vs. 37 ± 17%) and rapidly moving sperm (22 ± 14% vs. 16 ± 11%) were higher in treated than control samples ($p < 0.05$). A storage-dependent increase in DNA single strand breaks (TUNEL assay) was ameliorated by antioxidant addition. In conclusion, adding equal concentrations of SOD, CAT and GPX to a semen extender suppressed caspase-3 activation and preserved stallion sperm motility, suggesting a protective effect at the mitochondrial or axonemal level.

P 68 | Under-nutrition reduces the testicular growth of male goats exposed to artificial long days in autumn and winter

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In well-nourished bucks, the sexual activity is stimulated during the sexual rest by exposure to artificial long days. This study aimed at determining the testicular response of undernourished bucks exposed to artificial long days. The well-nourished bucks were fed 2.0 times, whereas the undernourished ones were fed 0.7 times of the maintenance requirements from September 1st to March 30th (n = 7 each). All bucks were exposed to 2.5 months of long days (16 h of light/day) from November 1st. Body condition (BC) and testicular circumference (TC) were determined every 2 weeks from September 1st to March 30th. Both variables were analyzed by a 2-way ANOVA (time and group) for repeated measurements followed by t-test for individual points comparisons. BC and TC changed throughout the study, and these changes differed between groups of bucks ($p < 0.001$). In well-nourished bucks, BC remained high from September (2.7 ± 0.1) to March (3.0 ± 0.2), whereas a significant decrease was observed in undernourished bucks (from 2.7 ± 0.1 to 1.6 ± 0.1, respectively; $p < 0.01$). In well-nourished bucks, TC decreased from September (27 ± 0.5 cm) to November (24 ± 0.8 cm). Then, TC increased to reach the maximum on 30 March 30th (29 ± 0.8 cm). In undernourished bucks, TC decreased from September (27 ± 0.5 cm) to January (23 ± 0.6), reaching then the maximum value on March 30th (25 ± 0.7). Therefore, TC was higher in well-nourished than in undernourished bucks from January to March ($p < 0.01$). It is concluded that in undernourished bucks TC gets lower than in well-nourished ones when exposed to artificial long days.

P 69 | Genome-Wide Association Study of reproductive traits in a gene pool breed of the Russian White Chickens

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Studies of candidate genes and SNP associations with reproductive traits provide a valuable source of information for managing small populations of gene pool breeds and their genomic selection. Genome Wide Association Studies (GWAS) were done using SNP chip genotyping technology in the Russian White chicken breed maintained in a genetic research collection of rare and endangered

poultry breeds. This breed was developed in the early 1930s by crossing local Russian chicken breeds and White Leghorns, with selection of the breed being started in 1954. An increased sustainability of growing chicks to low temperatures and the white color of embryo down are main breed's distinctive features. Understanding of the genetic background of these unique traits has become a vital part of the ongoing breeding program. For that purpose, the breed was genotyped using the Illumina 60K SNP Chicken BeadChip. Phenotypic data on age at first egg and pedigree were collected from 163 birds. Genomic data were obtained using the Plink 1.9 software. SNP quality control criteria were applied, with minor allele frequencies being less than 0.001, and Hardy Weinberg equilibrium, more than 0.0001. The GWAS analysis was performed using a mixed model approach employing EMMAX software. A significant effect ($p = 9.985225 \times 10^{-6}$) of a SNP, rs317931060, was detected. Two genes, FGF9 and TNFRSF19, were close to this SNP, with the distance being 280 and 314 Kb, respectively. Both genes are responsible for sex determination and cell proliferation and differentiation. (Studies were supported by a Russian Scientific Foundation grant (No. 16-16-04060) and by a FASO program for developing a bio resource collection.).

P 70 | Discrimination of early pregnancy and endometrial cyst by ultrasonographic assessment of uterine echotexture in mares

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The ultrasonographic image of a tissue depends on the histological structure of the tissue and is defined as echotexture. The present study was performed to demonstrate tissue differentiation of uterine ultrasonographic image by computer assisted analysis programs in mares with endometrial cyst and early pregnancy. A total of 32 thoroughbred Arabian mares aged between 10 and 15 years were included in the study, which were diagnosed with pregnancies of 13–15 days (group I, n:16) and endometrial cyst (group II, n:16). Images obtained during ultrasonographic examination were recorded and transferred to the computer. Later, the mean gray value (MGV), heterogeneity and contrast values were measured with a special program (ImajeJ 1.42q; NIH, USA-Image Processing and Analysis Java). The recorded images were assessed and obtained data were statistically analyzed by independent-samples T test. MGV from uterine echotexture values was higher in pregnant mares (78.90 ± 0.80) than the mares with endometrial cysts (65.83 ± 1.48) ($p < 0.001$). Heterogeneity obtained from echotexture parameter of pregnant mare (34.63 ± 1.40) was found to be higher than that of the mares with endometrial cysts (27.43 ± 1.36) ($p < 0.01$). When the contrast of the last echotexture parameter was evaluated, it was higher for pregnant mares (192.25 ± 1.76) than for mares with endometrial cysts (170.00 ± 4.60) ($p < 0.001$). In conclusion, it was determined that the mean gray value (MGV), heterogeneity and contrast measurement of echotexture parameters may be used for distinguishing the mares with early pregnancy from the mares with endometrial cyst.

P 71 | Evaluation of biosafety of equine amniotic membranes for allogeneic use – a pilot study using PCR screening on peripheric maternal blood and paired amniotic membranes**

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Transplantation of amniotic membranes (AM) is increasingly used for equine tissue reconstruction. However, there is currently no published data on systematic screening of donor mares for infectious diseases, in contrast to human medicine. To minimize the risk of iatrogenic donor-borne contamination, we questioned the biosafety of equine AM. We hypothesized that the processed amnion layer of AM is less exposed than peripheric maternal blood (PMB) to pathogens, aiming to assess the risk of vertical contamination of AM from mares potentially carrying common equine infectious agents. The study was conducted on 6 fetal membrane collections during foaling seasons 2016–2017. Systematic Coggins test and PCR screening for *B. caballi*, *T. equi*, *A. phagocytophilum*, *B. burgdorferi*, *Leptospira spp*, *Mycoplasma spp*, *R. equi*, equine arteritis virus, equine herpesviruses type 1, 2, 4 and 5 were performed on PMB. The amnion layer was isolated, processed and frozen down at −80°C. Mycoplasma PCR screening and bacteriological and fungal cultures were performed to exclude environmental contamination. In case of one or more positive PCR result(s) on PMB, a PCR analysis for the detected pathogen(s) was conducted on a biopsy sample of the associated AM. All PMB samples were PCR positive for at least one pathogen: 3 for EHV2, 3 for EHV2 and EHV5. All AM samples were negative for these specific pathogens. In conclusion of this pilot study, processed equine AM appear free of donor-borne contamination, even for vertically communicable pathogens such as gammaherpesviruses. More data is needed to assess the residual risk of contamination of amnion tissue when the mare is positive for one or more infectious agent(s). Also, emerging pathogens like hepaciviruses should be included in the future.

P 72 | Dynamic remodeling of Notch signaling proteins in bovine spermatozoa during the acrosome reaction

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Mammalian spermatozoa (Spz) must undergo capacitation in order to acquire fertilization competence. One of the late capacitation-associated events consists in the Spz ability to undergo the acrosome reaction (AR), which is a pre-requisite for Spz-oocyte fusion. Following our report first illustrating the presence of Notch signaling proteins in bovine Spz, here we evaluate their involvement during the AR. Viable frozen-thawed bovine Spz obtained following swim-up were incubated in TALP-Sperm with heparin and the AR was induced by the addition of a Calcium Ionophore (CaI) (0.7 mM). Spz were then processed for immunofluorescence using PNA and each of the anti-Notch antibodies. PNA allowed the differentiation of the following acrosome status: non-reacted (NR), reacting (R) and acrosome reacted (AR). Data were analysed using Fisher's exact test. NOTCH2, DLL4 and JAG1 proteins have a localization pattern related with the sperm acrosome status. As acrosome reaction proceeds, NOTCH2 and JAG1 proteins are re-localized from the apical (A) to the post-equatorial (PE) region ($p < 0.05$). DLL4 was only detected in acrosome intact or reacting Spz and was absent in acrosome reacted cells (the protein is lost with the acrosome). These dynamic protein patterns during AR suggest that NOTCH2 and JAG1 proteins are involved in the regulation of the AR, whereas DLL4 is possibly implicated in the acrosome stability. (Funding: FCT, UID/CVT/00276/2013).

P 73 | The effect of hormonal stimulation on the protein composition of carp spermatozoa

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Hormonal stimulation in common carp is a routine practice to enhance sperm production and control gamete maturation. Without hormonal stimulation, carp males either do not spawn at all or the collected milt is characterized by low and variable quality. The aim of the study was to compare proteome of spermatozoa from control males injected with PBS (n = 6) and hormonally stimulated males with Ovopel (n = 6). Flow cytometry and computer-assisted sperm analysis were used to evaluate changes in viability, ROS level and motility of spermatozoa. The spermatozoa proteins changed in abundance due to hormonal stimulation and were identified and quantified by two-dimensional difference gel electrophoresis (2D-DIGE) coupled with mass spectrometry. Hormonal stimulation increased sperm volume ($p < 0.01$), total sperm count ($p < 0.0001$) and sperm viability ($p < 0.05$). No differences were observed in sperm motility parameters and the number of ROS-positive cells. A total of 52 sperm proteins were found to be differentially regulated ($p < 0.05$; fold change 1.2) by hormone treatment. These proteins were associated with cellular movement, cellular assembly and organization, protein translational modification and protein folding. Moreover, protein ubiquitination, stress response and TCA were identified as the canonical pathways most affected by differentially abundant proteins. Differentially express proteins were localized to the cilia, axoneme, mitochondria and cytoplasm. Our results, for the first time indicated that hormonal stimulation is associated with changes in protein content of carp spermatozoa. This study contributes for unraveling molecular mechanisms of hormone action on sperm structure and functions in fish. (Supported by the National Science Centre, Poland 2016/21/B/NZ9/03620.).

P 74 | Mammary fibrosarcoma in a female dog with ovarian remnant syndrome – a case report

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Fibrosarcoma is a malignant tumor with rare involvement of the mammary gland in bitches. The ovarian remnant syndrome (ORS) is defined as the presence of functional ovarian tissue after an ovariohysterectomy. A 13-year-old, female dachshund was presented with vaginal bleeding, vulvar swelling and mammary gland tumor. Blood test revealed high level of 17β-estradiol and low concentration of progesterone. Abdominal usg showed adhesions between ovarian pedicles and the surrounding intestines. Cytology of mammary tumor revealed many spindle-shaped cells with pleomorphic nuclei, indistinct cell borders; intermixed with collagen fibers and epithelial cell clusters. During an exploratory laparotomy ovarian tissue in the area of the right ovarian pedicle was found and confirmed histopathologically. Moreover, histopathology supported by immunohistochemistry of the mammary tumor revealed a fibrosarcoma. The neoplasm was composed of pleomorphic spindle-shaped cells arranged in an interwoven pattern. The immunohistochemistry for cytokeratin (CK–), vimentin (Vim+), p63(–), S100(–), α-Actin(α-SMA–) confirmed its mesenchymal origin. To the periphery of the tumor tubular structures and cystic ducts of the mammary gland were present. Additionally, the expression of estrogen (ER) and progesterone (PR) receptors were examined. Only a low PR expression was noted in fibrosarcoma, whereas ER and PR had a high expression in mammary epithelium. Chest x-ray and the histopathology of inguinal lymph node did not reveal any metastasis. Our results demonstrated that ORS in dog might be the cause of mammary fibrosarcoma without hormonal receptor expression. (Support: National Science Centre, Poland, project DEC-2017/01/X/NZ5/01430).

P 75 | Real-time Ultrasound Elastography findings in the different stages of reproductive cycle in mare

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The real-time ultrasound elastography (RTUE) is an advanced technique allowing estimation of tissue deformities after sequential and fractional change in tissue. The aim was to evaluate the parameters of qualitative RTUE during different stages of reproductive cycle in the mare. The RTUE protocol, using ESAOTE MyLab Alpha with 3–11 MHz probe, was conducted on 6 Warmblood mares sequentially in winter anestrus, estrus and diestrus. The occurrence of follicles and CL and the uterine oedema was used to discriminate the different stages of cycle. Afterwards, the Average Percentage of Pixels of Each Color (APPEC) of uterine body was estimated and the grading assessment was performed based on a scale 1–4 (1 = mostly hard, 2 = intermediate hard, 3 = intermediate soft, 4 = mostly soft). No significant differences ($p > 0.05$) in APPEC parameters among mares in the same stage of cycle were observed. In estrus structure was mostly hard (scale 1: 44.8%±6.34), stiffer than in diestrus (scale 1: 32.6%±3.57) and anestrus (scale 1: 29.2%±4.72). In scale 2, larger share of intermediate hard structures in estrus (scale 2: 27.2%±4.21), less in diestrus (scale 2: 5.1%±1.32) and least in anestrus (scale 2: 1.7%±0.33) was obtained. The % of intermediate soft structures increased from estrus (scale 3: 7.2%±2.06) to diestrus (scale 3: 24.8%±2.27) and anestrus (scale 3: 44.7%±4.74). The content of the softest structures did not differ in estrus (scale 4: 22.8%±4.54), diestrus (scale 4: 34.8%±3.57) and anestrus (scale 4: 24.5%±2.80). RTUE provides detailed data of uterine structure in various stages of reproductive cycle according to differences in elastic properties and is a feasible supplement to the mare's uterus examination.

P 76 | Assessment of cell growth and differentiation markers in equine endometrial fibrosis

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Equine endometriosis is considered a result of recurring inflammation, directly connected with number of pregnancies and genetic predispositions. Deregulation of pathway of cell growth, differentiation, mitogenesis and motogenesis is suspected to be one of the causes of stromal and periglandular fibrosis. The aim of this preliminary study was to establish the expression of regulatory proteins: HGF (Hepatocyte Growth Factor), IGF1 (Insulin-like Growth Factor 1), bFGF (basic Fibroblast Growing Factor), VEGF (Vascular Endothelial Growth Factor) in cases of endometriosis. Biopsy samples were taken from Warmblood mares (n = 24; n = 6 in each biopsy group) and fixed according to HE and IF staining protocols. The expression of all markers was estimated using confocal microscopy and quantified with scanning cytometry. The quantitative results (mean±SD expression of immunopositive cells) were compared using one-way ANOVA followed by Tukey's multiple comparisons test. HGF expression was invariable in I (1.80%±0.30), IIA (2.22%±0.14) and IIB (2.16%±1.36) in opposite to decrease in III (0.46%±0.01). IGF1 expression was nearly equal in I (2.27%±0.49), IIA (1.10%±0.66), IIB (0.84%±0.94) and III (1.20%±0.07). BFGF expression was higher in I (10.89%±0.72) than in IIA (3.61%±0.67), IIB (3.13%±0.87) and III (2.02%±0.68). VEGF expression was not correlated with degree of fibrosis (I: 7.11%±1.76; IIB: 7.69%±4.92; IIA: 2.83%±0.57; III: 2.85%±0.25). The bFGF may be important in initial stage of fibrosis when the secretory function of endothelial cells is limited, whereas the HGF in the final stage of fibrosis, when cells growth, division, mobility and differentiation are terminally affected. We suggested bFGF and HGF as an interesting markers, possible to consider in further investigations.

P 77 | Effect of inbreeding on bull sperm head morphometry

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It is accepted that bulls' fertility is reduced by inbreeding, because it may also affect bull sperm quality. However, the underlying physiological mechanism of this effect is still unknown. The aim of this study was to investigate the influence of inbreeding on bull sperm head morphometry, and to relate possible changes in sperm head dimensions to inbreeding level. Thirty four Retinta bulls were grouped in two groups according to their individual inbreeding coefficient (F): (i) high inbreeding group (HI; $F \geq 13.5\%$; n = 16), and (ii) low inbreeding group (LI; $F < 12.2\%$; n = 18). Frozen semen samples (one per bull) were thawed and analyzed with the Sperm Class Analyzer automatic sperm morphometry analysis system (ASMA). At least 100 spermatozoa were assessed per sample. Sperm head morphometric values (length, width, area, perimeter, and ellipticity) were compared between groups using a Kruskal-Wallis ANOVA. Correlation

was performed by Spearman non-parametric analysis. Sperm head dimensions were significantly ($p < 0.01$) smaller in HI bulls compared to LI ones (length: 10.63 ± 0.02 vs. 10.99 ± 0.12 μm; width: 5.13 ± 0.01 vs. 5.39 ± 0.01 μm; area: 44.77 ± 0.13 vs. 47.80 ± 0.12 μm; perimeter: 27.34 ± 0.05 vs. 28.15 ± 0.05 μm), whereas the opposite was found for ellipticity (length/area: 2.07 ± 0.00 vs. 2.00 ± 0.00; $p < 0.001$). Significant ($p < 0.001$) relationships were observed between inbreeding level and length ($r = -0.15$), width ($r = -0.34$), area ($r = -0.31$), perimeter ($r = -0.27$), and ellipticity ($r = 0.23$). In conclusion, our results demonstrated that high inbreeding levels affect bull sperm head morphometric parameters.

P 78 | Detection of *Taylorella asinigenitalis* by real time PCR in different breeds of *Equus asinus* in Spain

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Taylorella asinigenitalis is a bacterium closely related to *Taylorella equigenitalis*. It was isolated for the first time in 1998 in the United States from donkeys and horses and has been later identified in several countries in Europe like Sweden, Italy and France. There are no data about the prevalence in Spain; therefore, the aim of this study was to determinate the presence of *T. asinigenitalis* in donkeys from different breeds and different areas in Spain. 221 swabs were taken from 106 donkeys (21 males and 85 females) from three different breeds (Majorera, Andaluza and Zamorano-Leonesa), in three Spanish provinces (Ávila, Badajoz and Zamora). Males were sampled from the penis, urethra and urethral fossa, while females were sampled from the clitoral fossa and sinuses. All swabs were transported to the laboratory in charcoal Amies medium and processed in less than 48 h where the DNA was extracted by using a commercial kit. A real-time PCR to identify *T. asinigenitalis* was used in all DNA extractions. *T. asinigenitalis* was identified in the three Spanish donkey breeds sampled. The prevalence for *T. asinigenitalis* in the study was 20.75%. The prevalence for males and females was 71.43% and 8.23%, respectively. The prevalence by breed was 91.67% in the Majorera breed, 3.85% in the Andaluza breed and 14.71% in the Zamorano-Leonesa breed, which matched the prevalence in the different provinces (Ávila, Badajoz and Zamora, respectively). *T. asinigenitalis* is present in *Equus asinus* with a varied prevalence in the Spanish provinces sampled, being widely extended in Ávila. However, further studies in other regions are necessary to confirm this prevalence throughout Spain.

P 79 | Modulating effects of dietary clinoptilolite (CPL) on progesterone (P4) and insulin-like growth factor 1 (IGF-1) blood concentrations in Holstein-Friesian cows during pregnancy and early lactation

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Objectives: To study the effects of dietary zeolite CPL on P4 and IGF-1 blood concentrations in Holstein Friesian (HF) cows during pregnancy and early lactation. Methods: Twenty HF-cows, aged 3–5 years and kept on a commercial farm near Đurđevac, Croatia were used in the study. They were assigned into two groups, each of 10 cows. The cows from the treatment group received 100 g/day of natural CPL modified by vibroactivation and micronization (Vibrasorb, Viridisfarm, Podpićan, Croatia). Blood samples were taken on days 90, 180, 210 and 240 of pregnancy, on day 10 before and day 0 after parturition, and on days 5, 12, 19, 26, 40 and 60 of lactation. Serum concentrations of P4 and IGF-1 were determined using ELISA. The obtained data were statistically analysed using the ANOVA method with repeated measurements. Differences were considered significant at $p < 0.05$. Results: The average values of P4 (6.25 ± 0.73 ng/ml vs. 6.00 ± 0.58 ng/ml) and IGF-1 levels (425.20 ± 59.36 ng/ml vs. 397.21 ± 41.23 ng/ml) were higher in the CPL-fed than in the control cows. During pregnancy, the highest level of P4 was recorded in the treatment group on day 90 (11.84 ± 1.78 ng/ml) and in the control group on day 240 (11.25 ± 1.69 ng/ml). In CPL-fed cows ovarian cyclity resumed on day 33 postpartum (PP), when the IGF-1 level was highest during the PP period, which was also higher ($p < 0.05$) than in the control cows (729.36 ± 190.39 ng/ml vs. 443.44 ± 141.91 ng/ml). Also, a consecutive increase of P4 levels was recorded from days 40 to 60 PP (5.84 ± 1.88 ng/ml and 7.87 ± 2.18 ng/ml). Conclusions: A dietary CPL preparation exhibited modulating effects on the endocrine status of dairy cows by increasing their P4 and IGF-1 serum levels during the PP period, which may influence their reproductive efficiency.

P 80 | Expression profiles of critical miRNAs in ovine endometrium during the peri-implantation

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The present study aimed to elucidate expression of critical ovine endometrial miRNA profiles during the peri-implantation. For

this purpose, endometrial samples were collected on days 12 (pre-implantation, n = 4), 16 (implantation, n = 4) and 22 (post-implantation, n = 4) of pregnancy, and on their corresponding days 12 (n = 4), 16 (n = 4) and 22 (n = 4) of the estrous cycle. According to our previous global endometrial miRNA profile data, ten miRNAs including oar-miR-218a, oar-miR-370-3p, oar-miR-379-3p, oar-miR-380-3p, oar-miR-411a-3p, oar-miR-411b-5p, oar-miR-485-5p, oar-miR-493-5p, bta-miR-1185, and gga-miR-1765 that had differential expression profiles between cyclic and pregnant groups were selected and validated by RT-qPCR in the ovine endometrium. Expression patterns of most of these 10 miRNAs were similar on day 12 and 22 (up regulation) while day 16 miRNA expression profiles displayed an opposing pattern (down regulation). Target genes of examined miRNAs that showed differences in the ovine endometrium were defined and found to be involved in cellular differentiation, adhesion, trophoblast/placental formation, implantation and endometrial secretions. According to those results, we could suggest that the ovine endometrium is very strictly regulated by miRNAs during the peri-implantation. (Supported by TUBİTAK 214O643).

P 81 | The influence of elevated temperature on the development of cattle embryos co-cultured with bovine oviduct epithelial cells (BOECs) in the context of embryonic genome activation

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Global warming causes a significant reduction in fertility in cattle, including early embryo development. In vitro studies indicate that bovine embryos from zygote to 8-cell stage are thermosensitive but that from 8-cell stage to blastocyst are thermotolerant, which is connected with the activation of the embryonic genome (maternal-embryonic transition-MET). The aim of this study was to evaluate the effect of elevated temperature on the development of cattle embryos from 8-cell to blastocyst stage and define the blastocyst cell numbers. Zygotes obtained in vitro were co-cultured with bovine oviduct epithelial cells (BOECs) to the 8-cell stage (72 h post fertilization) at control temperature (38.5°C) and from 8-cell to blastocyst stage (168 h post fertilization) both at control (38.5°C) and elevated temperatures (40.5°C and 41°C). The embryo development and the blastocyst cell numbers were analysed by Statgraphics 5.0 Centurion (USA). At the control temperature, the embryos developed normally from zygote to blastocyst stage (29.87 ± 2.35) but at elevated temperatures of 40.5°C and 41°C, the blastocyst rate was statistically significantly lower (17.41 ± 1.63; 14.44 ± 2.02) ($p < 0.001$). The mean cell number in blastocysts from the control group (132.2 ± 1.7) had a statistically higher significance than those from the groups at elevated temperatures of 40.5°C (117.5 ± 1.9) and 41°C (115.33 ± 1.8). In conclusion, in spite of the fact that cattle embryos after maternal-embryo transition are thermotolerant, this study demonstrates that elevated temperatures lead to a decrease in embryo development and quality. (Financed by COST 453/N-COST/2009/0).

P 82 | Trichostatin A-assisted epigenomic modulation of porcine bi-transgenic adult cutaneous fibroblast cells (ACFCs) gives rise to increased expression of human recombinant α1,2-FT and α-Gal A enzymes

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This study was conducted to explore whether trichostatin A-assisted epigenomic modulation (TSA-EM) can affect the expression of α1,2-rhFT and α-rhGal A immune system enzymes (ISEs) in ex vivo proliferating ACFCs derived from 2 × TG (hFUT2 × hGLA; n = 3) pigs produced for the needs of xenotransplantation. The ACFC lines at passages 2–3 were treated with 50 nM TSA for 24 h and the total protein was next isolated to analyse the expression of α1,2-FT and α-Gal A ISEs by Western blot. All cell cultures were independently repeated in triplicate. For both test 2 × TG and control non-transgenic (nTG) samples, the expression levels (ELs) of α1,2-FT and α-Gal A proteins in TSA+ cells were more than twofold higher (FUT2-2 × TG: 0.348 ± 0.017^{A}; GLA-2 × TG: 0.222 ± 0.011^{a}; FUT2-nTG: 4.508 ± 0.230^{A}; GLA-nTG: 0.722 ± 0.036^{a}) as compared to ELs in TSA− cells (FUT2-2 × TG: 0.174 ± 0.008^{B}; GLA-2 × TG: 0.103 ± 0.005^{b}; FUT2-nTG: 1.664 ± 0.083^{B}; GLA-nTG: 0.314 ± 0.016^{b}) [A,B $p < 0.01$; a,b $p < 0.05$; ANOVA and Tukey's HSD post hoc test]. In summary, TSA-EM of porcine 2 × TG and nTG ACFCs appears to result in enhanced transcriptional/translational (T/T) activities of incorporated hFUT2 transgene and endogenous pFUT2 gene. Increased abundance profile of porcine α1,2-FT protein in TSA+ nTG cells can arise from an approximately 80% amino acid sequence similarity of α1,2-pFT to homologous α1,2-rhFT. Moreover, TSA-EM of 2 × TG and nTG ACFCs has brought about elevated ELs of α-Gal A proteins. It is worth highlighting that pigs are characterized by lack of α-Gal A protein expression, which stems from species-specific silencing both alleles of pGLA gene. Therefore, TSA-EM of nTG ACFCs seems to trigger onset of T/T activity for pGLA gene. (Funded by grant No. INNOMED/I/17/NCBR/2014 and statutory activity No. 01-19-04-21.).

P 83 | New echotexture parameters to evaluate the testicular parenchyma in bulls

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We have developed several algorithms for analysation of testicular ultrasonograms, according to the distribution of black, white and grey pixels and also according to the size and density of hypoechogenic areas. We have performed several validation assays to evaluate the relationship between these echotexture parameters and bull epididymal sperm quality (assay 1, n = 31 bulls), ejaculated semen quality (assay 2, n = 45 bulls) and frozen-thawed sperm quality (assay 3, n = 47 bulls). The testicles were scanned, using an EXAGO (ECM, France) connected to a 7.5 MHz linear probe and images were analyzed by means of the new algorithms. In semen samples, we evaluated the main sperm features with main focus on sperm morphoanomalies. We established a cut-off value of maximum 15% major sperm abnormalities in the ejaculate (sperm head anomalies, intermediate piece formation anomalies, and proximal cytoplasmic droplets) as a picture of a mature semen sample. Analysis of data by ANOVA indicates that testicles producing subfertile samples differed significantly in 5 of 6 echotexture parameters from testicles producing fertile sperm samples ($p < 0.01$). It was also indicated that bulls producing immature ejaculates have significative differences in the mean density of hypoechogenic areas of the testicular ultrasonograms ($p < 0.05$). Logistic regression indicated that the density of hypoechogenic areas in the ultrasonogram of a testicle could predict the fertility or subfertility of an epididymal semen sample (assay 1). This parameter could also predict the maturity of a young bull (assay 2). Finally, in our third assay, 3 echotexture parameters were significantly related to the quality of frozen-thawed sperm. (This work was supported by Eureka E!11188 and IDI-20170220).

P 84 | Fine-tuning of estrus synchronization protocols to enhance the outcome of fertility rates in trans-cervically inseminated INRA180 ewes with or without mucus removal

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Estrus synchronization with progestogens is widely applied for controlling reproduction when performing artificial insemination in sheep. No information is available about these products regarding fertility mainly in a Moroccan prolific sheep and especially when the mucus is removed or not. The aim of the present study was to compare the efficacy of Chronogest® and Eazi-breed TM CIDR® G (controlled internal drug release device) vaginally inserted for 14 days for estrus synchronization combined with a fixed dose of PMSG (300 IU) with or without mucus removal on the success of artificial insemination in INRA 180 ewes. The mucus was removed from animals in standing position using a vaginal speculum. N = 60 ewes from INRA180 breed aged >2 years old were randomly chosen and assigned to five groups of 12 ewes (Group 1: CIDR with mucus removal, Group 2: CIDR with mucus, Group 3: Chronogest® with mucus removal; Group 4: Chronogest® with mucus and Group 5: served as a control without progestogen and PMSG and naturally mated). At CIDR and sponges removal, 300 IU PMSG were injected. The data of fertility rate were compared using Pearson's chi-squared procedures. The results showed that fertility of ewes lambing to the first service was 56.25%, 70%, 35.71%, 68.42%, respectively for Group 1, 2, 3 and 4 with no difference observed between group 2 and group 4. In group 5 the highest lambing rate was observed (100%). Whatever the device was, the groups with mucus had a higher lambing rate than those after mucus removal. It has to be concluded that CIDR-G and sponges gave good results in the presence of mucus in ewes more than 2 years old. This study has to be confirmed in a larger number of animals.

P 85 | Power and colour Doppler ultrasonography for the evaluation of the vasculature of ovarian abnormality in mare

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The aim of the present study was under taken to diagnosis of reproductive problems using color Doppler ultrasound in mare by monitoring ovarian structures vascularization. Mares ovarian structures (n = 21) were classified into normal preovulatory follicle, corpus luteum (CL), inactive ovary, granulose cell tumor (GCT) and anovulatory follicles. Results revealed that, the mean red color blood flow vascularization area of the dominant follicle was higher in corpus luteum (CL), granulose cell tumor (GCT), and anovulatory follicle than those obtained in the dominant follicle. In addition, the mean blue color blood flow vascularization area of the corpus luteum was significantly ($p < 0.05$) higher than those observed in the inactive ovary. The ovaries with GCT have the highest color (red + blue) blood flow vascularization as compared to other ovarian structures. In addition, the mean red and blue color blood flow vascularization area of the GCT was significantly ($p < 0.05$) higher than those observed in the inactive ovary. No significant difference could be detected between the ovarian granulose cell tumor and anovulatory compared to the corpus luteum and dominant follicles on the power Doppler blood flow. However the means of power Doppler blood flow were significantly ($p < 0.05$) higher in the corpus luteum than those obtained in the dominant follicles. It could be concluded

that Doppler ultrasound could distinguish between normal follicle, anovulatory follicle, functional corpus luteum and inactive ovaries.

P 86 | The effects of preanesthetic Sildenafil citrate usage on feto-maternal circulation in pregnant rabbits

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The effects of preanaesthetic Sildenafil citrate (SC) on fetomaternal circulation in two anaesthesia protocols were studied in rabbits. Thirty pregnant healthy New Zealand rabbits were divided in five groups. In Group 1 and 2, after 60 min of SC administration (33 mg, per os), they were anaesthetized with medetomidine (0.25 mg/kg)/ketamine (50 mg/kg) im combination, or propofol (8 mg/kg and 1 mg/kg/min) i.v. for an hour. There was only SC administration in Group 3 as control. In Group 4 and 5, following placebo, similar medetomidine/ketamine and propofol injections were done, respectively. Prior to SC, during anaesthesia, and 24 h pulsatil (PI) and resistance (RI) indices, fetal heart rate (FHR) on uterine and umbilical arteries from most caudal fetus, and maternal vital findings were recorded. The lowest uterine PI and RI were in Group 1 in all periods during 24 h; the highest PI was seen in Group 2 between 30–60 min ($p < 0.05$). Fetal pulsatility and resistance were higher in both propofol groups than medetomidine-ketamine group (Group 4) at 30 min ($p < 0.05$). The minimal fetal pulsatility ($p < 0.05$) and resistance ($p < 0.01$) were in Group 4. FHR were above 225 bpm in Group 1 and 4, and highest values were seen between 10-60 min ($p < 0.01$). High maternal pulsation and bradycardia were observed in propofol groups, but not significantly different. It is concluded that SC has an effect on decreasing the uterine vascular resistance during 24 h, improving uterine perfusion and maternal vitality without making any incompatibility on medetomidine-ketamine combination. Meanwhile, the increased feto-maternal risk in using sildenafil-propofol combination should be considered in pregnant rabbits.

P 87 | Accuracy of pregnancy specific blood or milk tests for late embryonic mortality diagnosis in dairy cattle

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The aim of this study is to investigate the extent to which embryonic mortality (EM) can be determined in cows using pregnancy-associated bovine glycoprotein serum(s) and milk (m) test (PAG), and pregnancy-specific protein B (PSPB-s) test. The samples were taken from 58 Holstein cows at 28, 30, 32 and 40 days after artificial insemination (AI). Ultrasonography (US) was performed on days 30 and 40 after AI. Measurements were carried out on 9 cows whose pregnancy was confirmed and 9 cows whose EM was determined. In PAG-s, there was a significant difference ($p < 0.05$, $p < 0.01$) between the measurements on 40th day when EM was detected and on the 28th and 32nd days of pregnancy. In the PAG-m and PSPB-s tests, there was a difference ($p < 0.05$) between the 32nd day and the 40th day when EM was detected. Significant differences in PAG-s ($p < 0.001$) and PSPB-s ($p < 0.01$) was obtained between animals that were pregnant on the 40th day and animals that had EM on the 40th day according to US observations. Sensitivity and specificity rates were determined as 100% from the pregnant animals on the 28th day and this ratio was found to be 22.2% and 44.4% and sensitivity of 57.1% and 77.8%, respectively, on the 40th day when EM was detected. No compliance was determined in the kappa test performed on the 40th day which is in between the tests and the US results. In the cows determined to be pregnant on the 30th day, the test results obtained on the day when EM was determined by the US method on the 40th day and the diagnosis of EM were not individually reliable in every animal. However, it is thought that in between 28th and 40th day, statistically significant difference ($p < 0.001$) and statistically significant difference ($p < 0.01$ and $p < 0.001$) between the pregnant and EM animals can be predicted.

P 88 | New echotexture parameters to evaluate the testicular parenchyma in rams

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We have developed several algorithms to analyze testicle ultrasonograms: EC1 (black pixels), EC2 (white pixels), EC3 (mean gray level of pixels), Density (density of hypoechogenic areas), Diameter (mean diameter of hypoechogenic areas) and Area (total percentage of hypoechogenic area). The aim of this work was to validate these algorithms in rams. The paired testicles of 39 rams were scanned, using an EXAGO (ECM, France) connected to a 7.5 MHz linear probe and images were analyzed by means of the new algorithms. In semen samples, we evaluated the main sperm features with the main focus on sperm morphoanomalies. We established a cut-off value of maximum 15% major sperm abnormalities in the ejaculate (sperm head anomalies, intermediate piece formation anomalies, and proximal cytoplasmic droplets) as a picture of a mature or fertile semen sample. The Pearson correlation showed a significant moderate correlation with all echotexture parameters (excepting EC2) and the percentage of primary morphoanomalies ($p < 0.01$). The same was true for the percentage of total abnormal spermatozoa. Rams producing more than 15% primary sperm anomalies, differed significantly in 5 out of 6 echotexture parameters (all except EC2), from rams producing semen with less than

15% primary anomalies ($p < 0.05$). Rams with more than 30% abnormal spermatozoa in their ejaculates had a lower density of hypoechogenic areas ($p = 0.008$). The most accurate cut-off points for detecting a ram with more than 30% abnormal spermatozoa in their ejaculate, was an Area in their testicular ultrasonogram inferior to 6.5% or superior to 13%. Sensibility was 100% and specificity was 77.4%. (This work was supported by Eureka E!11188 and IDI-20170220).

P 89 | The effect of *Saccharomyces cerevisiae* cell walls on serum concentrations of zearalenone, α-zearalenol, and β-zearalenol of dairy cows consuming zearalenone-tainted food

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Zearalenone (ZEA) is a non-esteroidal estrogenic mycotoxin produced by several fungi species of the genus Fusarium. This mycotoxin and its metabolites α-zearalenol (α-zea) and β-zearalenol (β-zea), may produce reproductive problems. Some compounds may prevent the absorption of mycotoxins by binding, and eliminating them through the faeces. The aim was to assess a preparation of Saccharomyces cerevisiae cell walls (CW) on the adsorption of ZEA, α-zea and β-zea by measuring their concentration in blood serum of dairy cows fed for 10 days with zearalenone-tainted food; measurements were made by high performance liquid chromatography. Twenty four animals were divided into 4 groups: i) Control cows (C) received no ZEA nor CW; ii) cows (Z) received ZEA (1000 mg/Kg live weight), and no CW; iii) cows (ZCW10) received ZEA (1000 mg/Kg), and CW (10 g/cow/day); iv) cows (ZCW20) received ZEA (1000 mg/Kg), and CW (20 g/cow/day). Blood samples were taken on day 0 and 10 of this experiment; data are expressed as logarithmic conversion. Control (0.76 ± 0.09) and ZCW10 (0.97 ± 0.05) showed similar values of ZEA which were smaller than Z (1.35 ± 0.18) ($p < 0.05$); in contrast, ZCW20 (1.09 ± 0.06) was no different from the other groups. With respect to α-zea, values of cows that received CW (1.02 ± 0.06) were different ($p < 0.05$) from those of cows that received no CW (C = 0.62 ± 0.09; Z = 1.35 ± 0.18). Regarding β-zea, value of cows that received ZCW10 (0.83 ± 0.03) was different ($p < 0.05$) from that of Z (1.11 ± 0.12), and C (0.39 ± 0.09); in contrast, ZCW20 (0.89 ± 0.07) was no different from Z, and ZCW10, but it was from C. Use of 10 g/cow/day of CW decreased serum concentrations of ZEA, α-zea, and β -zea, which may reduce the risk of reproductive disorders in cows. (Supported by UNAM PAPIIT IA204917.).

P 90 | Nutritional supplementation and exposure to sexually active bucks advance puberty in spring-born goat kids

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We determined in goats kept under extensive conditions, whether, the nutritional supplementation and exposure to sexually active bucks advances puberty in spring-born goats. Females were born on March 30 ± 3 days, and since 60 days old, they grazed natural vegetation from 10:00 to 18:00. On November 30th, goats were divided into 2 groups: one group (n = 8; 16 ± 0.2 kg) grazed natural vegetation, and the other one (n = 11; 18 ± 0.9 kg) received 600 g of a commercial concentrate (14% CP; 1.7 Mcal/kg) after grazing from December 1st to April 11th. On April 1st, the supplemented group was exposed to 2 males rendered sexually active due to 2.5 months exposure to long days (16 h of light/day) from November 1st. Body weight was determined once a month and ovulation was determined every two weeks by the identification of corpora lutea by using transrectal ultrasonography. Goats exposed to bucks reached puberty much earlier than isolated goats ($p < 0.0001$). Indeed, all goats exposed to bucks reached puberty at a mean (±SEM) of 376 ± 2 days old (April 11th ± 2 days), with a body weight of 25 ± 1 kg. In contrast, goats isolated from males reached puberty at 551 ± 4 days old (September 30th ± 4 days), with a body weight of 29 ± 0.5 kg. In conclusion, nutritional supplementation and exposure to sexually active bucks anticipates puberty in spring-born goats.

P 91 | Laser assisted embryo biopsy and embryo sexing in rabbit

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The preimplantation genetic diagnosis of embryos is a valuable method for genotyping embryos using embryonic cells obtained by micromanipulation. The technique could be use to determine the sex of livestock and detect the number of genetic diseases. Our aim was to develop a sex determination method for rabbit embryos with laser assisted biopsy. As micromanipulation requires mucin free embryos, one or two cell stage embryos were collected from superovulated donors and cultured in vitro until 8–16 cell stage. A single blastomere was isolated from the embryo trough a laser opened elliptic hole on the zona pellucida. Ninety five % of the biopsied embryos were developed to blastocyst stage. A SRY gene specific region was amplified

in a single cell nested PCR to detect the presence of the Y chromosome in the blastomeres. As a positive endogenous control, GAPDH gene was used. The sex determination was successful in 80% of the embryos. Out of 932 biopsied embryos 323 were determined as female which were transferred into the oviducts of asynchronic recipient females with laparoscopic technique. Seven successful pregnancy resulted litters where all newborns were female. We have developed a new single cell-PCR based sex determination method in rabbit. Our method can be used for modelling the long term effects of human laser assisted embryo biopsy in rabbit or for developmental and social biology experiments based on the same sex progeny.

P 92 | 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 bovine fed with *Pittosporum undulatum*. The animal 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 animals group (n = 4) was subjected to diet containing *P. undulatum* while the control group was not. During the oestrus cycle, peripheral blood samples were collected every 3 days and progesterone levels were analyzed by enzyme-linked immunosorbent assay (ELISA). After slaughtering, heifer's ovaries were recovered and oocytes were collected, in vitro maturated, 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. undulatun* 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). The adverse effect of nutrition on the heifers reproductive performance could be attributed to some *P. undulatun* compounds affected directly or indirectly on cyclooxygenase-2 (COX-2) activation, which may diminish follicular development through the inhibition of prostaglandins synthesis and oocyte maturation and, consequently, reduce the ability of oocytes to be fertilized and developed.

P 93 | Effect of *Toxoplasma gondii* on ram semen fertilizing capacity after experimental infection

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The aim of this study was to investigate the effect of *Toxoplasma gondii* on ram semen fertilizing capacity. Forty pre-pubertal 5 months old rams were divided in 4 groups (n = 10/group). Group A was controls; the rest received p.o. 5000 oocysts per lamb. Group B received no drug treatment. Group C received sulphadimidine (i.m. 33 mg/kg for 8 days; every 48 h) 2 months post infection (p.i.) and Group D received it twice (24 h p.i. and 2 months later). Blood samples were collected every 15 days to detect IgG Abs (ELISA). The study lasted 4 months up to sexual maturation of rams, when all of them were euthanized. Epididymal sperm samples were analyzed for concentration, kinetics (CASA), morphology/viability (eosin-nigrosin), membranes' functional integrity (HOS Test) and DNA integrity (Acridine Orange Test). Histopathological examination was performed on the testes and epididymides. In infected groups the antibody titres raised 2 weeks p.i. and remained high for 4 months (positivity threshold 0.595, max value 3.654 optical density). Higher values were noticed in curvilinear velocity (VCL) and rapid spermatozoa (%) in A vs. C group ($p < 0.05$). Viability and HOS+ spermatozoa (%) were higher in controls compared to other groups ($p < 0.05$). Abnormal sperm (%) was higher in groups C, D vs. A and C vs. B ($p < 0.05$). Sulphadimidine had no positive effect. Histopathology revealed similar findings with little variation among all infected groups, characterized mostly by increased interstitial connective tissue, non-purulent inflammation, and presence of seminiferous tubules with spermatogenic cell depletion, which increased gradually from D to C and B groups. In conclusion Toxoplasmosis negatively affected ram semen fertilizing ability, while sulphadimidine failed to alter this.

P 94 | Evaluation of transvaginal ultrasonography for early pregnancy diagnosis in Bulgarian White milk goats

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The present study aimed at evaluating the possibility of using the transvaginal ultrasonography for early pregnancy diagnosis in Bulgarian White milk goats. The experiment was carried out with thirty animals (aging 4–6 years, weighing 45–51 kg) after estrus synchronization by

intravaginal sponges and twice matings by fertile bucks 12 h apart. The serial examination by ultrasound scanner equipped with endocavity probe with frequency 6.5 MHz was performed 18, 23, 28 and 33 days after the last mating (Day 0 of pregnancy). Criterions for pregnancy and embryo vitality were visualization of enlarged uterine lumen (EUL), embryo (E) and cardiac activity detection (CAD). The possibility for a registration of the aforementioned criterions according to examination day was also registered. The obtained data were compared with the parturition ones and accuracy, sensitivity, specificity, positive and negative predictive value of the method were calculated. The results were processed by computer statistical software. For the first time EUL and E were registered in 75% and 58.3% of the pregnant goats (n = 24) on Day 18 while CAD was possible on Day 28. On Day 33, EUL, E and CAD were determined in 100%, 100% and 87.5% of the pregnant animals, respectively. The accuracy, sensitivity, specificity, positive and negative predictive value (96.7%, 100%, 83.3%, 96% and 100%) on Day 33 differed statistically ($p < 0.05$) than obtained values (76.7%, 85.7%, 55.5%, 81.8% and 62.5%) on Day 18. In conclusion, transvaginal ultrasonography can be recommended for early pregnancy diagnosis in goats not earlier than Day 33 after mating or artificial insemination. It is an easy feasible and hygienic method for examination of animals without a preliminary diet.

P 95 | Artificial long days applied individually to sexually inexperienced bucks does not affect their ability to stimulate sexual activity in seasonally anestrous goats

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Sexually inexperience and social isolation diminish sexual behavior. The aim of this study was to determine if sexually inexperienced bucks kept individually during photoperiodic treatment affect their ability to induce sexual response in anestrous goats. Males were isolated from females since weaning. When males were 10 months old, they were separated in two groups (n = 5). A group of males remained together in a pen (5 × 8 m), whereas the in second group, each male was placed individually in a pen (2 × 2 m). The two groups of males were subjected to artificial long days (16 h/light/day)/2.5 months. Males were exposed to anestrous females (n = 50 each) during 15 days. Male sexual behavior was registered for 3 days post-introduction into female groups. Analysis was done by Chi-square test for goodness of fit, and proportion of females displaying estrous behavior was compared by Fisher's exact test. On days 0, 1, 2, nudging and anogenital sniffing was higher in males kept in group ($p < 0.0001$), whereas mounting attempts did not differ between the groups of males ($p > 0.05$). On day 1 mounts with intromission did not differ between groups ($p > 0.05$), on day 2 it was higher in males in group ($p < 0.05$). The percentage of estrous behavior (≥92%) did no differ between females ($p > 0.05$). In conclusion, sexually inexperienced males placed individually during exposure to artificial long days induce sexual response in seasonally anestrous goats.

P 96 | Vasectomy in the wild deer**

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Deer are considered to be polyestric short day breeders. In these species, maturity has been observed to be biphasic. Testicle dropping occurs at the end of the first summer after whelping (August for the Roe deer, September for Red deer and October for the Fallow deer) becoming socially pubers. Despite its social maturity, the real time of the root and breeding occurs several years later depending on species (2 years for the Roe deer, 5–6 years for Red deer and 3–4 years for the Fallow deer). Nowadays, deer are becoming a new captive animal in several rural areas, and its management is becoming an important challenge because of its behavior. Because sterilization of deer turns into the antler changes, developing immature and non-developed mineralized protuberances, vasectomy is considered the best choice for reproductive control. One Red deer, one Raw deer and four Fallow deer of one year old were captured with a blow dart (Pistolgetta Standard P1, Ziboni tecnofauna). Chemical immobilization was obtained with a combination of medetomidine, tiletamine and zolazepam (IM). Induction and maintenance of general anesthesia was obtained with propofol (IV). Vasectomy was performed by and inguinal access and vas deferens were ligated with an absorbable monofilament (Monofil 0). A five centimeter of each vas deverens was removed to avoid possible recanalization of the ducts. Surgery was performed in around 40 min. Reversion of medetomidine was performed with Atipamezole. After surgery, Benzylpenicillin benzathin (12,500 UI/Kg) and streptomicin (2 g/Kg) were administered. No complications such as granuloma in the surgical area were observed during the follow up of every male. No sexual behavior changes were observed in the follow-up period.

P 97 | Prevention of postpartum diseases in sows

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The aim of the research was to study the efficiency of "Metramag-15" in postpartum diseases in sows. This is a complex drug with

antimicrobial, uterotonic, anti-inflammatory and general stimulating effects. 4 groups of sows were formed: in the 1st group (G1, n = 20) "Metramag-15" in a dose of 10 ml was injected IM the day of farrowing. In the 2nd group (G2, n = 20) a dose of 10 ml was injected twice (on the day of farrowing and 24 h later). Sows of the 3rd group (G3, n = 20) received a dose of 10 ml twice (on the day of farrowing and 48 h later). The 4th group (G4, n = 20) consisted of untreated control animals. The incidence of puerperal diseases in the early postpartum period (until day 8 after parturition) was 55%. Postpartum diseases were manifested as acute postpartum purulent metritis in 45% of the animals and the syndrome of metritis-mastitis-agalactia (MMA) in 10%. Using a single injection of "Metramag-15" as a prophylaxis (G1) or a double injection (G2) the manifestation of postpartum pathologies were reduced by 2.2 times for postpartum metritis and 2 times for MMA. The best preventive effect was found in G3 with a decrease in postpartum metritis by 5.5 times, and no cases of MMA syndrome. More piglets were weaned in G1 (88.9%), in G2 (92.3%), in G3 (89.5%) than in G4 (85.9%). The body weight of the piglets was 9.52 + 0.37 kg in G1, 10.23 + 0.27 kg in G2, and 10.35 + 0.20 kg in G3, which was increased by 1.5%, 9% and 10.3% compared to the control group. "Metramag-15" reduced the risk of postpartum complications and increased the viability of the offspring and its productive qualities.

P 98 | Identification of mutation in exon 8 of CLPTM1 gene in domestic dog *Canis lupus* familiaris

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According to 2003' WHO guidelines, orofacial clefts can be divided into cleft lip with or without cleft palate, cleft lip, cleft lip and palate and isolated cleft palate. Cleft palate is one of the most common congenital abnormalities found both in domestic dog and human and leads to a marked increase in newborn mortality. Currently, genetic background is considered the most important causative factor, the most likely candidate gene being CLPTM1 – *Canis lupus* familiaris Cleft Lip and Palate Associated Transmembrane Protein 1. Previous studies in dogs indicated 869 nucleotide substitution (T>A) of coding sequence 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 2 healthy and 6 affected French Bulldogs and 2 healthy and 6 affected English Bulldogs were used in our study. The isolated genetic material was amplified with polymerase chain reaction, followed by PCR-RFLP and the resulted product of 394 bp length was cut with specific AccI restriction enzyme. We found that both healthy and affected individuals of each breed were homozygous (restriction enzyme did not cut this sequence) for the synonymic T869A mutation in exon 8 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 99 | No evidence of an association between lethal recessive Osteopetrosis and performance in dairy cattle**

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To maintain or not maintain carriers of lethal recessive mutations in cattle breeding is an important question. It seems obvious to eradicate carriers from the herd; however, this may have a negative impact to the genetic merit of the farm if it is associated with positive effects on production traits and if so, could instead be carefully managed. The purpose of this study was to determine if carriers of lethal recessive genetic disorder Osteopetrosis (OS), in which affected calves are most often stillborn prematurely or survive less than 24 h, were associated with positive or negative effects on fertility, carcass and milk production traits. Genotypes and phenotypes in the form of predicted transmitting abilities (PTAs) of 14,939 dairy cattle were obtained from the Irish Cattle Breeding Federation. The PTAs were deregressed and using a weighted mixed animal model, were analysed in ASReml for an association with the SNP. The adjusted reliability cut-off was set at >20% resulting in the following n numbers from the 14,939 animal population. No association ($p > 0.05$) between polymorphism responsible for OS was observed in the fertility traits: calving interval (n = 2467), calving difficulty (n = 3237), gestation length (n = 12,688), or maternal calving difficulty (n = 2,157); carcass traits: carcass weight (n = 4,414) or culled cow weight (n = 5,111); and milk traits (n = 14,773): milk yield, milk fat yield, milk fat percent and milk protein percent. A tentative association ($p < 0.1$) between OS and increased milk protein yield (5.25 kg, s.e. 2.79) and decreased somatic cell score (−0.32, s.e. 0.19, n = 6155) was observed. The above results provide no evidence to support the maintenance of carrier animals of OS in order to achieve optimum breeding goals and maximise profitability on farm.

P 100 | A preliminary study on transcriptome analysis of boar spermatozoa differed in freezability

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In this study transcriptome sequencing was performed in fresh spermatozoa of three Polish large white (PLW) boars each with good and poor semen freezability (GSF and PSF, respectively). Total RNA was extracted from spermatozoa and RNA-Seq library preparation was used for paired-end sequencing on the NextSeq 500 system (Illumina). A total of 18,570 sperm gene transcripts were detected using the DEseq2 pipeline. RNA-Seq produced a total of 121 million sequenced reads of which an average 87.9% of the reads were uniquely mapped to the porcine genome assembly. Common transcripts were identified in the spermatozoa of the GSF and PSF boars, and were associated with spermatogenesis, reproduction and embryo development. Numerous differentially expressed genes (DEGs), detected in spermatozoa, differed between the freezability groups. Among the DEGs identified in spermatozoa, genes that were responsible for regulation of transcription (TCF15 and VGLL4), protein stabilization (HSP90AA1) and glycolytic activity (PFKFB2) were up-regulated, while the down-regulated genes were associated with protein glycosylation (ST8SIA4), fertilization (RNASE10 and ABHD2) and protein transport (ATP6V1E1). Preliminary findings on RNA-Seq of spermatozoa provided an overview of the DEGs that might be considered for further study on the freezability of boar semen. (Supported by a NCN project in Poland (2015/19/B/NZ9/01333).).

P 101 | Embryonic resorptions and neonatal mortality in a canine kennel with identification of uterine Mycoplasma

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A canine breeding kennel (mainly maltese and shih-tzu) with 26 breeding females had been suffering high rates of reproductive failure during 2 years in which embryonic resorptions in several bitches were observed by ultrasonography during mid-pregnancy diagnosis, as well as an overall 50% neonatal mortality rate with an average of 2.2 pups per bitch. Necropsy of puppies revealed severe hemorrhage in both thoracic and abdominal cavity and some signs of prematurity. PCR for *Brucella spp*, *Leptospira spp*, canine parvovirus, distemper, canine herpesvirus, Canine Minute Virus from vaginal discharge and tissue samples were negative. A semi-quantitative PCR for Mycoplasma revealed a positive 10^3 charge on a vaginal discharge in one bitch and in the organs of a dead puppy. It was decided to confirm diagnosis with a direct sample from a resorbing uterus in another bitch by explorative laparotomy. Histology report revealed a chronic endometritis, PCR for Mycoplasma was 10^4 positive. Mycoplasma is not considered as a normal flora in the canine uterus (Watts et al. 1996, J Small Anim Pract 37:54–60). After fluoroquinolone treatment (marbofloxacin) on all bitches, reproductive failure completely disappeared. Prior to this event, the breeder was using systematic preventive antibiotherapy of amoxicillin clavulanic acid 2 weeks before whelping on all the bitches. Although Mycoplasma is found on 51% of normal vaginal flora from clinically healthy bitches (Doig et al. 1981, Can J Comp Med 45:233–238), inappropriate use of antibiotics in this kennel may have induced a disruption of bacterial genital flora, allowing Mycoplasma to overgrow and become pathogenic.

P 102 | Testing of boar semen by DIC, portable and desktop CASA devices

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Boar semen samples were collected at a commercial artificial insemination (AI) center from five Hungarian Large White boars. Five diluted and cooled (17°C) semen samples from each boars were shipped to our lab (n = 25). The concentration and motility were measured by two CASA systems (Sperm Class Analyzer, SCA, Microptic-desktop CASA; Ongo Sperm Test, Microfluidlabs -portable CASA), while DIC (differential interference contrast) was used for morphological examinations. Each sample was gradually warmed up to 38°C and put into the Ongo Counting Chamber (with 10 μm depth). The concentration and motility were measured immediately and after one hour incubation at room temperature with SCA and Ongo (total sample size = 50) as well. The original samples were fixed in 10% formal buffered saline and wet mount was prepared for microscopic evaluation (Olympus BX61 with DIC beam). The agreement between CASA devices was studied with Bland-Altman analysis. The acceptance criteria has been set to ±10%. The sperm concentration showed −4.6 million/ml bias (confidence interval = −5.4, −3.8) agreement within ±8.5 million/ml limits. The total motility agreement calculated as −1.3% bias (confidence interval = −2, −0.4) within ±9% limits, while progressive motility has been found with −2.4% bias (confidence interval = −3.3, −1.8) within ±8.2% limits. All limits were within the preset acceptance range. Morphological examination by DIC was an easy way for qualifying spermatozoa without any artifact. Based on the above presented results, it looks Ongo and DIC can be used as acceptable precise methods for on-site sperm motility and morphology analysis in pigs. (This research was supported by FM (theme code TKOMPLEX).).

P 103 | Reproductive ultrasonography in Catalonian Donkey

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Ultrasonographic data on Jackass reproductive tract are almost nonexistent. A total of twelve exams were done on three Catalonian donkeys. B-mode Ultrasonography was used to explore and measure accessory glands and Doppler to assess the blood flow in three different location of the testicular artery: proximal pampiniform plexus (PPP), supra-testicular (ST) and testis periphery (TP). The Doppler parameters evaluated were Resistive Index (RI), Pulsatility Index (PI) and Total Arterial Blood Flow (TABF). Then semen was collected and analyzed. Observed mean results of three donkeys for the total number of sperm was 16933.38×10^6 while viability and morphological abnormalities evaluated by eosin-nigrosin stain were 82.85% and 13.51%. The mean measures for accessory glands were: Bulbourethral gland (18.5 ± 0.96 depth, 36.67 ± 1.03 length), Prostate (28.28 ± 1.20 depth), Vesicular gland (8.46 ± 0.39 depth), Ampulla (31.9 ± 1.33 diameter). Prostate was higher than in horse while Ampulla was significantly higher with anechogenic areas offering a glandular aspect. Both could be related to the high semen volume in donkey. Doppler parameters decreased significantly to the testis (PPP, ST, TP), PI (4.41 ± 1.45, 2.28 ± 0.08, 1.27 ± 0.04), RI (0.87 ± 0.01, 0.82 ± 0.01, 0.68 ± 0.01), TABF (0.55 ± 0.02, 0.46 ± 0.02, 0.23 ± 0.01) which results in a reduced temperature and oxygenation in testis. The TABF was lower in donkey in comparison to horses. The value of PI was negatively correlated with the sperm number and velocity. PI could be a good predictor for sperm motility and concentration in Donkey. Further studies are necessary to analyze the effect of testis perfusion on donkey spermatogenesis.

P 104 | MicroRNA expression profile in porcine oocytes aspirated from follicles of different sizes

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Oocyte developmental competence is acquired during folliculogenesis, closely linked with follicle size and regulated by complex molecular mechanisms. Several molecules are involved in these regulation mechanisms including microRNAs (miRNAs) that are essential for oocyte-specific processes during development. The objective of this study was to identify the expression profile of miRNAs in porcine oocytes aspirated from follicles of different sizes using RNA high throughput sequencing technology. Two small RNA libraries were constructed from oocytes aspirated from large (3–6 mm) and small (<2 mm) follicles and then sequenced on an Illumina NextSeq500. In total, 199 and 188 known miRNAs were detected in large and small oocyte groups, respectively with 171 miRNAs commonly expressed in both groups. A group of 36 miRNAs was abundantly expressed with more than thousands of sequence reads in both groups. Further analysis showed that 44 miRNAs were differentially expressed (DE) between both groups (>2 fold change) with 26 up- and 14 down-regulated miRNAs in large compared to small oocyte groups. The let-7 family were among the abundantly expressed miRNAs and were found to be up-regulated in large compared to small oocytes. Target gene prediction followed by KEGG pathway analysis revealed 61 pathways that were enriched with miRNA-target genes. Signaling pathways including TGF-beta, FoxO, and MAPK were targeted by DE miRNAs. In additions, oocyte meiosis pathway related genes were targeted by most of the up-regulated miRNAs in large compared to small oocytes. These results can help us to further understand how oocyte development is regulated by miRNAs. (This work was supported by projects CZ.02 .1.01/0.0/0.0/15_003/0000460 from OP RDE, VEGA 1/0022/15, VEGA 1/0327/16 and APVV-14-0001.).

P 105 | Isolation and purification of Cajal cells (CC) and interstitial Cajal-like cells (ICLC) from equine intestine and uterus

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Uterine contractile activity plays crucial role in: transport of reproductive cells and embryo, fertilization, recognition and maintaining of pregnancy, implantation and development of embryo. CC and ICLC found in many species have been proposed as pacemakers and propagators of electric signals regulating the contractions of smooth muscle. We have recently shown that in severe endometriosis the number of ICLC in myometrium decreases. Therefore we have formed a hypothesis that implantation of ICLC expanded in ex vivo culture may improve clinical features. For that purpose a reproducible and efficient isolation and purification protocol had to be established. The cells were isolated from intestine and uterine tissues, based on the presence of c-kit (CD117) surface marker. Antibody labeling was performed in intact tissue, and following extensive washing, the tissues were digested with collagenase and dispersed mechanically. Since some of the CD117 antibody was internalized by phagocytic cells (e.g. monocytes), these cells were additionally labeled with soluble fluorescent BSA as well as antibodies against F4/80 (macrophage marker) and CD45 (pan-leukocytic marker) conjugated with fluorochromes and paramagnetic beads. This allowed for immunomagnetic depletion of phagocytic cells, followed by fluorescence-based sorting in order to isolate CD117 +

/F4/80-/CD45- cells (CC or ICLC). The enrichment and purity were quantified by qPCR for increase in CD117 and low level of CD68, an immune cell marker. In conclusion, we have successfully isolated and purified equine CC and ICLC, which is a prerequisite for further functional characterization and expansion of the cells.

P 106 | The introduction of sexually active bucks during the luteal phase of the oestrous cycle did not modify the NEFAs and IGF-1 concentrations

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In previous experiments, we have described an increase in some metabolic/nutritional factors concentrations such as non-esterified fatty acid (NEFAs) and plasma insulin-like growth factor-1 (IGF-1) after a male effect. These increases could be associated with some modifications to the synthesis of certain reproductive hormones. The objective of the present study was to examine the evolution of the NEFAs and IGF-1 concentrations, after the introduction of males, during different stages in the luteal phase of the sexual cycle. Thirty-two does were divided into three groups. They were synchronised using a commercial intravaginal sponge treatment. Males were introduced with females at different times after sponge removal and they configured the different groups: 4 days introduction (n = 10, Group 4D) and 18 days introduction after sponge removal (n = 10, Group 18D) and a group without any contact with males (n = 12, Control Group). Oestrus was checked daily using bucks. Since sponge removal, plasma samples were obtained daily for NEFAs and IGF-1 determination during at least 24 days. No significant differences between the daily concentrations of the control group and the other groups were observed for NEFAs or IGF-1. Regarding the obtained results, it can be concluded that neither the introduction of males at different stages of the luteal phase of the oestrous cycle nor the moment of the oestrous cycle influence the concentration of both NEFAs and IGF-1. Therefore, the modifications of those parameters, observed on the previous experiments, could not be due to the synthesis of hormones as progesterone. (This study was funded by Grant AGL2016-75848-R from MINECO-AEI-FEDER (Spain).

P 107 | Transcriptomic changes in livers of adult rabbits conceived after embryo cryopreservation**

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Assisted reproductive technologies (ART) are currently used to overcome infertility in humans but also to promote reproductive efficiency in domestic animals. Although ART are still considered safe, increasing evidences holds that embryonic and fetal adaptation to suboptimal environments can predispose a series of metabolic diseases at adulthood stage. In this line, ART are associated with liver steatosis accompanied of transcriptomic and metabolomics changes. Nevertheless, our knowledge in this field is fairly incomplete. To assess this issue, we used RNA-seq to build a comprehensive transcriptomic profiling data set across liver tissue of adult rabbits derived from cryopreserved embryos (cryopreserved group: three-days-old embryos recovered, cryopreserved and transferred into recipient rabbit females) and non-cryopreserved embryos (control group). The cryopreservation process resulted in 652 differentially expressed liver transcripts ($p < 0.05$). PCA analysis showed two clusters associated to both experimental groups. The differential transcripts registered a major gene ontology enrichment in relation to lipid and sugar metabolism, such as fatty acid biosynthetic process (GO:0006633), long-chain fatty acid-CoA ligase activity (GO:0004467), glycolytic process (GO:0006096) or carbohydrate binding (GO:0030246). Furthermore, the most relevant Kegg routes affected were: glycerolipid metabolism, biosynthesis of unsaturated fatty acids, fatty acid metabolism, and those metabolic pathways in relation to digestive secretions. These results, taken together with other effects reported in the literature, suggests that embryo cryopreservation is not neutral at transcriptomic level. (This study was supported by Prometeo II 2014/036 and AGL2014-53405-C2-1-P.).

P 108 | Ovulation failure of lactating dairy cows under heat stress conditions decreased after inducing ovulation using hCG in a five-day progesterone-based fixed-time AI protocol

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This study compares effects after inducing ovulation using gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) at the end of a 5-day progesterone (P4)-based protocol for fixed-time artificial insemination (FTAI) on ovulation failure under heat stress conditions (HS). Heat stress was defined as herd environment with a temperature humidity index (THI) higher than 72. Protocol consisted of a P4 intravaginal device (PRID) fitted for five days, along with the administration of GnRH at PRID insertion and a double dose (24 h apart) of prostaglandin $F_{2\alpha}$ at PRID removal. Cows received either GnRH (GnRH group; n = 153) or 3000 IU hCG (hCG group; n = 256) 36 h after PRID removal and were inseminated 16–20 h later. The absence or presence of one or more corpora lutea at least 10 mm in diameter were assessed by ultrasonography 7–9 days after AI. Ovulation failure was defined as cows with no presence of corpus luteum 7–9 days after AI. Based on the odds ratio, the interaction treatment × THI had a significant effect on ovulation failure. The interaction implies that using GnRH cows with no HS as reference (5/48, 10.4%) hCG cows with no HS decreased 0.5

times the ovulation failure rate (51/130, 3.8%) ($p = 0.03$) whereas cows in the GnRH group increased 1.5 times the ovulation failure under HS (19/105, 18.1%) ($p = 0.04$). These results demonstrate the efficacy of hCG treatment to induce ovulation also during the warm season at the end of a 5-day P4-based protocol for FTAI.

P 109 | Cumulative testosterone levels in horses detected in hair: relationship with season, physiological features and stress hormones

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Hair is a matrix able to cumulate steroid hormones, mainly demonstrated with stress hormones in several species, including the horse. High hair cortisol concentrations have been related to chronic stress environments or situations. There are not studies assessing testosterone concentrations in hair in relation to cortisol levels, season and age. This study was conducted to characterize the seasonality of concentrations of hair testosterone (HTC) in relation to hair cortisol concentrations (HCC), season and age. Five male horses aged from 5 to 11 years old were submitted to monthly hair sample extraction through 15 consecutive months by shaving a concrete area of the abdomen to the level of the skin at each sampling time. Biochemical validation tests for cortisol and testosterone determination in horse hair were performed by enzyme immunoassay with reliable results following precision, specificity, accuracy and sensitivity criteria. Surprisingly, the Spearman's correlation coefficient for HTC and HCC was 0.36 ($p < 0.01$) showing a low positive correlation when it is well known that stress negatively affects the reproduction function. Average HTC was 6.90 ± 0.57 pg/mg. There were no significant differences in HTC among seasons ($p > 0.05$; spring: 8.30 ± 1.02 pg/mg, summer: 7.67 ± 1.37 pg/mg, winter: 6.99 ± 1.61 pg/mg, autumn: 6.62 ± 1.06 pg/mg). The levels of HCC were higher in summer and spring (3.48 ± 0.23 pg/mg, 3.29 ± 0.27 pg/mg, respectively) than in autumn and winter (2.31 ± 0.31 pg/mg, 2.28 ± 0.17 pg/mg, respectively; $p < 0.001$). Both hormones, HTC and HCC decreased significantly with age ($p < 0.001$). Our study showed that cortisol and testosterone concentrations can be measured in hair of adult horses which may be useful as a non-invasive indicator of horse well-being.

P 110 | Renal blood flow and perfusion in bitches with pyometra – preliminary study**

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Pyometra is a common uterine disease that results in sepsis; acute kidney injury (AKI) is a frequent complication of these entities. AKI may be a consequence of blood perfusion alterations, endothelial dysfunction, infiltration with inflammatory cells, thrombosis, tubular obstruction or deposition of immune complexes in the glomeruli. Therefore, the objective of this study was to compare the renal blood flow and cortical perfusion from healthy versus pyometric bitches by spectral Doppler and Contrast Enhancement Ultrasound (CEUS), respectively. The left kidney from 6 pyometra and 6 healthy bitches were evaluated by ultrasound, searching for renal artery Doppler fluxometric parameters (Systolic velocity SV, diastolic velocity DV, resistivity RI and pulsatility indexes PI) and cortical hemodynamic CEUS parameters (contrast peak CP, time to peak TP, mean transition time MTT and area under curve AUC). These parameters were statistically compared by Kruskal-Wallis test ($p < 0.1$) and presented as median ± IQR. The SV and DV in pyometra (73 ± 36; 21 ± 11 m/s) were lower ($p = 0.016$; 0.004) than in healthy bitches (131 ± 35; 40 ± 11 m/s), while PI and CP (1.7 ± 1.4; 36 ± 24%) were higher ($p = 0.078$; 0.016) than in healthy group (1.3 ± 0.4; 22 ± 5%). The other parameters were not different ($p > 0.1$; IR = 0.7 ± 0.1; TP = 13 ± 10; MTT = 21 ± 21; AUC = 825 ± 1380). These results indicate a decrease in renal blood flow in pyometra, with a compensatory increase in renal arterial resistance (increase in PI), while the renal perfusion was less affected (TP, MTT and AUC) and even showed an increase indicated by CP. This study demonstrates a compensatory renal response induced by infection hemodynamic alterations and presents the CEUS as a promising technique for detection and monitoring renal blood perfusion alterations.

P 111 | Effect of two diluents upon semen quality in Dorper rams

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The aim of this study was to investigate the possible effect of two diluents upon some variables defining semen quality. Dorper rams (2–4 years, n = 4) in northern Mexico (25°N) were exposed during December and January to one estrogenized female (2 mg of estradiol cypionate im), and semen was collected using an

artificial vagina. After each collection, the samples were immediately evaluated for volume, motility and viability. Subsequently, to each semen sample two diluents – either egg yolk citrate (CY) or Optydil, (OP) were added. In each of the diluted samples, three different conservation stages were considered [1) fresh semen (SF), 2) semen refrigerated for 2 h (SR), and 3) frozen semen (SC) to evaluate the response variables motility, viability and the membrane sperm integrity. Sperm variables were analyzed by the GLM procedure of SAS while the obtained means were compared by t-test among groups. In the SF group, no differences ($p > 0.05$) regarding motility score and sperm membrane integrity was observed among diluents (CY = 4.2 ± 0.25 vs. OP = 4.1 ± 0.19 years CY = 67% vs. 59%; respectively), however, the OP diluent generated the highest viability ($p < 0.05$) (87% vs. 73%). In the SR group, the CY diluent generated not only the greatest motility (4 ± 0.17 vs. 3 ± 0.19, $p < 0.05$) but also the highest sperm membrane integrity (60% vs. 54%, $p < 0.05$). In the SC group, no differences ($p > 0.05$) occurred between diluents in any of the evaluated variables. Results demonstrated that the use of the CY diluent generated reduced functional alterations in the sperm membrane when the semen was kept refrigerated for 2 h. When the semen was frozen, no differences in the evaluated variables between both diluents were observed.

P 112 | Blue-LED light stimulation improves the quality of canine sperm

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Sperm photostimulation is a new experimental technique that might improve the boar sperm quality and fertility. The aim of this study was to determine the effect of different blue-LED light stimulation procedures on canine sperm quality parameters. Semen was obtained from 4 Beagle dogs (5 years of age) by digital manipulation. Ejaculates were pooled, centrifuged (1000 g/5 min) and resuspended in Tris-fructose-citrate cooling medium. Then, semen samples were exposed to two different procedures of blue-LED light stimulation (450–500 nm) and control (without photostimulation); single light period of 30 min (L) and sequential light periods separated by a darkness periods of 5 min each one (L/D: 15/5/15). Sperm motility (CASA system), viability (eosin/nigrosin), acrosome integrity (rose bengal staining) and DNA damage (toluidine blue staining) were evaluated after 0 h and 24 h of storage at 4°C. Data were analyzed by GLM test. L/D photostimulation significantly increased ($p < 0.05$) kinematics parameters of spermatozoa, the percentages of viability and acrosome integrity compared to the control sperm samples after 0 h of refrigeration. However, after 24 h of conservation only the acrosome integrity values were significantly higher ($p < 0.05$) in L/D samples compared to control. D photostimulation showed no differences between L/D and control samples. Sperm DNA integrity didn't show any change along the study. In conclusion, light/dark periods of blue-LED photostimulation improve canine sperm quality. (Supported by DGA and Fondo Social Europeo (IA2).).

P 113 | Anatomical and histological study on true bilateral hermaphroditism in a Spain domestic pig – a case report

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This study describes a true bilateral hermaphroditism case in a domestic sow (Landrace x Large White) in Spain. The female genital organs were obtained from a slaughterhouse in Murcia (Spain) and fixed into 10% formalin. Gross and histological studies were performed. The gonads contained an ovotestis on each side in which both testicular and ovarian structures were noted. However, the ovarian part was larger on the left than on the right, and vice-versa for the testicular part. Next to the ovotestis both epididymes but not the oviducts were observed. The uterus was formed by 2 uterine horns, a body and a cervix. Next to the uterine horns 2 ducts included in the mesometrium were present. Vagina, vestibule, vulva and clitoris were normal. The histological testicular part of the ovotestis consisted of a well-developed seminiferous tubule containing seminiferous tubular epithelial cells, and Sertoli and interstitial cells. However, spermatogenic cells were not identified. The epididymes appeared to be normal, lined by pseudostratified columnar ciliated epithelium without any spermatozoa within the lumen. Related to the ovarian part, ovarian stroma, ovarian follicles in various stages of development and luteal cells in corpus luteum were observed. The uterus section had well-developed uterine endometrial glands and also revealed that the 2 ducts included in the mesometrium corresponded to the ductus deferens. In conclusion, true hermaphroditism is rare in domestic animals but has been reported most frequently in swine. Unfortunately, genetic analysis could not be done. From the authors' knowledge, this is the first case of true bilateral hermaphroditism in domestic pigs reported in Spain.

P 114 | Protein array-based strategies for identifying ram sperm capacitation-associated changes in apoptotic signalling molecules**

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We have already shown a relationship between capacitation and apoptosis in ram spermatozoa through the Rafs/MEK1/2 pathway

and the c-Jun N-terminal and p38 MAP kinases. Moreover, melatonin has a direct effect in modulating sperm capacitation and it is involved in apoptosis-related pathways in some somatic cells. The objective of this study was to determine whether certain proteins involved in the process of apoptosis vary their expression during ram sperm capacitation, and the possible effect of melatonin. Semen from nine Rasa Aragonesa rams was collected and in vitro capacitation was induced incubating swim-up-selected spermatozoa without (control) or with cAMP-elevating agents (cocktail sample), plus two concentrations of melatonin (100 pM and 1 μM). To assess the protein expression, microarrays of proteins designed for the simultaneous detection of 19 signalling molecules involved in the regulation of apoptosis were used (12856S, Cell Signaling Technology). Data were compared by ANOVA test. In order to optimize the microarray signal, a range of 0.1–0.7 mg/ml protein concentration was loaded. We proved that the highest intensity signal and more evident differences were obtained with 0.7 mg/ml protein without reaching saturation, so it was the chosen option for carrying out the experiments. Despite there is a tendency for melatonin to decrease the expression of certain proteins such as ERK1/2, HSP27, JNK, PARP and Caspase-7, no significant differences between treatments were observed, probably due to the high variability among signalling. More experiments should be done for greater reliability of the results. (Grants: AGL-2014-57863-R, DGA 2016-A26, BES-2015-072034.).

P 115 | Anesthetic and surgical procedures for MRI, functional MRI and removal of different type mammary gland tumors in laboratory rats

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Laboratory rats were presented for MRI, functional MRI and oncological surgeries to collect material for further histopathological evaluation during different types of research about physiology, pathology and therapy of mammary gland tumors. Balanced type anesthesia was performed for long, multiple, highly complicated operations, reoperations and functional MRI diagnostics during research. The balanced general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with facial mask following intramuscular premedication (medetomidine 0.25 mg/kg; Cepetor 1 mg/ml CP-Pharma, Germany, butorphanol 0.5 mg/kg; Butomidor 10 mg/ml, Richter Pharma AG, ketamine 80 mg/kg; Bioketan 50 ml VetoquinolBiowet PL), preemptive analgesia (meloxicam 1.0 mg/kg; Metacam 5 mg/ml; Boehringer Ingelheim Vetmedica) and intravenous induction (etomidate 0.3 mg/kg; Etomidate-Lipuro 2 mg/ml, B Braun Melsungen AG, Germany) was performed and polyionic balanced crystalloid (SolutioRingeri Lactate, Fresenius Kabi Poland) at dosage 10 ml/kg/h with synthetic colloid at dosage 10 ml/kg/h (Gelofusine, AesculapChifa Poland) was given to maintain both normotension and normovolemia. No general side effects were observed. The procedure allows for optimal safe management of the precise, multiple, traumatic surgeries and functional MRI necessary for surgical and experimental planning and therapy monitoring. The goals of anesthetic management should focus on using a balanced, multimodal approach with different techniques.

P 116 | Bovine fetal gender determination: positioning of the fetus (left/right) by gender

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The determination of fetal gender is achieved thanks to the identification of the genital tubercle (GT). In cattle, the accuracy of this exam is very high: an error margin of 0.01% has been calculated. The objective of the present study was to test the hypothesis that the prevalence of male fetuses is higher in the right horn while female fetuses are more prevalent in the left horn. The test was carried out between January 2014 and January 2018 in three commercial dairy farms, each with about 280 lactating cows. The test included nulliparous heifers and cows. A total of 4335 sexings were performed, of which 4200 were evaluated. The test was carried out on pregnant cows between the 55th and 110th day of gestation, with a portable ultrasound unit Imago® (ECMFrance) with a 5.0–7.5 MHz linear probe. The diagnosis of fetal sex was carried out on the basis of the position of the GT. 2103 males were diagnosed (50.07%) and 2097 females (49.93%). 59.37% (1.245) of female gestations were located in the right horn, while 40.63% (852) of the female fetuses were located in the left horn. 59.63% (1.254) of the male gestations were located on the right horn, while 40.37% (849) of the male were located in the left horn. No statistical difference was found due to parity. On the basis of these results, the initial hypothesis stating that male gestations are more prevalent in the right horn while female ones are more prevalent in the left horn, could not be validated for the female sex, while it was confirmed for the male sex. Male gestations are actually found in greater numbers in the right uterine horn.

P 117 | Adoption of orphan foals by lactating mares

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Raising orphan foals is a hard and expensive challenge; foals often develop poor social skills making them unsuitable for normal

equitation. We describe a simple technique to induce maternal behaviour and adoption in barren lactating mares after weaning of their own foals. Mares were sedated slightly and a single injection of cloprostenol (0.5 mg IM) given, when mares start to sweat, manual vaginal stimulation was applied and the foal was introduced to the mare and allowed to nurse. We present four clinical cases, orphan foals were 12 h to 4 days old. Nurse mares were 7–22 years old, with 4–5 month old foals at feet; their own foals were abruptly weaned and the mares were treated as described; all mares accepted the orphan foals immediately. Three of the mares were treated with sulpiride (1 mg/Kg BID IM) in an attempt to increase milk production. Our method is adapted from a previously described technique to induce adoption in non-lactating mares; but induction of lactation can take up to 7 days and failure can still occur. Our technique has the advantage of providing immediate results, being less money and time consuming. Pharmacological treatment and parturition simulation under our protocol seem to elicit maternal behaviour in these mares despite of trauma from weaning. Moreover, the adopting mares seem to prolong their milk production period during the five to six extra months needed for this second suckling period. (Porter et al. 2002, Physiol Behav 77: 151–154; Daels and Bowers-Lepore 2007, Proc Am Assoc Equine Pract 34).

P 118 | Equine oviductal fluid can be used as coadjutant for protein tyrosine phosphorylation induction in equine sperm

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Sperm capacitation is a crucial event preceding fertilization. Our aim was to assess the role of equine oviductal fluid (eOF) on function of stallion spermatozoa. Stallion spermatozoa were incubated at 30 × 10^6/ml for 4 h at 37°C in Modified Whittens (MW) medium added with bicarbonate, calcium and PVA (MW+++; pH 7.25) in presence of different doses of eOF ranging from 0.0625 – 1% (v/v) (n = 3; 1 ejaculate/stallion). We evaluated total motility (TM), progressive motility (PM), curvilinear velocity (VCL) and lateral head displacement (ALH); acrosomal status by flow cytometry (PNA+/PI-) and overall protein tyrosine phosphorylation (PY) by western blotting. The results were analysed by one-way ANOVA and are presented as mean ± SEM. No significant differences were observed for TM in % (54.0 ± 4.2 for control to 57.7 ± 5.8 for 1% eOF), PM in % (18.0 ± 2.9 for control to 19.7 ± 3.3 for 1% eOF), VCL (93.7 ± 3.0 for control to 93.3 ± 2.9 μm/s for 1% eOF) and for ALH (3.7 ± 0.3 μm for control and 1% eOF). The percentage of PNA+/PI- spermatozoa did not change (4.3 ± 0.3 for control to 5.4 ± 1.4 in the 1% eOF treatment, p > 0.05). In two from three ejaculates eOF at 0.0625% and 0.125%, but not at higher concentration (0.25, 0.5 and 1%), increased PY when compared against control. Therefore, eOF can effectively induce a raise in PY, which is dose and stallion dependent and is not associated with the induction of acrosome reaction or hyperactivation. (Funded by: AGL2015-73249-JIN and AGL2017-84681-R (AEI/FEDER/UE) from the Spanish Ministry of Economy, Industry and Competitiveness; UAX-S 1.010.809; and IB16159 from Junta de Extremadura (FEDER/UE).).

P 119 | Results of breeding soundness evaluation of beef bulls in the province of Cáceres (Spain)

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Breeding soundness evaluation of bulls is used to reduce the risk of using subfertile animals in herds because they can adversely affect pregnancy rates. We wanted to know the rate of bulls with an unsatisfactory score based on physical exam (including evaluation of scrotal circumference and testicular tone) and seminal characteristics, following American Society of Theriogenology guidelines. 27 beef bulls, under conditions of natural mating, from 13 to 100 months old and belonging to 14 extensive beef farms in the province of Cáceres (Spain) were evaluated. After a general physical examination, semen was collected by electroejaculation. Semen volume, concentration, total sperm number, objective motility (CASA system) and sperm morphological abnormalities (eosin/nigrosin stain) were assessed. Correlation analysis (Spearman correlation test) has revealed a moderate positive association between age and scrotal circumference ($r = 0.61$, $p < 0.01$), between age and percentage of normal sperm ($r = 0.44$, $p < 0.01$), and a high correlation between testicular tone and sperm concentration ($r = 0.70$, $p < 0.01$). 7 bulls (25.9%) were rated "unsatisfactory potential breeder" after breeding soundness evaluation as they did not fulfill the minimal requirements. 4 bulls with unsatisfactory rating were declared unfit in two or more tests. This examination has thus proved to be an essential tool in the detection of bulls with abnormal breeding potential.

P 120 | Placental characteristics in American minihorses

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Although gaining popularity, data describing physiological obstetrical characteristics in the American minihorse are scarce. Knowledge of normal foal weights, sizes and placental characteristics is essential

in understanding obstetrical challenges. Only mares (N = 58) with an uncompromised (peri-)parturient period and with normal placental thickness were considered to describe these characteristics. Pregnant, at term mares weighted 134.30 kg (98–172, σ = 19.408), had a height of the withers of 89.30 cm (72–104, σ = 5689), a thorax circumference of 122.74 cm (σ = 15.482) and a body length of 120.78 cm (σ = 8.604). They gave birth to foals weighing 10.74 kg (5.75–16.00, σ = 2.33), measuring 54.66 cm (σ = 7080) at the height of the withers and having a thorax circumference of 51.125 cm (σ = 4.9593). The placenta was expelled in 58.9 min (σ = 102.479). Total weight of the placenta was 1.2 kg (σ = 0.400), length of the umbilical cord was 41.81 cm (22–59, σ = 9.284), with 2.7 torsions (σ = 2.204). In 3 (5.17%) cases a transfixation was seen and in one occasion a vitelline sac remnant. On average, foals' birth weight was 9.44% of the mares post-partum weight (113, 34 kg, σ = 16.94), with the placenta weighing 11.21% of the foals weight. A significant correlation (Pearson correlation test, SPSS) between parity and weight of the foal (0.341, p = 0.05) was seen and furthermore the placenta weight was correlated with the mares' parity (0.507, p = 0.01) and foal's birth weight (0.376, p = 0.05). Although limited in number, the data of the minihorse population shown in this study can be helpful to differentiate normal vs. abnormal findings in post-partum examination of American minihorses.

P 121 | Does Hydatid cysts of Morgagni cause infertility in sows?

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Cysts of the paramesonephric duct origin are common in mammalians and are located on the fimbria of the oviduct. The term used for this neoplasia is Hydatid cyst of Morgagni and is most often a benign, noninflammatory condition of the fallopian tubes. This cystic degeneration is a possible cause of unexplained infertility in humans. Due to reproductive failure in a sow-pool system, three genital tracts of pluriparous crossbred Large White x Landrace sows were send for post-mortem examination. During the examination, a Hydatid cyst of Morgagni on the left paramesonephric duct with a diameter of 5 cm was found in one sow without other pathognomonic lesions of the genital tract. The fluid of the cyst was evaluated for further diagnostics, revealing an extremely low cellularity with a clear background. Nucleated cells consisted predominantly of macrophages and/or cyst-lining cells displaying minimal vacillation. Furthermore, a low number of lymphocytes and neutrophils was detected. No evidence of malignancy was found. In addition, a histopathological examination was conducted. The wall of the cyst consisted of a single layer of columnar epithelial cells resting on a basal membrane with connective tissue and a low number of smooth muscle cells. The present report describes the first detection of a Hydatid cyst of Morgagni in a sow with fertility problems. Like in other mammalians, a Hydatid cyst of Morgagni might be a possible cause of unexplained infertility in sows. Therefore, further investigation is necessary to evaluate the presence of Hydatid cysts of Morgagni in the genital tract of sows and to prove the relevance for reproductive failure in sow herds.

P 122 | Placental development during early pregnancy in sheep: Nuclear estrogens and progesterone receptor mRNA expression in the utero-placental compartments

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To examine the mRNA expression of nuclear estrogen (ESR1 and 2) and progesterone (PGRAB and B) receptors in different compartments of the uterus and placenta, tissues were collected in exp. 1, on days 16, 20 and 28 after mating (NAT) and on day 10 after estrus (non-pregnant controls [NP]); and in exp. 2, on day 22 of NAT, and pregnancies established after transfer of embryos generated through mating of FSH-treated ewes (NAT-ET), in vitro fertilization (IVF), or in vitro activation (IVA; parthenotes). In exp. 1, ESR1 expression in endometrial stroma (ES), endometrial glands (EG) and myometrial blood vessels (MBV), ESR2 in endometrial blood vessels (EBV), PGRAB in ES, and PGRB in ES, EG and MBV was greater ($p < 0.0001$–0.02) in pregnant than NP depending on day of pregnancy. Day of pregnancy affected ($p < 0.001$) expression of ESR1 in MBV, ESR2 in EBV and MBV, and PGRAB in ES. In exp. 2, ESR1, PGRAB and PGRB in EG, but not in other compartments, was greater ($p < 0.02$–0.09) in NAT-ET than NAT, and PGRB greater ($p < 0.02$) in NAT-ET than the IVF. These data demonstrated that the ESR and PGR expression was different in pregnant vs. NP ewes in selected compartments, and was affected by pregnancy stage or embryo origin in selected compartments. Thus, sex steroid hormone mRNA expression is differentially regulated in a spatio-temporal manner in uterus and placenta, and is affected by application of assisted reproductive technology in sheep. (Supported by NIH grant 1R03HD076073-02 to LPR and ATGB.).

P 123 | Reproduction problems in meat pigeon breed

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As Wrocław Meat Pigeon is a new and numerically small Polish breed, maintaining its genetic diversity is extremely difficult.

The aim of our study was to determine the causes of reproductive problems in a non-inbred flock. Genomic DNA was isolated with phenol-chloroform method from the blood of 23 pairs of parental generation (P) and 22 of F1 (from 8 pairs of P generation). PCR was performed with 10 pairs of STR (Short Tandem Repeats). Expected (HE), and observed heterozygosity (HO), Polymorphism Information Content (PIC), Hardy-Weinberg exact test (HW) were calculated by Cervus 3.03. software (Kalinowski et al. 2007, Mol Ecol 16:1099–1106). We determined Probability of Exclusion (PE) and Combined Probability of Exclusion (CPE) together with the genetic distance between sex and generations by Genetix 4.05.2 software. The number of identified STR alleles was lower than reported by other authors. CPE, estimated at 0.985, suggests that the selected STRs can be used for parentage control. In P generation the genetic distance between sexes was greater in parents of F1 (0.204) than in birds that did not produce offspring (0.067). F statistic results were higher in birds without progeny (FIS = 0.153, FIT = 0.141; FST = −0.011) than in F1 parents (FIS = 0.106; FIT = 0.166; FST = 0.067). As FIS indicates the relative deficit of heterozygotes and its plus value informs about the occurrence of inbreeding (Liu et al. 2008, Int J Poult Sci 7:1237–1241), we conclude that it could have been the reason for reproduction problems in the flock.

P 124 | Comparison of the hatching results in two breeding lines of Japanese quail (*Coturnix japonica*)

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Embryogenesis in Japanese quail proceeds faster than in other poultry species and lasts for 16–18 days. Apart from being commercially bred for meat and eggs, this species is also used for experimental purposes. Regardless of the purpose of reproduction, issues related to hatchability and chicks quality are of great importance, especially when we expect chicks to hatch simultaneously, be more uniform, active, of similar healthy appearance, body weight, feed conversion ratios and low postnatal mortality rates. The narrower the hatch window is, the more uniform chicks are and this boosts both productivity and welfare. Improvement in this field is far more advanced in domestic chicken than in other poultry species. The study was conducted on the total of 264 Japanese quail eggs, produced by birds from two lines: K (control) and S (selected for increased body weight on the 28th day of age). Eggs layed by 7-month-old females from either line (12 nests/line) were collected in two consecutive weeks and incubated together in two hatches until the last hatching chick. Higher egg weight ($p < 0.001$) and chick body weight ($p < 0.001$) was observed in S line (12.32 g in S vs. 10.68 g in K and 8.83 g in S vs. 7.17 g in K, respectively). The results demonstrated higher reproductive parameters for K line as compared with S line (egg laying 91.7% in K vs. 67.3% in S, $p < 0.001$; hatchability from fertilized eggs 86.6% in K vs. 77.4% in S, $p = 0.030$). Hatching time was significantly extended in S line in comparison to K line ($p < 0.001$). The peak of hatching in K line was in the middle of the 17th day, while in S line it moved to the midnight between 17 and 18 days of incubation. Results obtained in this study revealed that the reproductive performance is reduced in the S line compared to the K line.

P 125 | Equine sperm cryopreservation efficiency optimization by colloidal centrifugation

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Due to low fresh sperm quality in PRE horses, only few stallions can be incorporated into cryopreservation protocols. In this study we investigated if the colloidal centrifugation prior to cryopreservation could increase the number of stallions or ejaculates suitable for this procedure. A total of 17 ejaculates from six PRE horses were used. Fresh ejaculates with ≥60% progressive motility (PM), 6 from the 17 ejaculates, were classified as Suitable for Cryopreservation (SC) and those with <60% PM, 11/17, were classified as Non Suitable for Cryopreservation and were subjected to two different single layer colloidal centrifugation protocols (with extended or raw semen). Samples with PM ≥60% after colloidal centrifugation (6/11) were classified as acceptable after colloidal centrifugation (ACC). Both SC and ACC samples were subjected to a standard freeze-test with three different freezing extenders (BotuCrio®, INRA-Freeze® and Lac-EDTA). After thawing, spermatozoa motion characteristics were evaluated using a CASA system at 5 and 30 min post-thawing. The effect of centrifugation and freezing extender treatment on the different motility variables were determined by ANOVA and Duncan tests, $p < 0.05$. We did not find significant differences in motion characteristics between the two different colloidal protocols and no important differences were found in post-thaw sperm quality between colloidal and simple centrifugation processing techniques. Performing colloidal centrifugation prior to cryopreservation the percentage of suitable ejaculates increased from 35% (6/17, SC) to 71% (12/17, SC+ACC). Additionally, colloid centrifugation technique could be used as a valuable clinical technique to obtain high quality samples after thawing also from ejaculates with low PM.

P 126 | The presence of bucks and the body condition are modulators of the seasonal reproductive activity in Blanca Andaluza goats

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It has been studied under different latitudes that presence of males or the body condition (BC) could be modulators of the seasonal ovulatory activity. However, little information is available about the change in the ovarian activity of Mediterranean does kept in the absence of bucks with high or low constant BC. The objective of the present study was to examine the effect of the presence or absence of males on the annual pattern of ovulatory activity depending on the female's BC. Fifty six Blanca Andaluza does were divided into five groups. A group of 10 does were completely isolated from males between the 18th of December and the 17th of December of the following year (YEAR group). Four other groups were established containing does that had been in contact with males until February 3rd (n = 12, FEBRUARY group), March 19th (n = 12, MARCH, group), April 30th (n = 11, APRIL group) or June 11th (n = 11, JUNE group). Half of the does in each group had a low BCS (≤2.50) and the other half had a high BCS (≥3.00). Ovulatory activity was monitored via the progesterone concentrations determined in blood samples collected twice per week, for one year, in the YEAR group. In the other groups samples were taken during three weeks before males isolation. The presence of the males delayed the onset of seasonal anoestrous ($p < 0.05$), and the end of reproductive activity was delayed in the higher BCS does ($p < 0.05$). The present results indicate that the presence of males and the BCS are modulators of the seasonal reproductive activity in Blanca Andaluza goats. (This study was funded by Grant AGL2012-31733 from MINECO-AEI-FEDER (Spain).).

P 127 | Short term effects of multiple biopsies on equine endometrium

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Collection of endometrial biopsies (EB) in mares is assumed a safe procedure. However, reliability of a single EB for assessment of uterine functionality is critically discussed. As part of a lager study, we aimed to evaluate adverse short term effects of multiple EB (mEB) on uterine health. Twelve healthy broodmares (4–12 years) with 0–2 previous pregnancies were daily examined in estrus for general and reproductive soundness. On day (D) 1 and D21, bacteriological (B), cytological (C) and histopathological (hEB) evaluation were done. Additional B and C were performed on D4. All hEB readings were done by an experienced, blinded pathologist. On average 8 EB (7.5 ± 2.7) with a total of 1 g per mare were collected equally distributed over uterine body and horns on each D1-3. No mare showed signs of discomfort during the study. By D3/D4, 2 mares had mild hemorrhagic vaginal discharge. All mares developed mild, but 2 mares moderate intrauterine fluid on D3/D4. On D4, neutrophilic infiltrates were noticed in 7 mares (<60 PMN per 200 cells); mild mixed bacterial growth occurred in 9 mares, but needed no treatment. Comparing D1 and D21, hEB results revealed no change in score ($p = 0.19$); mild PMN influx was noted in hEB, but was not confirmed by C. Collection of mEB has probably no immediate adverse effect on the endometrium, other than mild inflammation. Biopsies 1 year later could shed light on endometrial degeneration; however, fertility and live foal data is likely to provide better evidence of procedural harmlessness.

P 128 | Mammary gland biopsy does not affect colostrum yield of sows: a pilot study**

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As litter size has increased, the amount of colostrum ingested per piglet has decreased. Thus, increased colostrum yield of sows becomes more important in order to ensure that each piglet receives a sufficient amount. In this regard, biopsy of mammary gland tissue in sows for analyzing gene expression or RNA profiling may provide a tool for studying factors affecting colostrum yield. The aim of the study was to investigate whether mammary gland biopsy before parturition itself affects colostrum yield of the sow. A total of 12 multiparous sows (parity 2–7) were used for this experiment. Biopsies were performed in 8 sows three days before the expected farrowing date and the other sows (n = 4) served as controls. On the day of biopsy, sows were moved into individual crates and udders were disinfected three times with a povidone-iodine (7.5% Betadine; Leiras, Helsinki, Finland) solution. With an automatic biopsy needle (14 gauge; 10 cm length, 22 mm penetration depth, Monopty 121410; Bard Oy, Finland), we took biopsies from the lateral-caudal part of the 1st, 3rd, and 5th pairs of the mammary gland. Colostrum intake was assessed based on piglets' bodyweight at birth and 24 h after birth. Data were analyzed by MIXED model. There was no difference in the colostrum yield between the biopsy group and the control group (n = 4; 4249.9 ± 711.2 vs. 3347.0 ± 1005.7 g; $p = 0.5$). In addition, colostrum yield of sows within the biopsy group did not differ from that of their previous parity (4249.9 ± 630.3 vs. 4697.3 ± 727.8 g; $p = 0.7$). Our experiment suggests that biopsy of porcine mammary gland before parturition does not affect colostrum yield. Thus, this method can be effectively used in studies on the effects of nutrition, environment, and other factors on mammary gland function.

P 129 | Association of POU1F1 gene polymorphism with reproduction and production traits in Khalkhali goat

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Litter size and milk are important traits in goat that have high economic value for farm industry. Because of low heritability in reproduction traits, we could not get a noticeable response to phenotype selection. Therefore, genetic information of effective gene could be used to enhance selection response. This study was carried out to investigate the association of pituitary specific transcription factor 1 (POU1F1) gene with litter size and milk composition traits in Iranian indigenous Khalkhali goat using polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP). A total of 120 unrelated individuals were randomly collected in 4 different flocks at the North West of IRAN. Animals were bred with ad libitum access to pasture grass (37°36′58″N 48°31′31″E) and grazed on natural pasture. The exon 4 of POU1F was amplified using specific primer and genotyped. Different identified pattern of sscp were sequenced. The analysis lead to identify 3 single nucleotide polymorphism in four populations including G→C, A→C, A→G. Association analysis indicated that the mutation A→C has an significant effect on litter size, milk protein and fat percentage in Khalkhali goat ($p < 0.05$). The three phenotype (AA, AC, and CC) were identified with a frequency of 0.52, 0.40 and 0.08, respectively, and the genotypes were distributed according to the Hardy–Weinberg disequilibrium. The frequencies of litter size for different genotype were 1.77, 2.15, and 1.25 for AA, AC and CC respectively. Here we conclude which allele A is useful for twining trait and could be used for potential marker-assisted selection programs in goat breeding and production.

P 130 | Diagnosing subclinical endometritis in dairy cows by uterine secretions

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Subclinical endometritis (SE) is defined as a clinically inapparent inflammation of the endometrium and is reported to impair fertility in dairy cows. A gold standard for its diagnosis has not been set. Uterine cytology and histopathology are mainly used as diagnostic devices. A new approach to illuminate the pathogenesis and facilitate the diagnosis of SE is the examination of uterine secretions (US): A novel device including among others a highly absorbent Merocel-swab allows for consecutive collection of cytological, histological and US samples from bovine uteri. It was applied to 108 dairy cows between 43 and 62 days p. p. following gynaecological examination. Detection of cytokines in US was conducted by AlphaLISA. In vivo sampling of US was feasible and generated samples of good quality. Cows were assigned to groups according to their uterine health status. 83 animals displayed no signs of endometritis (E.NEG). Cytological and histopathological examination revealed low agreement, hence animals with SE were differentiated into SE(cyto) and SE(histo) ($n = 7$ and $n = 13$, respectively). One animal with SE(cyto+histo) as well as four animals with signs of clinical endometritis were excluded from the analysis. SE(cyto) showed significant higher concentrations of cytokines IL1B, IL8 and IL17A in US compared to E.NEG, whereas no significant differences were found for IL6 and IL10. SE(histo) presented no differences for the analysed cytokines compared to E.NEG. In conclusion, a method to sample US was successfully established in dairy cows. IL1B, IL8 and IL17A are potential diagnostic markers for SE(cyto) in US. Further assessment of US might contribute to a better understanding of pathomechanisms leading to chronic endometrial inflammation. (Supported by FBF.).

P 131 | Effect change of temperature about morphometric head sperm in goat tropical

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The aim of this study was to evaluate the effect of temperature changes on the spermatic morphology of the head in goat semen, evaluated in 56 ejaculates of 7 male goats of recognized fertility using the Morphometric Semen Analysis (ASMA) system. The parameters of morphometry were analyzed with a principal component analysis (PCA) where they showed the highest variance, thus reducing the number of variables. Fresh: Subpopulation 1 (SP1) consisted of long and wide spermatozoa (SPZ) (35.12%), SP2 with SPZ of lower ellipticity (36.81%), SP3 in SPZ of average measurements (9.58%) and SP4 in small SPZs (18.48%). Cooling: SP1 (32.93%) were SPZ long, SP4 (18.54%) small, SP3 (12.88%) were similar to SP1, SP2 (35.66%) SPZ not hydrodynamic (very wide). Post thawing: SP2 (35.66%) wide SPZ, SP1 (30.92%) long and elliptical (hydrodynamic), SP3 (14.43%) similar to SP1, SP4 (21.53%) they were small cells. The minimal effects of the sperm cryopreservation process on the morphometry in goat semen were observed in the frequency of SPZ distribution in SPs and the characteristics of their forms for each subpopulation; the structure of the SP was maintained after the period of refrigeration and cold storage. In conclusion, cryopreservation did not modify specific parameters such as SPZ distribution within SPs (less than 5% of changes).

P 132 | Assessment of different staining protocols for the evaluation of nuclear maturation in mare oocytes

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The oocyte meiotic competence has been evaluated using fluorescent stains, as it is imperative to determine the effectiveness of assisted reproduction techniques. The aim of this study was to compare different protocols including Hoechst 33342 to find the most efficient methodology for the assessment of the nuclear status of equine oocytes after in vitro maturation. Oocytes from 11 mares were recovered from slaughterhouse ovaries by follicular scraping and matured for 42 h at 38.2°C and 5% CO_2. After maturation, oocytes were denuded with 80 IU hyaluronidase/ml and stained with: P1) 2.5 μg/ml Hoechst 33342 (H, Sigma-Aldrich) for 24–48 h at 22°C; P2) 10 μg/ml H for 10 min at 38.5°C; P3) 10 μg/ml H and 50 μg/ml propidium iodide (PI, Sigma-Aldrich) and P4) 2.5 μg/ml H and 50 μg/ml PI. In P3 and P4, each fluorochrome was incubated for 5 min at 38.5°C. Oocyte nuclear stage was assessed under an epifluorescence microscopy. Stained oocytes were classified as: G1 = excellent; G2 = good; G3 = moderate; G4 = bad, according to visualization quality to recognize the oocyte nuclear stage. Percentage of stained oocytes were compared between protocols by Chi-square. P4 protocol resulted in the highest ($p < 0.001$) percentage of oocytes with excellent visualization level than any other protocol (P4: 92.3% vs. P3: 20.0% vs. P2: 0.0% vs. P1: 0.0%). In conclusion, a combination of 2.5 μg/ml of Hoechst 33,342 with 50 μg/ml of PI (P4) combined with 10 min of incubation at 38.5°C was the best staining protocol for determining the nuclear maturation properly. (This study was supported by project AGL2013-42726-R.).

P 133 | Calving problems in cattle: Dystocia in the daily veterinary practice

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In cattle, calving assistance rates are varying between 10% and > 50%. The aim of this study was to collect cases of dystocia in 4 rural veterinary practices in Switzerland over a period of 12 months and to describe the causes for veterinary assistance. During 12 months, data of n = 573 parturitions were collected. The age of the cows involved was from 2 to 17 years with a median gestation length of 288 days. A uterine torsion was found in 31.8% of the cases with the direction of the rotation anti-clockwise in 94%. A feto-maternal disproportion was recorded in 21.8% of the cases. Uterine inertia was found in 179 cows with an increasing risk in higher parity. In 61% of the cases assistance was done by forced extraction, as the calves were found in correct position. A cesarean section was performed in 14 cases, a fetotomy in 17 cases. As to medical support, 199 of 573 cows received a calcium infusion. In 98 cases, a tocolyticum was used and in 71 cases, Dinoprostone was used to enhance uterine contraction and to ripen the cervix. Overall, 651 calves were born, therefrom 481 were singletons and 85 were twin births (history of n = 7 calves missing). Twins were twice as often in posterior presentation as single-born calves. As to gender, 61.2% of the calves were male and 38.8% were female. 66.1% of the calves were alive, 25.4% were dead, 5.1% were weak and 3.4% died during the parturition process. The percentage of dead calves was higher in twins than in singletons. The vitality was influenced by gestation length, parity and the time elapsed since the rupture of the amniotic sac. In this study, calf vitality was neither influenced by sex of the calf nor breed of the parents.

P 134 | Incidence of uterine, vaginal and rectal prolapses in Spanish breed pig herds

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Prolapses in breeding pigs can occur in the uterus, vagina or rectum, and can be followed by death of individuals, or raise at least animal welfare issues. The objective of this study was to assess the relative incidence risk for each type of prolapse in subgroups defined by parity, season of service and weeks after service. Data included records from 155,238 pigs in 144 herds. Producers were required to record a removal reason, including types of prolapse. The case-control matching was carried out and hazard models were fitted to the data. There were 1227 prolapse cases recorded, of which 8.2, 2.8 and 9.3% were uterine, vaginal and rectal prolapses, respectively. The remaining did not have any specific prolapse type recorded. The incidences of uterine and vaginal prolapses were 31.9–35.5 times higher at 16 and 20 or more weeks after service, than during the first 14 weeks ($p < 0.01$), whereas the rectal prolapse incidences were 11.4–51.2 times higher at 16 and 20 or more weeks after service, than during the first 14 weeks ($p < 0.01$). The incidences of uterine and vaginal prolapses were 2.8–3.0 times higher in parity 4–5 sows than in gilts ($p = 0.01$), whereas there was just a trend for the rectal prolapse to be different between parity groups ($p = 0.09$). The incidences of uterine and vaginal prolapses were 2.5 times higher in pigs serviced during winter than in those during spring ($p < 0.01$). However, there was no seasonal variability in the incidence of rectal prolapse ($p = 0.22$). These results show that the incidence of prolapses are influenced by parity, season and weeks after service. Consequently, producers should pay more attention to pigs exposed to high risks, while trying to identify prolapse cases at an early stage.

P 135 | Study of sperm capacitation on thawed sexed semen in Iberian red deer**

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Sexed sperm and in vitro fertilization (IVF) are posed as valuable techs for better production efficiency. However, IVF in cervids is not deeply studied. In small ruminants (sheep and goat) estrus sheep serum (ESS) is used for sperm capacitation, a non-defined substance also used in deer. The aim of this work was to assess sperm functionality in different time points of incubation in modified synthetic oviductal fluid (SOF) enriched with 20% (v/v) of ESS (SOF-ESS) by the evaluation of their structural components and motility. Thawed sexed semen of three deers was incubated in SOF-ESS for 1, 5, 15, 60 and 120 min. As negative control, a sample incubated in SOF without ESS and with 0.1% (w/v) of polyvinyl alcohol (SOF-PVA), was also evaluated at 0 min. Higher motility ($p < 0.001$) and velocity ($p < 0.05$) and greater population with active mitochondria (Mitotracker+) ($p < 0.01$) at 1, 5 and 15 min were shown (37 ± 3, 47 ± 3 and 39 ± 3; 106 ± 7, 107 ± 7 and 115 ± 7; 25 ± 2, 22 ± 2 and 22 ± 2) comparing to the samples incubated in SOF-PVA (29 ± 3; 81 ± 7; 13 ± 2). A deterioration of the samples incubated 120 min in SOF-ESS was evident for motility ($p < 0.001$), velocity ($p < 0.01$), Mitotracker+ ($p < 0.001$) and intact acrosomes ($p < 0.05$) (11 ± 3; 65 ± 7; 7 ± 2; 18 ± 3) in relation to 1 and 15 min (37 ± 3 and 39 ± 3; 106 ± 7 and 115 ± 7; 37 ± 3 and 39 ± 3; 26 ± 3 and 27 ± 3). As performed, SOF-ESS activates thawed sexed deer sperm at very early incubation times eliciting motionless and degraded populations at 120 min of incubation likely due to the high metabolic activity triggered by the ESS since its role as capacitation inductor. (This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2013-48421-R).).

P 136 | Influence of laser radiation on the sperm of bulls

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The purpose of the study was to study the effect of laser radiation, used in separation of sperm, according to the quality of spermatozoa in sex-separated sperm. Ejaculates received from n = 15 bull were included in the study. Sperm collection was carried with the standard protocol. The received ejaculates were divided into three parts: I-control; II–the part in which the hoechst33342/PI was added and subjected to diffused laser radiation; III-a part which was subjected to the focused laser radiation. The parameters of the laser beam in terms of photosensitizing action were identical to the beams, used in installations for the separation of sperms by sex. Sperm quality was studied with CASA (SpermVision; Minitube). Statistical analysis of the obtained data was performed using Microsoft Excel 2010. The sperm concentration in freshly prepared ejaculate amounted to 0.86 billion/ml. The content of abnormal sperm with twisted flagella was 6.8 ± 1.05%. As a result of technological processing with the use of an installation with a scattered beam the content of sperm with twisted flagella was 10.7 ± 1.52% ($p < 0.05$). The proportion of sperm with flagellum pathology in the samples processed using technological equipment with a focused beam increased more than 7 times and amounted to 50.3 ± 8.66%, ($p < 0.001$). The increase in the frequency of occurrence of this pathology negatively affected the activity of sperm. The content of progressive motile sperm (PR) in freshly prepared ejaculate was 85.7 ± 2.85%, 62.4 ± 3.96 and 59.13 ± 4.01% in I, II and III experimental samples. Thus, it can be concluded that the use of laser installations and dye Hoechst33342/PI leads to an increase in the proportion of abnormal sperm and a decrease in the content of progressive mobile sperm.

P 137 | Evaluation of microbiological risk factors associated with mastitis in cows

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The task of the study was to assess the hygiene of milking cows as a source of bacterial contamination of milk at the initial stage of its production in the Ural region of Russia. Selected samples were collected from milking Equipment, milking gloves and milk for the detection of Mastitis pathogens. The investigations were carried out by the polymerase chain reaction method, using the Vet-Septoskrin, n = 38. In 89.47% of the sampling, a pathogenic and conditionally pathogenic microflora was isolated, whereas in 10.53% of the cases no microflora was detected. In the monoculture of the microflora in all samples, *E. coli* was represented in 5.88%. In 94.12% of the bacterial culture *S. epidermidis*, *S. saprophyticum* and *S. haemolyticus* (43.75%), *Staphylococcus spp.*, *E. coli* (37.50%), *Staphylococcus spp.* and *Streptococcus agalactiae* (12.50%), *Streptococcus agalactiae*, *Staphylococcus aureus* and *Staphylococcus spp.* (6.25%) were found in association Microflora of milk in 15.79% is represented by monoculture (*Staphylococcus aureus*, *E. coli*), in 84.21% – associations of bacteria in the culture were found. In the structure of associations of cultures of bacteria 43.75% were on *Staphylococcus spp.*. Mixed species were selected in 56.25%: *E. coli* and *Staphylococcus spp.* (18.75%), *Staphylococcus aureus* and *Staphylococcus spp.* (12.50%), *E. coli* and *Streptococcus agalactiae* (12.50%), *Staphylococcus aureus*, *E. coli* and *Staphylococcus spp.* (6.25%), *Staphylococcus aureus* and *Streptococcus agalactiae* (6.25%). The results showed unfavorable milking conditions, which is a potential source of

milk contamination by microorganisms and explains the presence of mastitis pathogens. Therefore, it is necessary to implement a quality policy aimed at hygiene in the whole chain of primary production milk.

P 138 | Assessment of utility of Computed Tomography (CT) in evaluation of equine ovaries architecture

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Imaging diagnostic is most common way of qualitative and quantitative assessment of ovarian structures. Ultrasonography is oftenly used in ovaries imaging in vivo with few limitations in tissue architecture determination. Aim of this preliminary study is to evaluate possibilities of computed tomography (CT) in examination of fresh ovaries collected from slaughterhouse (n = 12), scanned up to 2 h after collection. The volume of ovary, volume of follicles, count of follicles and density of follicles were measured on the ovary in anestrus. Scans were performed on multi-slice CT scanner (750 Revolution CT, GE Healthcare, Waukesha, WI, USA), with following parameters: amperage: 550 mA, rotation: 0.8/s (He), voltage: GSI-QC (Dual Energy). Mean volume of ovary was 82.5 ± 9.4 cm^3, while stroma's volume was 42.1 ± 1.7 cm^3. Follicles in ovaries shown in CT were divided into 3 groups, basing on follicles' size in largest diameter: >20 mm, ≤20 mm and <10 mm. Medium number of follicles from each group was: 6.1, 8.3 and 2.9, while mean volume of follicles from each group was: 4.91 ± 1.47 cm^3, 3.85 ± 2.52 cm^3, and 2.32 ± 1.65 cm^3, respectively. Differences ($p < 0.0001$) in maximum radiodensity of each follicle was stated, with highest values (in HU, Hounsfield Units) in biggest follicles (44.01 ± 4.82), intermediate in follicles ≤20 mm (37.38 ± 6.02) and lowest in small follicles (30.17 ± 2.48). However, no differences were found in average radiodensity of follicles: 21.58 ± 1.25, 22.15 ± 1.99 and 22.73 ± 2.21, respectively. Even in anestrus the growing follicles demonstrated differences in maximum radiodensity. CT provides data about 3D structure of ovaries, which may be used in further studies covering properties of ovaries.

P 139 | Expression of proinflammatory cytokines IL-1β, IL-6 and TNFα in the retained placenta of mares

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Cytokines released during parturition are involved in the molecular mechanisms of uterine contractions, expulsion of the fetus and the fetal membranes. We hypothesized that in mares altered inflammatory response results in the fetal membranes retention (FMR). Within 2 h of foal expulsion samples of the allantochorion and the endometrium were collected from 16 mares with FMR and 31 mares without FMR. Samples were analyzed for mRNA expression analysis of IL-1β, IL-6 and TNFα by Real Time PCR. Data were normalized to β-actin and analyzed using Student's t-test (GraphPad Prism 7.03). Results showed an increase in mRNA expression of IL-1β in the endometrium (0.032 ± 0.007 vs. 0.014 ± 0.004; $p < 0.05$) and IL-6 in the allantochorion (0.007 ± 0.001 vs. 0.002 ± 0.0004; $p < 0.0001$) of mares with FMR in comparison to mares that expelled the fetal membranes physiologically. In contrast, there were no changes in TNFα mRNA expression between the 2 groups of mares. Moreover TNFα was barely detectable. Based on the above results it seems that the inflammation outbreak is involved in the mechanism of the placenta release. Increased expression of IL-6 in the allantochorion and IL-1β in the endometrium might be a local immune response which is focused on detachment of the fetal membranes since these cytokines stimulate activated T and B cells and acquire immune response which is thought to take place during parturition. Low expression of TNFα mRNA suggests that this cytokine is not involved in the fetal membranes expulsion. Further work is needed to define the exact role and timing of the inflammatory activation in the fetal membranes during equine parturition. (Supported by NSC grant 2015/19/N/NZ5/00655).

P 140 | Freezing semen from a donkey with 6 different extenders

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Semen freezing is an area of growing interest in the donkey industry. Six different freezing extenders were used to freeze semen from a 16 year-old Pyrenean donkey collected 3 times a week during 3 weeks using an INRA AV. Week 1: 8 mares were inseminated to proof 100% fertility; week 2: semen was frozen using INRA Freeze, EquiPlus Freeze 1-Step and 2-Step; week 3: Ghent, Botu-Crio and Cryo3. Sperm concentration and motility were checked prior to extending 1:1 in INRA 96. Each ejaculate was divided in three equal parts, centrifuged at 600 g for 10 min, the supernatant removed and the correct volume of each extender added to the pellet to get a final sperm concentration of 100 × 10^6 sp/ml. The specific recommendations from all 6 freezing extenders were respected. Ten 0.5 ml straws of each freezing extender were identified, refrigerated at 4°C followed by exposure to the liquid nitrogen vapor on a rack prior to plunging in liquid nitrogen. All straws were preserved in a liquid nitrogen

tank for 1 month before being analyzed after being thawed in a 37°C water bath for 30 s, with exception of the BotuCrio freezing extender that was thawed for 1 min at 37°C. All post freezing semen evaluations included a full Computer Assisted Sperm Analysis, a flow cytometer to evaluate viability and mitochondrial activity and a Hypo Osmotic Swelling Test (HOST). With an average volume of 61 ml gel free semen, a sperm concentration of about 245 × 10^6 sp/ml and a pH of 7.2 at the time of the semen collection, our results show that the post thaw motility, viability, mitochondrial activity of the sperm and the HOST was the least decreased with the Gent extender. More research is needed using different donkey breeds to conclude that Gent extender is superior to freeze donkey semen.

P 141 | Effective prevention of pseudopregnancy in the bitch

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In non-pregnant bitches, there are cases of pseudopregnancy (PP) lasting two months or more. PP is also associated with an increased risk of mammary gland neoplasia. The aim of the study was to assess the usefulness of cabergoline (CB) and bromocriptine (BR) for prevention of the occurrence of PP symptoms. A total of 56 females of different breeds were divided into 4 groups of 14 females (I-KB-5 μg/kg for 14 days, II-KB-7.5 μg/kg 14 days then 5 μg/kg for 14 days, III-BR −5 μg/kg for 14 days, IV– 7.5 μg/kg for 14 days followed by 5 μg/kg for 14 days). Treatment was started with the onset of peripheral blood progesterone concentration decrease and clinical enlargement of the mammary gland, on average 14 ± 6 days after beginning of cytological diestrus. Bitches were evaluated for: no signs of PP after treatment (NPP), recurrent symptoms of PP after treatment (RPP), occurrence of vomiting (V), cytological symptoms of proestrus (CP). Group I: NPP-10, RPP-4, V-2, CP-0, group II: NPP-14, RPP-0, V-4, CP-1, group III: NPP-8, RPP-6, V-6, CP-0, group IV: NPP-14, RCC-0, V-6, CP-0. NNP occurred most often in group II ($p = 0.012$), least often in group III ($p = 0.004$). RPP occurred in group III ($p = 0.004$), and least in group II and IV ($p = 0.012$). The use of CB and BR are effective in the prevention of PP, but if used for more than 2 weeks may lead to symptom recurrence, BR also induced more side effects (V). The use of both drugs in a decreasing dose for 4 weeks turned out to be the most effective. Only in one case, after use of CB, symptoms of cytological proestrus appeared (at day 27 of treatment). The use of low drug doses most often caused vomiting at the beginning of treatment, but required regular administration.

P 142 | Effect of second prostaglandin F2 alpha injection during the Ovsynch+Controlled internal drug release (CIDR) protocol on pregnancy rate in Simmental cows

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The main objective of this study was to evaluate the effect of a second prostaglandin F2 alpha (PGF) injection during the Ovsynch+controlled internal drug release (CIDR) protocol on pregnancy to the timed artificial insemination in Simmental cows. All cows (n = 90) in the groups were randomly assigned and synchronized with the 7 day Ovsynch+CIDR protocol. Cows received 100 g of gonadorelin diacetate tetrahydrate (GnRH, IM, Ovarelin®, CEVA, Turkey) and a CIDR (CIDR 1380®, Zoetis, Turkey) on Day 0, and 25 mg of dinoprost tromethamine IM (PGF, 5 ml Dinolytic®, Zoetis, Turkey) at CIDR removal on Day 7 (Group 1, n = 50). Cows received a second dose of dinoprost 24 h after the first treatment (Group II, n = 40). A second injection of GnRH was applied 56 h after the first PGF injection. Fixed timed artificial inseminations (FTAI) of all cows were carried out 16 h following the second injection of GnRH. All pregnancies were determined with transrectal ultrasonography (7.5 MHz rectal probe, Sonosite®, USA) between 28–30 days following FTAIs. Pregnancy rates in group 1 and group 2 were 46.0% and 55.0%, respectively. Pregnancy rates did not differ between treatment groups ($p > 0.05$). In conclusion, second PGF injection in Ovsynch+CIDR protocol showed a tendency to increase the pregnancy rate, but future studies on second PGF injection must be carried out with a higher number of cows.

P 143 | Castration influence on rat thyroxine level

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Castration is the most common surgical procedure in companion and domestic animals. However, its influence on animal's physiology remains unclear. The aim of this study was to evaluate changes in thyroxine (T4) serum level in growing rats caused by castration. Fifty eight 8-week-old Wistar rats (30 males and 28 females) were divided into five groups: study group 3, 7, 30 (S3, S7, S30), and control group 0 and 30 (C0, C30). On day 0, study group rats underwent castration (orchiectomy in males, ovariohysterectomy in females) in general anesthesia with the use of isoflurane. Control group stayed intact. Rats from both groups were kept in breeding

conditions with 12-h day/night system and with the access to fresh water and fodder ad libitum. Study group rats blood samples were collected on the day 3, 7 and 30 after castration. Control group rats blood samples were collected on day 0 and 30. Blood serum T4 level was evaluated with the use of commercial immunoenzymatic tests (Pointe Scientific, Poland). In intact rats T4 level remained at 59.3 ± 17.45 ng/ml through the whole experiment. On day 3, a significant decrease (29.63 ± 8.72 ng/ml) of T4 level was noted in castrated rats in both males and females ($p < 0.001$). On day 7, T4 level increased back to its previous range and remained the same up to 30 days after castration. In summary, castration seems to have a significant influence on rats thyroid function. This phenomenon needs further research. A new study concerning sex hormones receptors in rat thyroid and adrenal glands is in progress.

P 144 | Cysteine supplementation pre-freeze and post-thaw improves integrity and reduces oxidative stress in cryopreserved ram spermatozoa

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The cryopreservation of ram spermatozoa generates reactive oxygen species (ROS), which induces oxidative stress and impairs sperm function. Cysteine, a non-enzymatic antioxidant protects sperm membrane lipids, reducing the effect of ROS. This study determined the optimal concentration and timing of cysteine supplementation to improve sperm integrity in cryopreserved ram spermatozoa. Nine ejaculates collected from three Texel rams were cryopreserved individually in Tris-citrate-glucose cryodiluent supplemented with cysteine (0, 0.5, or 1.0 mg/ml) pre-freeze (PF) or post-thaw (PT) generating seven treatments: 1) control 0 mg/ml, 2) PF 0.5 mg/ml, 3) PF 1 mg/ml, 4) PT 0.5 mg/ml, 5) PT 1.0 mg/ml, 6) PF + PT 0.5 mg/ml and 7) PF + PT 1 mg/ml. The motility, viability, acrosome integrity, penetrability through artificial cervical mucus and ROS production were measured at 0–3 h post-thaw. Data were analysed by repeated measures ANOVA. Motility ($p < 0.001$), viability ($p < 0.001$), penetrability ($p < 0.001$) and % sperm with <50% ROS ($p < 0.001$) was greater in samples treated with cysteine compared to the control. Samples treated PF+PT had greater motility, viability, penetrability and lower ROS than other treatments at almost all time points. Furthermore, motility and penetrability were greater and ROS production lower in 1 mg/ml PF+PT than 0.5 mg/ml PF+PT. This study highlights the need to reduce oxidative stress during freezing (PF) and after thawing (PT) to retain sperm integrity. Cysteine at 1 mg/ml PF+PT is most beneficial and may aid the development of cryopreservation and thawing protocols to facilitate assisted reproductive technologies in sheep.

P 145 | Endometritis-pyometra complex and fibroadenomatosis in a 4.5 months old Sphinx queen

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Endometritis-pyometra complex (EPC) and fibroadenomatosis together are very rare complaints of intact queens. No such a case has been reported so far. A 4.5 months old Sphinx queen was referred to the WULS Veterinary Clinic in Warsaw, Poland with signs of anorexia, lethargy, elevated body temperature (39.6°C), polydipsia, significant enlargement of all mammary glands and purulent vaginal discharge. According to the owner the cat had the 1st heat 14 days ago. The blood tests revealed elevated leucocytes (21,000 G/l), AST (52 U/l) and AP (212 U/l), P4 was 12.2 ng/ml and E2 was 84.4 pg/ml. The ultrasound of the uterus showed enlargement of the uterine horns with a variable diameter from 12 to 16 mm without any visible cystic changes in the uterine wall. Ringer's solution with vitamin B complex, aglepristone (Alizin, Virbac; 15 mg/kg) on the day 1, 2, 7, 14, 21 and 28 together with cephalexin (Ceporex, Intervet; 10 mg/kg) during 5 days and tolfenamic acid (Tolfine, Vetoquinol; 4 mg/kg) for 3 days were applied as a treatment. On the second day, the animal's general condition was improved together with an increase of vaginal discharge. After 5 days, a gradual decrease in the size of mammary gland was observed. Vaginal discharge disappeared after 7 days. On the day 14 of treatment, clinical signs of heat appeared, which spontaneously disappeared after another 5 days without mating. Complete recovery of mammary glands occurred 4 weeks after the onset of treatment. In conclusion, the use of progesterone receptor blocker effectively helped to treat both diseases that were determined by the biological influence of the progesterone in a young queen. In such a case surgery does not seem to be the treatment of choice.

P 146 | Effect of intrauterine infusion of thyme oil and Dimethyl sulfoxide on healing of clinical endometritis in cows

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In the present study, it was aimed to determine the effect of the intrauterine application of thyme essential oil (TEO) and Dimethyl sulphoxide (DMSO) on healing and Tumor Necrosis Factor Alpha (TNFα) levels in cows with clinical endometritis (CE) 30–35 days post partum. The study was performed in 33 cows with CE (defined by rectal and vaginal examination). The cows were randomly allocated into three groups. Cows in Group 1 (GI, n = 11) were treated with an intrauterine infusion of the 120 ml mixture prepared from 20 ml

TEO (Mindivan, Turkey), 12 ml of DMSO (Emplura®, Sigma-Aldrich, USA) and 88 ml of distilled water (DW). Cows in Group 2 (GII, n = 11) were treated with an intrauterine infusion of 20 ml TEO mixed with 100 ml DW. In the third Group (GIII, n = 11), cows received intrauterine infusion of 12 ml of DMSO in 108 ml DW. Fourteen days following treatment, rectal and ultrasonographic examinations were performed to check and determine clinical improvement status. Blood samples of all cows was immediately collected prior to the treatments and on day 14 following treatment. The best clinical improvement was in GI (90.9%), whereas cows in GII and GIII had a clinical improvement of 72.7% and 54.5%, respectively. The mean levels of TNFα prior and following treatments in GI, GII and GIII were 382.39 ± 14.76 pg/ml vs. 285.10 ± 7.18 pg/ml; 411.53 ± 18.58 pg/ml vs. 306.49 ± 7.63 pg/ml; 285.88 ± 9.16 pg/ml vs. 221.13 ± 8.15 pg/ml, respectively. In all treatment groups, the levels of TNFα decreased by 25.39%, 25.54% and 22.5%, respectively, compared to before treatment. It was concluded that, the combined application of thyme oil and DMSO may be more effective in the treatment of cows with clinical endometritis.

P 147 | Uterine torsion in cattle: puerperal course and effects of NSAID administration

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Uterine torsion is known to be a precursor for diseases in the puerperium with negative impacts on subsequent performance. The aim of the study was to investigate the course of the puerperal period after uterine torsion and the benefit of a single administration of the NSAID meloxicam. The study presents 114 cases of uterine torsion documented under field conditions. Half of the cows were treated with meloxicam before re-torsion (group T), while the other half received a placebo (group P). The cows were examined during delivery, 2 h post partum (p.p.), 2, 12, 21 days p.p., 3 and 6 months p.p. Serum samples for analysis of the inflammatory mediator PGE2 were taken before therapy, 2 h and 2 days p.p. The mean PGE2 serum concentration 2 h p.p. was significantly lower in group T (513.6 pg/ml) than in group P (1654.4 pg/ml; $p < 0.001$). 2 days p.p. the mean value of PGE2 still tended to be lower in treated cows than in the controls ($p = 0.063$). The initial milk yield was 8.9 ± 2.8 kg per milking; it declined with an increasing degree of torsion ($p = 0.010$) and with the presence of lacerations ($p = 0.001$) without group differences. There were also no differences between groups T and P in the incidence of retained fetal membranes (10.7% of all cows), metritis 2 days p.p. (25.9%) and metritis 12 days p.p. (40.4%). In 81.9% of the cases a re-insemination was tried; 81.4% of these cows became pregnant (insemination index = 2.76). In conclusion, the administration of meloxicam significantly reduced the serum levels of PGE2, but it showed no benefits to the general condition, genital health and subsequent fertility of the animals.

P 148 | Morphological characteristics of oocytes collected from pubertal and prepubertal wild Felids

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To adapt ART programs for the protection of wild cats it is necessary to determine similarities and differences between oocytes from domestic and wild cats. In case of wild Felids, mortality of offspring is high and there is a possibility to obtain material from very young, prepubertal animals. The aim of this study was an analysis of morphological characteristics of oocytes collected from pubertal and prepubertal Felids. Ovaries were collected post mortem from both adult (4–6 years) domestic cats and wild Felids (Lynx, Serval, Pallas cat) as well as from young (0–2 months) domestic cats and *Felis manul*, *Panthera tigris altaica*. Oocytes were classified into 4 morphological groups. Oocytes with light and dark cytoplasm were counted and the diameter of the oocytes was measured. The average number of oocytes harvested from matured domestic cats was 52. A similar number of oocytes was derived from adult Servals (54), while from the Pallas's cat only 18 oocytes were retrieved. The lowest number of oocytes was collected from lynx (5). No oocytes were obtained from newborn domestic and Amur tiger kittens on the first day of birth, while in the case of older domestic and Pallas's cat kittens (1–2 months), 54 respectively 48 oocytes were collected. Oocytes obtained from kittens were characterized by a good quality and without cytoplasmic defects. In adult cats no significant differences between the number of oocytes with dark cytoplasm (77–87%) were detected. Significant differences were observed between the number of oocytes with dark cytoplasm in adult (83%) and prepubertal (56%) Pallas's cat. Ovaries of prepubertal wild cats can be a source of oocytes for gametes bank and ART. (Financed by NCBiR PBS3/B8/16/2015.).

P 149 | New method of gonadorelin application for treatment of cows with follicular cysts

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The aim of the research was to study the effectiveness of different ways of gonadorelin ("Fertagyl") administration in the therapy of follicular cysts in cows. Cysts were diagnosed by ultrasonography using an «Easi-Scan 4» scanner. 4 groups of animals were formed, having a cyst on one of the ovaries (n = 12 in each group). In the 1st group, gonadorelin was injected at a dose of 5.0 ml IM (control). The 2nd group was

injected with a dose of 2.5 ml IM, the 3rd group 5.0 ml IV, and the 4th 2.5 ml IV. On the 11th day, ultrasound examination of the ovaries was carried out to detect luteal tissue. For cows whose ovaries did not react to the 1st administration of gonadorelin, the injection of the drug was repeated, and they were again examined 11 days later. Cows with signs of luteinization were injected cloprostenol twice with a 12-h interval at a dose of 3.0 ml. Cows were inseminated in estrus. Immediately after AI, all animals were injected with a surfagon (a synthetic LH-RH agonist) in a dose of 25.0 μg. The presence of pregnancy was diagnosed by ultrasonography 30 days after AI. Administration of 5.0 ml of gonadorelin IV had a more pronounced therapeutic effect. After the 1st intravenous injection of gonadorelin, all cows responded by forming luteal tissue on the ovaries. In the 1st induced estrus, 33.3% (n = 4) of the cows could be inseminated, whereas in control group only n = 2. Within 4 months, 83% of the cows became pregnant in this Group (n = 10), whereas in the control Group only n = 8, and the duration of days open was shorter by 5 days. Thus, intravenous administration of gonadorelin was a more effective way of treating follicular cysts in cows.

P 150 | Circulating miRNAs as potential molecular biomarker of early pregnancy in ewes

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In this study, the aims were to evaluate plasma miRNA profiles during the critical stages of peri-implantation and to determine possible candidate marker miRNAs in order to develop an early pregnancy diagnosis method in ewes. For this purpose, plasma samples were collected from a total of 24 ewes 12 (pre-implantation, n = 4), 16 (implantation, n = 4) and 22 (post-implantation, n = 4) days after mating, and on their corresponding days of 12 (n = 4), 16 (n = 4) and 22 (n = 4) of the estrous cycle. Firstly, global miRNA profiles in plasma were determined by microarray technology. Following the analysis of global microarray results, 10 miRNAs that had differential expression profiles between cyclic and pregnant groups were selected and validated by RT-qPCR in plasma. By these results, global miRNA profiles in the plasma during the peri-implantation stage of pregnancy have been reported for the first time in ewes. An important number of miRNA showed a differential expression pattern especially between day 16 and 22 of pregnancy. Selected 10 miRNAs had similar profiles in both array and RT-qPCR. On day 22 of pregnancy, 3 miRNAs (oar-miR-493-5p, oar-miR-485-5p, gga-miR-1765) had greater expression levels determined in plasma by RT-qPCR. In conclusion, plasma profile of miRNAs appears to be regulated intensively during the peri-implantation stage of pregnancy in ewes and this could be considered to develop an early pregnancy diagnosis method. Due to multiple interaction among miRNA and their target genes, more detailed studies are warranted. (Supported by TUBITAK 214O643).

P 151 | The lack of intra-family phylogenetic consanguinity between scriptaid-treated somatic cells and nuclear recipient oocytes does not result in developmental failure of caprine-porcine cloned embryos

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The present research was carried out to ascertain whether inter-family and inter-genus, i.e., caprine-porcine, nuclear-transferred (C/P-NT) embryos can acquire and retain the competences to complete their ex vivo development to blastocyst (B) stage. To generate C/P-NT embryos (Group I/GI), adult goat peripheral blood-derived fibroblast-like cells (AGPB-FLCs) that had been epigenetically modified by exposure to 350 nM scriptaid (SCPT) were microinjected under zona pellucida of enucleated in vitro-matured pig oocytes. To create intra-species (P-NT) embryos (Group II/GII), pig ooplasts were subzonally injected with SCPT-exposed porcine nuclear donor cells. Efficiently fused and electroactivated oocytes were cultured to morula (M) and B stages for 6–8 days. Among 231 C/P-NT embryos assigned into GI, 172 (74.5%)^{A} underwent cleavage divisions. The percentages of embryos that progressed to M and B stages were 62/231 (26.8%)^{A} and 26/231 (11.3%)^{A}, respectively. In GII, out of 148 P-NT embryos, 139 (93.9%)^{B} displayed cleavage activity, but 104 (70.3%)^{B} and 52 (35.1%)^{B} developed to M and B stages, respectively (A,B $p < 0.001$; Chi-square test). Summing up, although inter-family and inter-genus taxonomic incompatibilities have been found between donor specimens of AGPB-FLCs (*Capra aegagrus hircus*) and nuclear recipient oocytes (*Sus scrofa domesticus*), epigenetically reprogrammable cell nuclei inherited from AGPB-FLCs that had been subjected to SCPT-assisted modulation did not fail to direct the development of C/P-NT embryos to reach M and B stages. Nonetheless, C/P-NT embryos were characterized by M/B formation rates that turned out to be significantly lower than those noticed for their P-NT counterparts. (This work was supported by grant number BIOSTRATEG2/297267/14/NCBR/2016.).

P 152 | The course of parturition and PPARGC1A/HaeIII genotypes of dairy cattle

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Identification of genes, which are responsible for genetic diversity of individual functional traits considered economically important, having

the potential to be used in breeding programs, is a very important subject in the field of dairy cattle genetics. The aim of the study was to determine the relationship between PPARGC1A/HaeIII polymorphism and the course of parturition of Polish Holstein-Friesian black-and-white cows. The study included 1007 cows that were kept in the loose barn and fed doses of the system total mixed ration. The course of parturition was evaluated on a 5-point scale: 1 – spontaneous parturition, 2 – easy parturition, 3 – difficult parturition, using greater force, 4 – difficult parturition (dystocia), 5 – spontaneous abortion). Identification of genotypes of individuals was performed using PCR – RFLP. Statistical analyses were made using the Duncan test. The highest proportion of spontaneous parturition (41.3%) showed the cows with genotype TC (for other genotypes: TT 35.5% and CC 36.9%). However, the most easy parturition were recorded in homozygous cows CC (61.2%). The largest proportion of difficult parturition (dystocia) showed the cows with genotype TT, and it was 4.5% (for other genotypes: TT and CC −1.9%). The consequence of difficult parturition is the increase of the calving index, extension of the service period and the interpregnancy period. In our own studies, there was no statistically significant relationship between individual genotypes and the course of parturition of the analyzed cows.

P 153 | The viability of *Danio rerio* embryos and larvae after GFP fluorescent gene microinjection

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The aim of the study is to compare the viability of transgenic embryos and larvae of zebrafish obtained after the introduction of the GFP gene versus controls. The plasmid pCEEGFP containing the reporter gene GFP under the control of the human elongation factor, with the enhancer of human cytomegalovirus was used in the work. Fish males and females were placed in the spawning tank at night at a temperature of 29°C for production of fertilized eggs. The plasmid with GFP gene or water was injected into blastodisk region of fertilized eggs. The analysis of embryonic stages both in the control and in the experimental groups showed the presence of normal and different forms of abnormal development through one day of cultivation after the introduction of the reporter GFP gene and water. After 24 h of cultivation, non-viable organisms were identified in all embryo groups studied. A total of 335 embryos were analyzed, 60 in the control group, 111 after the water microinjection, and 164 after the introduction of the pGEEGFP genetic construct. The main critical period of survival of fish embryos is on day one. The average survival rate of embryos and larvae for 7 days was 67.4%, whereas after microinjection of distilled water was 44.1% and 40.6% after introduction of the GFP gene. It has been shown that the introduction of water or genes has a negative effect on the viability of embryos and larvae of fish. Viability indicators of the early stages of development of *Danio rerio* can serve as a marker for the effectiveness of genetic constructs. Transgenic lines will be in demand for studying the expression of genes of different cell types.

P 154 | Mycotoxin eliminator «Elitox» in last-trimester pregnant cows application impact on immune blood profile of offspring

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Nonspecific immunity of new born play an important role for growth, development and productivity. The aim of this study was to investigate if treatment with the mycotoxin eliminator «Elitox» (combination of hydrated sodium calcium aluminosilicate, biopolymer chitosan and enzymes) in last-trimester pregnant cows, had an impact on the white blood cell profile of calves. The research was conducted in North-Western region of Russian Federation and in biochemistry and physiology department laboratory of FGBOU VO «SPbSAVM» on white-and-black cattle. Experimental group included 10 newborn calves born from cows which received mycotoxin eliminator «Elitox» in the last-trimester of pregnancy (dosage – 10 g per day). Control group included 10 newborn calves born from cows not given mycotoxin eliminator «Elitox». Blood samples were taken two times – at the age of 2 and 4 weeks, respectively, from a jugular vein. White blood cell count was higher in experimental group in comparison with control group (by 50% at the age of 2 weeks ($p \leq 0.05$) and by 20% at the age of 4 weeks ($p \leq 0.05$)). Consequently, the treatment with the mycotoxin eliminator «Elitox» in last-trimester pregnant cows might have an impact on white blood cell blood profile of their calves. Further studies with larger groups of calves are needed.

P 155 | The efficiency of ovarian responses to superovulation protocol and embryo quality in sheep in Kuwait

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The aim of this study was to evaluate the effectiveness of a superovulation protocol through ovarian response and embryo quality in a local sheep breed in Kuwait. Ten sheep received an intravaginal progesterone-releasing device (CIDR) for 14 days (Day 0 = day of insertion). On Day 12, a superovulatory pFSH (Pluset®) treatment was given. A total of 200 mg pFSH was administered in eight i.m. injections given twice a day at decreasing doses (40, 40, 30, 30, 20, 20, 10, 10 and 10 mg, respectively). On Day 14, CIDR was removed, and 200 IU of eCG and 0.25 mg cloprostenol administered (i.m.). A ram was left with the ewes for one day on Day 16. Ovarian response was assessed while counting Corpora lutea and follicles by laparoscopy 6 days after natural mating. Embryos were collected and morphologically evaluated under stereomicroscope; grade I to III morulae and blastocysts were considered

viable. In seven superovulated ewes 8–16 CLs were found. Embryos were collected only from these 7 ewes with good superovulation response. A total of 36 embryos and 6 unfertilized ova were recovered. The total number of viable embryos was 22 (17 morulae and 5 blastocysts). One ewe with high superovulation response (11 CLs) yielded 0 embryos and one ewe a total of 19 embryos (including 12 viable ones; 7 morulae and 5 blastocysts). Under this MOET program ewes with good superovulation response yielded an average of 3.1 viable embryos. Such a low efficiency may be the result of the fact that the females were treated at a young age (11 months). Further trials are needed to investigate possible sources of improvement. (Authors acknowledge financial support from The Federal Agency for Scientific Organizations (FASO Russia), project No. AAAA-A18-118021990006-9.).

P 156 | The effect of late follicular or early luteal bovine oviduct fluids on frozen thawed bull sperm parameters in non-capacitating medium

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The present study was designed to investigate the effects of late follicular (LF) or early luteal (EL) bovine oviduct fluids (BOF) on frozen thawed bull sperm parameters in non-capacitating medium. Frozen samples from 5 fertile bulls (Asturiana de los Valles) were thawed and diluted in Tris, Citric acid and Fructose (TCF) extender with LF or EL BOF (final concentration 1%, treatment groups) or without it (control group). After 20 min of incubation at 37°C, motility parameters were determined by CASA. Flow cytometry was used to evaluate viability, acrosome and capacitating status (Propidium Iodide-PSA-FITC, M540-Yo-Pro) within 2 h incubation period being measurements made at 0, 1 and 2 h. The percentage of total live sperm was higher in the BOF-LF (47.73 ± 1.00) and BOF-EL (45.19 ± 1.16) groups compared to control group (40.08 ± 0.76, $p < 0.05$), and spontaneously acrosome reacted sperm rates were different between BOF-LF (12.17 ± 0.38), BOF-EL (8.03 ± 0.47) and control groups (5.29 ± 0.41, $p < 0.05$). The spontaneously capacitated sperm rates were also different between BOF-LF (9.60 ± 0.34), BOF-EL (5.89 ± 0.33) and control groups (3.60 ± 0.31, $p < 0.05$). Total and progressive motility parameters were higher in BOF-LF group (respectively 48.82 ± 1.52 vs. 43.95 ± 1.42, 39.83 ± 1.49 vs. 35.59 ± 1.32) compared to control group ($p < 0.05$). In conclusion, the addition of LF or EL BOF in non-capacitating medium enhanced the sperm viability as well as the ability of sperm to undergo spontaneous capacitation and acrosome reaction. Late follicular BOF increased total and progressive motility. (Supported by Fundación Séneca 20040/GERM/16, MINECO AGL2015-66341-R and The Scientific and Technological Research Council of Turkey (TUBITAK) Science Fellowships and Grant Programmes Department 1059B191601224.).

P 157 | Testing of haplotypes impacting fertility in Russian Black and White cattle

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Haplotypes affecting fertility in dairy cattle are actively accounted in genetic selection programs of many countries since 2011. Russian Black and White cattle (RBW) was created in 1820s and is still the most numerous breed in Russian Federation (approx. 8 million animals). This breed was created by crossing local cows with German, Estonian and Lithuanian Ost Frisian bulls until 1959, when pure breeding program was started. Today, as farmers are focusing on milk production traits and less on functional traits, the number of matings between RBW cows and Holstein bulls is increasing daily. Leningrad area present today the highest milk production per cow in Russia, with intensive use of US and Canadian bulls. The aim of the study was to identify the frequencies of Holstein Haplotypes (HH) screening in local RBW cows. Analysis was performed using Illumina IDBv3 SNP chip on 603 cows from 13 herds, born in 2010 to 2014. Only 40 animals (6.6%) did carry different haplotypes. Distribution of HH among carriers was 52.5% for HH1, 42.5% for HH3 and 5% for HH4. Ancestry checks shows high frequency (64%) of US Holstein genetics in pedigrees. The phenotypic analysis of fertility data showed that cows with HH get 1st calf at a later age (plus 15 days) and success rate after first insemination is 5% lower when compared to HH free animals. Negative effect of HH on milk yield, fat and protein EBV's using GLM was detected in HH4 (−296.1, −9.5 and −7.9 kg) and HH1 (2.2, −0.2 and −1.1 kg), respectively. Results show that it may be relevant to include testing for HH haplotypes in the RBW selection program and using HH-free Holsteins to reduce number of carrier animals in population and to avoid a decrease of fertility performance in RBW. (FASO State Assignment AAAA-A18-118021590138-1).

P 158 | Effect of duration of cultivation on the developmental competence of bovine oocytes that have not finished growth phase in vivo

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Oocytes that have finished growth phase in vivo (Brilliant cresyl blue (BCB)+ oocytes) have significantly higher blastocyst development in vitro than growing (BCB-) oocytes (Alm et al. 2005, Theriogenology 63:2194–2205). The aim of the study was to evaluate the development competence of BCB- oocytes at prolongation of the time of cultivation to 30 h. Before IVM COCs were incubated in 26 μM BCB (B-5388) solution for 90 min. Then oocytes were divided into

BCB-(colorless cytoplasm) and BCB+ (colored cytoplasm). Compact cumulus oocyte complexes (COCs) were cultured 24 h in TCM 199 + 10% (v/v) FCS + 10 ng/ml recombinant bovine somatotropin (Monsanto) with 10^6/ml granulosa cells. Medium were supplemented with 10 IU/ml hCG after 15 h of cultivation. All chemicals were purchased from Sigma-Aldrich (Russia). After IVF embryos were cultured by standard protocols up to Day 8. After IVM chromatin of 464 oocytes (in 5 replicates, 18–23 oocytes/group) was evaluated by Hoechst 33258. In total 87% (119/137) and 89% (114/128) of BCB+ oocytes reached MII after 24 and 30 h of cultivation, respectively. Forty nine % (49/101) of BCB- oocytes reached MII after 24 h, the level of matured oocytes significantly increased after 30 h of cultivation [73% (72/98), $p < 0.01$, χ^2 test]. No significant differences were observed between the percentages of blastocyst that developed from BCB+ oocytes independently of duration of cultivation. The BCB- oocytes yielded a higher proportion of late morula and blastocysts by the prolongation of the time of cultivation to 30 h [12% (15/121) vs. 31% (41/133), $p < 0.01$, χ^2 test]. In conclusion, prolongation of the duration of cultivation to 30 h improved the development competence of BCB- oocytes. (Funded by FASO Russia, #181180215901329.).

P 159 | Influence of enterosorbent "Zoo-Verad" on reproductive characteristic in cows

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The aim of the research was to study the influence of "Zoo-Verad" enterosorbent on the reproductive parameters of cows. 2 groups of cows were formed with n = 30, each: the control group (CG) received only the basic diet, in the experimental group (EG) "Zoo-Verad" was added to the main diet at the rate of 0.1% of the mass of the dry matter of the main diet a month before the proposed calving for 7 days with a break of 7 days. The results of the study showed that "Zoo-Verad" feeding helped to accelerate the expulsion of afterbirth, 3 cases were noted in the EG, which is 57.1% less than in the CG. In the EG, postpartum endometritis was recorded in two cows, mastitis in three cows, which is 66.7% and 50% less frequently than in cows in the CG. The period from calving to first insemination in experimental animals averaged 62.5 ± 1.21 days, while in animals from the CG it averaged 78.4 ± 1.10 days, which is 15.9 days (or 20.3%) less. Service period in the EG averaged 78.9 ± 2.06 days, and in the CG – 82.0 ± 2.19 days. The duration of the intercalving period in the EG was 367.5 ± 6.41 days, and in the CG 389.0 ± 4.25 days, which is 5.5% more. The obtained data allow to draw the conclusion that the animals fed with "Zoo-Verad" enterosorbent showed better reproductive parameters and resistance to diseases of the post partal period.

P 160 | Analysis of the level of thyroid hormones in cows in different periods of lactation

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Thyroid hormones affect secretory processes in the mammary gland, stimulating increased milk production and increased synthesis of milk proteins and fat. They also stimulate the differentiation of ovarian follicles, and increase the production of luteinizing hormone, which contributes to the successful insemination of cows. This study analysed circulating thyroid hormone concentrations triiodothyronine (T_3) and thyroxine (T_4) in cows during lactations 1–3. Lactations were divided into: colostral period (G1, n = 25), increase of lactation (G2, n = 25), lactation peak (G3, n = 25) and decline of lactation (G4, n = 25). The maximum level of thyroid hormones was in the G1 group: the level of T_3 = 2.4 ± 0.08 nM and T_4 = 71.1 ± 3.8 nM. In G2 group: T_3 = 2.3 ± 0.1 nM, and T_4 = 63.2 ± 3.5 nM. Cows in G1 and G2 are sequentially rearranging their metabolic processes aimed at activation of lactogenesis. The minimum values were obtained in the G3 group: T_3 = 1.9 ± 0.1 nM, T_4 = 51.7 ± 2.5 nM. In G4: T_3 = 2.2 ± 0.1 nM, T_4 = 60.9 ± 3.5 nM. The lowest concentration of hormones in G3 can be explained by the adaptation of the organism to the intensive synthesis of milk components, the reduction of lactation and the overcoming of the negative energy balance period. At the same time, the relative increase in the level of thyroid hormones in G4 can be explained by the intensive development of the fetus during this period. The analysis of the functional activity of the thyroid gland is relevant for assessing breeding qualities of animals. Further studies are planned to determine the concentration of thyroid hormone levels at different stages after calving.

P 161 | New echotexture parameters to evaluate the testicular parenchyma in stallions

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The aim of this work is to investigate the relationship between semen quality in stallions and the following testicular echotexture parameters: EC1 (black pixels), EC2 (white pixels), EC3 (mean gray level of pixels), Density (density of hypoechogenic areas), Diameter (mean diameter of hypoechogenic areas) and Area (total percentage of hypoechogenic area). Three transversal ultrasound scans were performed per testicle, in a total of 34 stallions. Ultrasonograms were done using an EXAGO scanner (ECM, France) connected to a 7.5 MHz linear probe. Afterward, a semen sample was collected per stallion, sperm motility was analyzed

by CASA and a sample was analyzed by microscopy at 1000× to investigate sperm morphoanomalies. The percentage and type of sperm abnormalities of each sample were determined. A total of 200 spermatozoa were counted per sample. As described by Love et al. (2015) (Love et al. 2015, Theriogenology 84:1587–93), we established a threshold of maximum 47% total abnormal sperm for fertile stallions. There were no differences between ipsilateral testicles in the echotextural parameters ($p > 0.05$). The Pearson correlation showed a significant moderate negative correlation between the EC2, EC3, density of hypoechogenic areas in the ultrasonogram and the percentage of primary morphoanomalies ($p < 0.01$). The same was true for the percentage of total abnormal spermatozoa. Analysis of data by ANOVA indicates that testicles producing subfertile samples differed significantly in EC2, EC3, Area and Density ($p < 0.05$). (This work was supported by Eureka E! 11188 and IDI-20170220.).

P 162 | Effect of a single dose of a GnRH analogue (lecirelin) on ovulation in pure breed Spanish (PRE) mares

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In this work, we examined the efficacy of a single injection of lecirelin on ovulation in PRE mares compared with the standard ovulation treatment with human chorionic gonadotropin (hCG). Once the estrus was detected and the presence of a preovulatory follicle (≥35 mm diameter at ultrasound), a total of 95 PRE mares were randomly injected im. with 2500 u.i of hCG (Veterin Corion, Divasa) (n = 38) or 100 μgr of lecirelin (Dalmarelin, Fatro) (n = 57). Ultrasound follow-up of the ovaries was done every 12 h until ovulation. There was a tendency to have significant differences ($p = 0.07$) between hCG and lecirelin, in the meantime between treatment and ovulation: 44.2 ± 14.6 h for hCG vs. 50.1 ± 16.7 h for lecirelin, but the median ovulation time was not different between both hormones: 40 h. Dividing the mares according to diameters of preovulatory follicles: less than 40 mm (n = 23), 40 mm (n = 49) and more than 40 mm(n = 23) there were significant differences ($p < 0.05$) between the 3 groups, for the time elapsed until ovulation. Mares with larger follicles ovulate logically earlier. When comparing treatments only in the mares with a 40 mm preovulatory follicle, the time elapsed to ovulation was not different ($p > 0.05$): 42.3 ± 13.8 h for hCG vs. 47.9 ± 16.1 h for lecirelin. In the group of mares with smaller follicles, the hCG treatment caused earlier ovulation, but differences were not significant ($p > 0.05$). Globally, there was no difference between treatments in the percentage of mares ovulating within 48 h after treatment.

P 163 | Reproductive and productive parameters in hair sheep under confinement system in the tropic of Veracruz affected by the type of mating

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The aim of this study was to assess the type of mating in hair sheep under confinement system on pregnancy and kilograms of lamb produced in a productive cycle in the tropic of Veracruz. Sheep were assigned randomly in three types of mating: 1) Continuous mating (n = 248), rams remained all the productive cycle, 2) Postpartum mating (n = 248), started at 45 days postpartum and lasted for 60 days and 3) Postweaning mating (n = 248), started 15 days after weaning and lasted for 60 days. Pregnancy was diagnosed by ultrasonography at 30 days postbreeding. Pregnancy rate was analyzed by chi-square test. The body weight at birth and at weaning of the lambs was analyzed by ANOVA and Bonferroni post hoc test. Lower pregnancy rate was observed in continuous mating (38.7%) showing significant differences ($p < 0.05$) compared to the postpartum mating (80.1%) and postweaning mating (82.1%). The type of mating also influenced ($p < 0.05$) the productive parameters (mean±SD). Kilograms of lamb at birth were significantly lower in continuous mating (567.7 ± 87.8) compared to postpartum mating (795.9 ± 77.0) and post-weaning mating (893.3 ± 98.6). Likewise, kilograms of lamb at weaning were significantly lower in the continuous mating (2454.4 ± 422.6) compared to mating postpartum (3735.7 ± 356.6) and mating postweaning (4120.9 ± 436.5). In conclusion, it is advisable to have 60 days controlled mating management, starting 45 days after parturition or 15 days after weaning to obtain a better pregnancy rate and more kilograms of lamb per sheep per productive cycle.

P 164 | Prolonged duration of farrowing in sows is related to a delayed start after decline of progesterone

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We hypothesised that in sows, a prolonged duration of stage II of labour, the period of foetal expulsion, may be related to the length of stage I of labour. The length of stage I was defined as the time between the start in decline of progesterone and birth of the first piglet. The decline in progesterone sets off a number of events that prepare the sow for stage II of labour, such as cervical dilation and increased oxytocin secretion. Data were obtained from 59 nulliparous sows, from which 8-h blood samples were obtained for progesterone measurements, in the last 3 days before farrowing. Parturition was induced with a luteolytic dose at d 113 of gestation (2 ml of alfaprostol, Alfabédyl, Céva Santé Animale, Libourne, France). The decline in progesterone (P4) before farrowing was fitted for individual sows

to a quadratic function, P4 = a.t. 2 + b.t + c, with t = time relative to farrowing in h, and the correlation between actual and predicted progesterone was 0.95. The start of decline in progesterone was arbitrarily defined as when the slope in the predicted progesterone profile equalled −0.07 ng/ml/h, and the median for this point was −52 h. Of the sows with a stage I longer than the median, 62% had a longer than average duration of farrowing (stage II). Of the sows with a stage I shorter than the median, only 36% had a farrowing duration longer than the median (Chi-square; p = 0.05). We therefore conclude that a delay in stage I events, may increase the risk of prolonged duration of foetal expulsion, stage II of farrowing.

P 165 | Screening of seed male birds' sperm

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Poultry breeding farms are facing the problem of decreasing reproductive function of seed male birds that results in their culling. The purpose of the research is screening of male birds' sperm. The results of the research done on the native sperm have shown the following changes: an increasing number of the defected sperm – 58% (normally 5%); decreasing germinating power of sperm – 51% (normally 70%); content of normal sperm – 39.4% (normally 90%), that means low quality of the sperm resulting in decreasing hatchability. Microbiological research done on the sperm have discovered opportunistic pathogenic microflora: *Stenotroph.meltophilia* (100%) and *Candidasp.* (80%) while the norm is not more than 10%. The results of electron microscopy of the sperm have shown swelling of mitochondria, clearing of matrix, and partial or total destruction of crista. Some sporadical sperm have been identified, such as: the ones with binominal spermatic filaments, in the state of high-grade destruction, and with abnormalities, whereas cytoplasm vacuolation, granularity of chromatin and abnormality of spermatic filaments have been identified. Such sperm with exaggerated sperm necks cannot penetrate an egg membrane and range and speed are influenced in a negative way. The main reason for alteration of mitochondria is connected with negative changes in ATF production. The II type of swelling mitochondria caused by increased penetration of inner membrane has been identified. When the content of calcium sparks exceeds the norm, they penetrate mitochondria and form the precipitate of calcium phosphate that results in nonreversible calcification of mitochondria, damage of inner and outer membranes, and destruction of mitochondria.

P 166 | Age-related changes in binding characteristics of growth hormone receptors in the granulosa layer of hen preovulatory follicles

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Ovarian aging is considered as the main reason for the decline in the laying intensity in aged domestic hens. Growth hormone (GH) acts as an endocrine or paracrine/autocrine regulator of the avian ovarian function. The aim of the present work was to compare plasma GH levels and binding characteristics for GH receptors (GHR) in the granulosa layer of preovulatory follicles in young hens with long clutch (YLC), old hens with long clutch (OLC), and old hens with short clutch (OSC). To this end, the three largest yellow follicles (from F1 to F3) of the hens sacrificed at 1.5 and 14.5 h after assumed ovulation were used. Equilibrium dissociation constants (Kd) and maximum binding capacities (Bmax) for GHR were determined by Scatchard analysis of saturation curves using a radioreceptor assay. Plasma concentrations of GH were determined by RIA. Regardless of the hen age, reproductive status, and stage of the ovulatory cycle, Bmax for GHR increased 1.3–1.4 times (at least $p < 0.05$) with follicular enlargement from F3 to F1. Concurrently, the respective Kd changed only in the case of OLC hens at 1.5 h after ovulation, decreasing 1.2-fold ($p < 0.05$) during the transition from F3 to F1 follicles. Furthermore, Bmax for GHR in the OLC group rose ($p < 0.01$) between the beginning and middle of the ovulatory cycle from 234 ± 11 to 296 ± 17 fM/10^6 cells (F1) and from 176 ± 12 to 235 ± 14 fM/10^6 cells (F3). At the same time plasma GH levels were similar in all compared groups. These findings indicate that the sensitivity of granulosa cells to GH increases with follicular maturation despite the hen age and reproductive status. By contrast, ovulatory cycle-adjusted changes in the cell sensitivity may be associated with maintaining the reproductive function in aged hens.

P 167 | Biochemical status of cows in the dry period in connection with reproductive performance and milk productivity

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The aim of the research was to study the biochemical profile of the Holstein cows in the second half of the dry period in connection with the period from calving to the first AI (1AIP) and the milk yield for 100 days in lactation. At the beginning of the dry period (60 days before calving), 20 cows were divided into two groups. Group I

(n = 10) received the usual diet and group II (n = 10) the same diet supplemented with sugar rich fodder (glucose-50–55%, fructose -25–35%, maltose -10–15%, sucrose-10–20%) 150 g per cow/day. After 40 days, blood samples were taken. The concentrations of glucose and cholesterol were higher in group II (3.89 ± 0.05 mM vs. 3.68 ± 0.07 mM, p<0.05 and 2.96 ± 0.12 mM vs. 2.09 ± 0.12 mM, p<0.001). The 1AIP tended to decrease in the cows of group II (80 ± 9 days vs. 105 ± 10 days, p = 0.075). The milk yield during the first 100 days of the subsequent lactation was higher in group II (4415 ± 167 kg vs. 3660 ± 242 kg, p<0.05). A negative correlation between the peripheral glucose concentration in the second half of the dry period and the 1AIP (r = −0.62, p < 0.05) was found in group I. In group II there was a tendency to a negative correlation of the cholesterol concentration and the 1AIP (r = −0.641, p = 0.056). Results of the study suggest that an improvement of the energy metabolism in the second phase of the dry period is associated with an increase in the subsequent milk yield and tends to decrease the period from calving to first AI. (Authors acknowledge financial support from The Federal Agency for Scientific Organizations (FASO Russia), project No. AAAA-A18-118021990006-9.).

P 168 | Could Doppler ultrasound of testicular arteries be a good indicator of oxidative DNA damage of canine sperm?

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Assessment of testicular artery blood flow by Doppler ultrasound is an important diagnostic technique that has been recently included in the breeding soundness examination of male dogs. In addition, alkaline comet assay permits to quantify sperm oxidative DNA damage. This preliminary study aimed to evaluate the relationship between testicular artery blood flow and oxidative DNA damage of canine spermatozoa. Semen collections and ultrasound examinations were performed in five dogs (3–11 years old). Sperm DNA damage was assessed and scored from 0 to 4 (depending on the percentage of DNA present in comet's tail), using an alkaline comet assay with and without a Formamidopyrimidine DNA glycosylase treatment. Several testicular artery blood flow parameters were measured in the supratesticular and marginal arteries of both testis: Systolic Peak Velocity (SPV), End-Diastolic velocity (EDV), Resistive Index and Pulsatility Index. Data were analysed by T-test and Pearson′s correlation coefficient. Mean SPV values of the left supratesticular artery were significantly higher (p < 0.05) than of the right (30.7 ± 7.07 vs. 19.0 ± 3.37). In the left testis, SPV of the marginal artery was significantly lower (p < 0.05) than in the supratesticular artery (18.1 ± 7.5 vs. 30.7 ± 7.07). A strong positive relationship was observed between EDV of the marginal artery and total DNA damage of spermatozoa (r^2 = 0.88; p < 0.05) as well as with oxidative DNA damage (r^2 = 0.83; p < 0.05). Our results suggest that Doppler ultrasound of testicular testis can be a promisingly predictive technique of oxidative DNA damage of spermatozoa, although more studies are required to confirm this conclusion.

P 169 | Anesthesia concerns for CT imaging (computed tomography) and surgery of the reproductive system in bitch with atypical Ovarian Remnant Syndrome (ORS)

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Imaging of the reproductive tract in dogs relies mainly on ultrasound (US); however, CT has an invaluable role to play when diagnosis cannot be satisfactorily delineated by US 6 year old mixed breed bitch, ASA II, with painfull abdomen and lumbar region, leucocytosis, emaciation and episodes of vomiting was presented to the clinic two years after ovariohysterectomy surgery. The outcome of the ultrasound examination was equivocal as bilateral focal lesions with hyperechoic components were found at the region of ovarian pedicles but there was no ovarian tissue detected. Advanced diagnostic imaging (CT) of reproductive system before further, immediate surgery was performed. Main concern of emergency anesthetic plan to maximize patient management success was to maintain normotension, isovolemia and adequate cardiac output sufficient to maintain renal perfusion. The general inhalational anesthesia (sevoflurane in oxygen; Sevoflurane Baxter) with fentanyl CRI (fentanyl 6 μg/kg/h, Fentanyl) with endotracheal intubation following intravenous premedication (midanium 0.1 mg/kg; Midanium 5 mg/ml) and induction (etomidate 1 mg/kg; Etomidate-Lipuro 2 mg/ml) was performed and polyionic balanced crystalloid (Solutio Ringeri Lactate) at dosage 10 ml/kg/h with synthetic colloid at dosage 10 ml/kg/h (Gelofusine) was given to both hydration and alkalization to avoid postcontrast (Ultravist 370) dialysis. Inflammatory granuloma around suture and residual ovarian tissue were resected and right nephrectomy as well as segmental pancreatectomy was performed. No general side effects were observed. The procedure allows for optimal safe management of the CT necessary for surgical planning and further surgery. The goals on anesthetic management should focus on using a balanced, multimodal approach.

P 170 | New device for deep cervical artificial insemination in gilts

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Post-cervical artificial insemination (PCAI) has been implemented in the last decades in porcine industry, which deposited the sperm in the body of the uterus instead of the classical cervical deposition (CAI). However, PCAI has some limitations in gilts (Hernandez-Caravaca 2017, Theriogenology 90:147–152) mainly because of the difficulty in introducing the cannula through the cranial part of the cervix. The aim of this study was to evaluate the reproductive performance using a new AI catheter specially designed for gilts (Deep cervical AI, Dp-CAI) in which the sperm is deposited deeply in the cervix (8 cm cranial to CAI deposition). The experimental groups were: (1) CAI: gilts (142.31 ± 8.27 kg; n = 1071) inseminated using 2.5×10^9 sperm/85 ml; (2) Dp-CAI: gilts (142.35 ± 8.06 kg; n = 1010) inseminated using 1.5×10^9 sperm/45 ml. Data for farrowing rate (%) and number of piglets born (total and live) were analyzed by Chi-square and Mann–Whitney tests ($p < 0.05$), respectively. The Dp-CAI method was successfully applied in 88.90% of the gilts. The results showed differences in all the parameters analysed [CAI vs. Dp-CAI: Farrowing rate: 90.6% vs. 87.5%; Total piglets born: 12.63 ± 1.41 vs. 13.11 ± 3.48 and piglets born alive: 12.28 ± 1.48 vs. 12.00 ± 3.50, $p < 0.05$). Moreover, the fecundity index (total number of piglets born per 100 inseminations calculated by farrowing rate × total piglets born) was higher while using Dp-CAI method (CAI: 1144.10 ± 127.7 vs. Dp-CAI: 1147.25 ± 305.2, $p < 0.05$). In conclusion, the use of Dp-CAI device could be applied in gilts with a high degree of success reducing conventional sperm doses without impairing reproductive parameters. (Supported by MINECO (AGL2015-66341-R) and Fundación Séneca (20040/GERM/16).)

P 171 | Tissue composition differences in the cranial cervix of nulliparous and multiparous sows

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Post-cervical artificial insemination (PCAI) -semen deposited in the body of the uterus-, is replacing the traditional cervical insemination (CAI) because its high reproductive performance with 2–3 times lower sperm concentration. However, PCAI is hardly used in nulliparous sows due to the difficulty of passing through the cranial part of the cervix. Changes related to ageing and/or the succession of pregnancies may affect the tissue composition of the cervix. Thus, we hypothesized that the tissue is significantly different in nulliparous and multiparous sows and the differences might be related to the problems found in nulliparous with regards to the progression of the cannula during PCAI. Histological cross-sections from the cranial portion of the cervix of nulliparous (n = 17) and multiparous sows (n = 11) were stained with Masson trichrome, and the % of collagen, muscle fibres, fundamental substance and capillaries quantified with a digital morphometry software (SigmaScan Pro 5.0). Either ANOVA or Mann–Whitney tests were performed for statistical analysis at 95% of confidence. Multiparous sows showed higher collagen content either in endometrium (56.34 ± 19.46 vs. 39.43 ± 20.15%; $p < 0.001$) or myometrium (21.56 ± 11.75 vs. 7.52 ± 7.36%; $p < 0.001$); and less muscle fibres (10.25 ± 6.30 vs. 16.93 ± 11.77%; $p < 0.01$) and fundamental substance (15.44 ± 5.19 vs. 19.76 ± 11.46%; $p < 0.05$) in endometrium. No differences in capillaries were found. In conclusion, ageing and/or the number of parturitions increases the collagen content and decreases the muscle fibres in the cranial portion of the porcine cervix so that consequent changes in the tensile properties could be related with the easier progression of the cannula during PCAI. (Supported: MINECO (AGL2015-66341-R), Fundación Séneca (20040/GERM/16))

P 172 | Quality of porcine sperm selected by sorter technique for its binding to OVGP1-Cherry

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It is known that oviductal proteins play an important role in the interaction of gametes. Among them, oviductin (OVGP1) has been identified as a major component of oviductal fluid. OVGP1 has been detected bound to the sperm membrane and it has been demonstrated that treatment with OVGP1 increases sperm-oocyte binding (Yang et al. 2015, PlosOne 10:24). In previous studies, we have been able to select spermatozoa by binding to a fluorescent-fusion recombinant protein (OVGP1-Cherry) using cytometry techniques (López-Úbeda et al. 2017, Reprod Dom Anim 52 Suppl. 4:78). The aim was to evaluate the quality of selected sperm subpopulation (OVGP1-Cherry −; OVGP1-Cherry +,) assessing (viability, motility and acrosomal integrity) compared to control sample (sperm incubated without OVGP1-Cherry). Sperm incubated in presence of OVGP1-Cherry protein and separated into two subpopulations (OVGP1-Cherry + or −), by Cell Sorter (Sony SH800Z) using 561 nm excitation laser, were incubated with YO-Pro1 to analyse its vitality or PNA-FITC to assess the sperm acrosomal status. An aliquot was used for the study of motility by CASA system. Initial results show that sperm bound to OVGP1 had statistically ($p < 0.05$) higher viability levels (OVGP1-Cherry +: 97.1 ± 0.9) than de other groups (Control: 46.6 ± 1.0; OVGP1-Cherry −:64.8 ± 5.5). Acrosomal integrity, no statistical differences ($p > 0.05$) were found between the groups, although the spermatozoa bound to the OVGP1 protein showed a higher staining

(Control: 68.4 ± 21.0; OVGP1-Cherry − :66.2 ± 17.4 and OVGP1-Cherry +: 79.9 ± 12.8). CASA analysis shows that the protein does not modify sperm motility and sperm after sorting exhibit motility. In conclusion, OVGP1-Cherry protein selects sperm with superior vitality. (Supported by MINECO-FEDER (AGL2015-70159-P).)

P 173 | Case report of an uncommon benign luteoma in an ovarian remnant of a spayed bitch

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Sex cord stromal ovarian tumours (SCS) as granulosa cell tumour (GCT) are common in the bitch ovary. However, luteoma is an unusual benign SCS ovarian tumour and rarely reported in the bitch (Ichimura 2010, J Vet Med Sci 72:229–234). An ovariohysterectomized 11 years old mixed breed bitch was presented with a persistent oestrus signs during 6 months. The bitch was spayed 7 years ago. Vaginal cytology showed intermediate and superficial cells. Hematological and biochemical analysis were normal. On abdominal ultrasound examination, a cyst structure caudally of the right kidney was seen. Abdominal Computed Tomography (CT) confirmed the presence of a cavitary mass ventro-caudally to the right kidney (6 × 5 × 6.7 cm) with soft tissue attenuation and peripherally enhancing. Based on findings, presumptive diagnosis of cyst and/or tumour in ovarian remnant were made. After laparotomy, a large cystic structure containing serosanguinous liquid in a remnant right ovary was observed. Histopathologic study revealed a luteoma SCS ovarian tumour. The bitch recovered uneventfully. This case presented abnormal vaginal bleeding, which is the most usual clinical manifestation of GCT and luteomas in an ovarian remnant. There are several reports regarding GCT in spayed bitches (Spoor 2014, Vet Clin Pathol 43:109–10), however, to our knowledge only one report has been published previously about presence of luteoma in a spayed bitch.

P 174 | Specific PDE10 inhibitor improves chilled canine sperm quality

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Nowadays, there is increased interest for the use of chilled canine semen. The main problem of semen preservation is related to decrease in sperm quality and fertility during storage and transport. The phosphodiesterase (PDE) inhibitors may increase the intracellular level of cAMP and subsequently effect sperm motility and capacitation status. The objective of this study was to test the effect of a specific PDE10 inhibitor (PDE10 I) on sperm quality after storage for 48 h at 4°C. The ejaculates were obtained by digital manipulation from five 4–5 years old healthy dogs. Sperm samples were extended in Tris-fructose-citrate medium, chilled (0 h, 24 h and 48 h) and then incubated with different concentrations of PDE10 I (0 μM, 2.5 μM, 10 μM and 20 μM) during 20 min at 37°C. Sperm motility (CASA), viability and acrosome integrity (PI/FITC-PNA) were evaluated after 0 h, 24 h and 48 h of preservation at 4°C. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. The results showed that PDE10 I had no effect on sperm quality after conservation for 0 h and 24 h. However, concentration of 2.5 μM significantly increased ($p < 0.05$) kinematics parameters of spermatozoa without impairing viability and acrosome integrity compared to control at 48 h. The supplementation with 20 μM significantly decreased ($p < 0.05$) all sperm quality parameters evaluated. In conclusion, the utilization of 2.5 μM concentrations of PDE10 I improved canine sperm quality after 48 h of refrigeration. (Supported by DGA and Fondo Social Europeo (IA2).)

P 175 | Additional seminal plasma on boar seminal doses mitigates the deleterious effect of uterine fluid

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Artificial insemination is widely used in farms with an intensive pig production. To optimize the production, the ejaculate is diluted in commercial extender, and in consequence the concentration of seminal plasma (SP) is reduced. The SP plays a critical role on spermatozoa functionality in the female reproductive tract. In fact, sperm quality is affected by uterine fluid (UF) but not in presence of SP. Therefore, the aim was to compare the effect of UF on boar seminal doses quality, in presence/absence of additional SP. Three different experimental groups were analyzed: (1) Control: seminal dose; (2) UF: seminal dose +20% of UF; (3) UF-SP: seminal dose +20% of UF +20% of SP. Sperm motility [analyzed by CASA system: total motility (%), straight line velocity (VSL), linearity of the curvilinear path (LIN)], viability and acrosome damage were analyzed. As results, the total motility was greater in UF-SP group than UF ($p = 0.008$) (UF-SP: 90.3 ± 2.7% vs. UF: 84.7 ± 2.7%), without differences with control (87.7 ± 2.7%). The VSL was greater in UF than control ($p = 0.004$) (UF: 36.3 ± 2.0 μm/s vs. control: 29.8 ± 2.0 μm/s), without differences with UF-SP. The LIN was greater in UF and UF-SP than control ($p < 0.0001$ and $p = 0.03$, respectively) and it was greater in UF than UF-SP ($p = 0.003$) (UF: 57.9 ± 2.1% vs. UF-SP: 52.4 ± 2.1% vs. control: 48.4 ± 2.1%). The acrosome damage was greater in control and UF than UF-SP ($p = 0.007$ and $p = 0.01$, respectively) (control: 4.2 ± 0.6% and UF: 4.0 ± 0.6% vs. UF-SP: 2.1 ± 0.6%), without differences between those. The viability was not affected by UF. In

summary, the addition of SP to commercial seminal doses mitigates the deleterious effect of UF increasing total motility and decreasing acrosome damage. (Supported by MINECO (AGL2015-66341-R) and Fundación Séneca (20040/GERM/16).)

P 176 | Low sperm concentration affects boar sperm quality in semen doses even in presence of high amounts of seminal plasma**

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Reduction of seminal plasma (SP) is regarded as critical for sperm quality in boar semen doses with a lower sperm concentration. The aim was to examine the interactive role of sperm concentration and seminal plasma content on sperm quality in liquid stored semen doses. In Experiment 1, semen (n = 8 boars) was diluted to 18×10^6 and 10×0^6 sperm/ml ad 100 ml Beltsville Thawing solution (BTS). Additionally, in one 10×10^6/ml sample, the extender was supplemented with homologous SP up to the same SP concentration as reached in 18×10^6/ml doses (3–14%). After 72 h of storage, motility assessed with computer-assisted semen analysis and the proportion of viable sperm with high mitochondria membrane potential assessed by flow cytometry were lower ($p < 0.05$) in both 10×10^6/ml groups (i.e. with and without substituted SP) compared to the 18×10^6/ml group. In Experiment 2, semen (n = 8 boars) was centrifuged and four variants of semen doses containing 18×10^6 or 10×10^6 sperm/ml in BTS with either 10% or 0.5% SP were prepared. After 24 h and 144 h of storage, motility was lower ($p < 0.05$) in the 10×10^6/ml group compared to the 18×10^6/ml group both with 10% SP (24 h: 82 ± 7% vs. 85 ± 4%; 144 h: 51 ± 35% vs. 82 ± 17%) and 0.5% SP (24 h: 77 ± 6 vs. 84 ± 5; 144 h: 74 ± 6 vs. 82 ± 5). Plasma membrane and acrosome integrity did not differ. In conclusion, low sperm concentration affects sperm quality regardless of the amount of seminal plasma present in the semen dose.

P 177 | N-acetylcysteine addition to canine sperm freezing extender does not affect its post-thaw quality

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Increased reactive oxygen species (ROS) production during cryopreservation impairs sperm post-thaw quality. Our objective was to alleviate this oxidative burst supplementing a commercial canine sperm freezing extender with N-acetylcysteine (NAC). Ejaculates were collected from 6 healthy dogs and evaluated for concentration and total motility (>70%). Each ejaculate was split in 3 aliquots, centrifuged at 700 g for 10 min and diluted in a commercial freezing medium (CaniPlus Freeze®) to reach a final concentration of 100×10^6 sperm/ml. The aliquots were supplemented with 0, 1.25 or 2.5 mM of NAC. Spermatozoa were packed in 0.5 ml straws, cooled at 4°C (1 h), placed above liquid nitrogen vapors (20 min) and stored. After thawing, total motility (TM) was evaluated using a CASA system while mitochondrial membrane potential (MMP), sperm viability and ROS production were assessed by flow cytometry using JC-1, SYBR-14/PI and MitoSox, respectively. To compare pairs of values a one way ANOVA was used. All results are expressed as mean ± SEM in % for 0, 1.25 and 2.5 mM of NAC, respectively: TM (45.7 ± 5.5 vs. 40.9 ± 5.8 vs. 34.9 ± 4.7); viability (51.4 ± 7.5 vs. 52.2 ± 7.5 vs. 47.7 ± 7.5); high MMP (54.0 ± 6.0 vs. 51.8 ± 6.4 vs. 52.2 ± 6.0), and sperm producing ROS (88.9 ± 2.5 vs. 85.4 ± 4.2 vs. 87.9 ± 3.6). No significant differences were observed between treatments ($p > 0.05$). Hence, more dosages need to be tested in order to clarify if sperm freezability can be further improved in dogs by the use of NAC.

P 178 | Gene expression in blood as potential biomarkers of early pregnancy in dairy cattle**

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An early pregnancy test, valid before day 21 post artificial insemination (AI) would have widespread application in dairy herds and potentially increase herd reproductive efficiency. Changes in gene expression in different tissues have been described previously. The aim was to identify differently expressed genes and describe their profile in blood during early pregnancy. Dairy cows (n = 25) were enrolled for a control oestrous cycle and the subsequent cycle following AI. Pregnancy was confirmed by ultrasound at day 35 after AI. Whole blood was collected in Tempus RNA tubes on days 15, 17, 19 and 21 of both cycles. Total RNA was extracted and reverse transcribed into cDNA. Relative expression of 14 genes were analysed by real-time qPCR. Following gene normalisation, data were analysed using a paired *t*-test and fold changes were calculated. At day 15 of pregnancy, genes 7 and 10 had greater expression compared with expression levels on the equivalent day during the non-inseminated oestrous cycle ($p < 0.05$). At day 17, genes 5, 7, 9 and 10 showed greater expression during pregnancy ($p < 0.05$), however, all fold changes were below 1.5. At days 19 and 21 of pregnancy, 13 of the 14 genes exhibited increased expression ($p < 0.05$) and fold changes were 1.5 or higher for genes 2–6, 8, 9 and 13 at day 19; and genes 1–9 and gene 13 at day 21. Sensitivity and specificity for the genes with a fold change >1.5 ranged from 59% to 91% and 41% to 86%,

respectively, at day 19; and 83–100% and 52–100%, respectively, at day 21. A panel of different genes evaluated were sufficiently sensitive and specific to be used as a biomarker of early pregnancy in cattle.

P 179 | Study of the renal development of canine fetuses by high definition ultrasound (HD) – preliminary results

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Ultrasonography is an important technique for fetal evaluation in dogs. Recently, High-definition (HD) ultrasonography has been included in veterinary obstetrics. The objective of this study was to evaluate the renal development in canine fetuses, by ultrasound (HD) in brachycephalic pregnant bitches. Twelve clinically healthy, brachycephalic, multiparous pregnant bitches were selected. From the 7th day of pregnancy till parturition, daily examinations were performed using HD (ACUSON S2000/SIEMENS with matrix and multifrequential transducer of 18.00 MHZ). The presence of the kidney was identified as a hyperechoic structure at the 27th day of pregnancy, without differentiating the renal regions. At the 37th day of pregnancy, the renal pelvis was visible as an anechoic structure. Renal diverticula and the distinction between the cortex and medulla (1:1) were clearly visible at day 38, the renal cortex being visible as a more hyperechoic structure relative to the medulla (Figure 1). Our results show that when compared to conventional two-dimensional ultrasound, HD achieves a better image in terms of quality and precocity for gestational examination of the pregnant bitch. Some authors report visualization of the kidney at 39–47 (Nyland & Mattoon, 2015), 41–43 gestational days (Kim & Son, 2007). High-definition ultrasonography is a safe and efficient technique for fetal monitoring in dogs. These data help to a promising examination in the gestation and contribute to the diagnosis of abnormalities and facts not yet elucidated in the area.

P 180 | Vitrification modifies expression of genes associated with cell cycle, DNA replication and DNA repair in porcine blastocysts**

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Vitrification is the preferred method for porcine embryo cryopreservation. Although good farrowing rates (70–75%) have been obtained after transfer of vitrified blastocysts, the loss of pregnancies is higher (10–20%) than that for fresh embryos (<2.5%). Since DNA-damages and alterations of cell cycle-genes have been related to abortions in humans, the aim of this study was to investigate the expression of genes related to these biological functions in vitrified porcine blastocysts. Vitrified (N = 15) and fresh (N = 15) blastocysts were analyzed using an Affymetrix microarray. A total of 846 genes were up-regulated and 625 down-regulated in vitrified blastocysts compared to controls. The five target pathways evaluated were significantly ($p < 0.05$) modified due to vitrification. The 8.7%, 6.25%, 5.7%, 5.6% and 4.7% from mismatch repair, base excision repair, DNA replication, cell cycle and nucleotide repair KEGG-pathways respectively, were modified in vitrified blastocysts compared to controls. Most of the altered genes were down-regulated. Up-regulation was only recorded for Lig I and PAR from mismatch and base excision repair and DNA replication pathways. In cell cycle pathway four genes were up-regulated ZBTB17, CDKN1, TGFB and Smad2,3. The up-regulation of TGFB/Smad2,3 signaling may be of importance since it induces cellular arrest. Alterations of this pathway have been also correlated to developmental defects. This, together with the alterations of DNA replication-repair pathways, which may cause increase of the DNA-alterations in vitrified blastocysts, may be related to an increased pregnancy loss. (Supported by MINECO-FEDER (AGL2015-69735-R) and Seneca Foundation (19892/GERM/15).)

P 181 | Ultraviolet light reduces bacterial contamination without impairing sperm quality of chilled canine semen

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Nowadays, resistances of bacteria to antibiotics are a worldwide problem; therefore it's necessary to develop alternative methods to control bacterial contamination in semen samples. The aim of this study was to determinate the effect of ultraviolet light (UV, 340–380 nm) irradiation on canine sperm quality, DNA integrity and total microbiological count. Ejaculates from 4 Beagle dogs (5 years of age) were obtained by digital manipulation. Semen samples were pooled, extended in a cooling medium (Kenney) and centrifuged at 700 g 10 min. Sperm pellets were re-suspended in the same cooling medium and irradiated with UV (experimental group) or not (control) into petri dish at 37°cm during 5 min (preliminary studies were performed) under sterile conditions. Then, sperm samples were kept at 4°C and evaluated 0, 24 and 48 h later. Total and progressive sperm motility, viability, acrosome integrity and DNA damage were assessed. In addition, bacterial contamination was determined after culture on blood agar plates for 24 h at 37°C. Data were analyzed by GLM test. The results showed that UV irradiation significantly reduced ($p < 0.05$) the sperm bacterial contamination without causing changes in sperm quality parameters. Neither significant effect on sperm DNA integrity during UV irradiation nor along refrigeration period was observed. In conclusion, sperm UV treatment at 37 cm during 5 min might be an

alternative method to control sperm contamination without detrimental effects on sperm quality. (Supported by Fondo Social Europeo (IA2).)

P 182 | Tyrosine phosphorylation pattern in ejaculated and epididymal mouflon (*Ovis musimon*) sperm associated with capacitation process**

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Capacitation is a series of biochemical and physiological changes that sperm must undergo to fertilize the egg. Among others, this process is associated with an increase in protein tyrosine phosphorylation (PTP). Although widely studied in domestic small ruminants, capacitation is poorly known in wild species. The aim was to evaluate the PTP pattern of frozen-thawed mouflon epididymal and ejaculated sperm. Straws were thawed and diluted in TALP medium supporting capacitation (CAP) (supplemented with bovine serum albumin and $NaHCO_3$) or not (NCAP). Samples were incubated at 40×10^6sperm/ml during 3 h at 39°C and 5% CO_2. Sperm PTP was assessed by Western blot (WB) at 1 h and by indirect immunofluorescence (IIF) at 0, 1, 2 and 3 h of incubation. Statistical analysis was performed by one-way ANOVA followed by T-Tukey test. Results obtained by WB showed a higher PTP in ejaculated sperm incubated in CAP than in NCAP ($81.8 \pm 2.9 \times 10^6$ vs. $64.68 \pm 5.0 \times 10^6$ relative amount; $p < 0.05$) while there was no effect of medium in epididymal sperm. Regarding the IIF results, there was a higher PTP in ejaculated sperm tail in CAP than in NCAP at 1, 2 and 3 h (10.8 ± 3.4 vs. 1.4 ± 1.4, 9.2 ± 2.8 vs. 1.2 ± 0.8 and 14.8 ± 3.7 vs. 2.6 ± 1.7%, respectively; $p < 0.05$). There was also an effect of time in CAP with higher tail PTP at 3 h than 0 h (14.8 ± 3.7 vs. 2.0 ± 2.0%; $p < 0.05$). However, epididymal sperm did not show different PTP distribution neither between media nor time effect. In conclusion, ejaculated sperm responded to capacitation milieu by an increase in PTP, but not epididymal sperm. Further studies are required to evaluate if the contact with seminal plasma could explain the differences between ejaculated and epididymal sperm capacitation. (Supported by "EU-H2020 MSCA, REPBIOTECH 675526" and "MINECO AGL2014-52081-R".)

P 183 | Testicular volume in the Miranda Donkeys: accuracy of ultrasonographic measurements

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Ultrasonography is considered one of the most accurate method for obtaining measurements of testicular length, width and height. The aim of the present work was to compare ultrasonographic (US) determinations of testicular size with real (R) measurements that were obtained after orchiectomy. Eight Miranda donkeys were studied, being six colts and two adults, age range 4–48 months and weight median of 154.75 kg. Ultrasonographic evaluation of testicular dimensions was performed before orchiectomy, with measurement of length (USL), width (USW) and height (USH). After orchiectomy, the same measurements were obtained (i.e. RL, RW and RH). Testicular volume (TV) was then obtained through the Lambert formula for ellipsoid $TV = L \times W \times H \times 0.5233$. Data analysis was performed with SPSS 25.0 software, with a p value < 0.05 as statistically significant. Medians of USL, USW and USH (cm) were 3.4, 2.75 and 2.19 (left testicle) and 3.34, 2.55 and 2.14 (right testicle), respectively; and medians of RL, RW and RH (cm) were 3.80, 2.40 and 2.60, and 3.50, 1.90, 2.00, respectively. There were no differences between medians of US-total TV and R-total TV (16.490 cm^3 and 19.425 cm^3, respectively; $p = 0.069$). Analysis of paired medians of US testicular to R measurements revealed significant differences in measurements of W of the right and TV of the left testicle ($p = 0.036$ and 0.050, respectively; Wilcoxon signed-rank test). Measurements by both methods showed no differences between the right and the left testicle. Strong and significant correlations were found between body weight, age and TV, as well as between both methods of measurements ($p < 0.01$). Testicular US evaluation method shows strong and significant correlations with real testicular dimensions in donkeys.

P 184 | The non-invasive system of surface temperature monitoring during perinatal period in mares

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The perinatal period is critical for decisions that can affect the future health of the foal and future reproduction of the mare. All problems

during the foaling require immediate detection and treatment, which is problematic due to variable length of gestation (335–342 days) and changeable time of parturition. A method leading to prediction of parturition date is needed in the reproductive management of mares. One valuable method is internal temperature monitoring, yet it requires an invasive system based on implantation of electronic sensors. We demonstrate a non-invasive, telemetric system for long-term monitoring of uninterrupted surface temperature. In six mares surface body temperature was monitored for 7 days before parturition. In telemetry, the thermal sensor was positioned on the vulva (VT) and bottom surface of the tail (TT) using a high adhesion tape and tail protector in own modification, respectively. No difference ($p = 0.064$) between VT and TT was stated, with mean values of 35.4 ± 1.7°C and 36.1 ± 1.4°C, respectively, with a strong positive correlation between VT and TT ($r = 0.81$; $p < 0.001$) in each horse. The measurement in VT and TT at 0 hr and −1 hr were significantly lower ($p < 0.01$) from temperatures at the same time in other days (1–6) and a decreasing trend between −15 hr and −2 hr was shown. Low positive correlations between the ambient temperature (AT; 16.5 ± 3.9°C (9.0–24.4°C)) were stated (AT vs. VT; $r = 0.23$; $p < 0.001$ and AT vs. TT; $r = 0.27$; $p < 0.001$). Obtained results corresponded with previous research using internal temperature measurement. Presented telemetric system is non-invasive, easy to apply and provides rapid readings of temperature.

P 185 | N-acetylcysteine addition to Puro Sangue Lusitano sperm freezing extender does not affect its quality post-thaw

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Sperm cryopreservation in horses induces an overproduction of reactive oxygen species (ROS) and decreased fertilizing ability. Our aim was to evaluate the effect of N-acetylcysteine (NAC) addition at different concentrations (1 mM or 2.5 mM) to a sperm freezing medium composed by INRA 96® extender added with 2.5% (v/v) dimethylformamide, 2.5% (v/v) glycerol and 2% egg yolk (v/v). Three to seven ejaculates of 4 Puro Sangue Lusitano stallions were used ($n = 17$). Total motility (TM) and progressive motility (PM) were assessed by CASA. Viability, mitochondrial membrane potential (MMP) and ROS production were evaluated by flow cytometry using propidium iodide PI/SYBR-14, MitoTracker and MitoSOX, respectively. A one way ANOVA was used to compare pairs of values. No significant differences were found. The results are expressed as the mean in % ± standard error of the mean (SEM) for control, 1 mM or 2.5 mM of NAC, respectively: TM (31.4 ± 1.8 vs. 30.3 ± 1.4 vs. 30.0 ± 1.3); PM (9.4 ± 1.1 vs. 9.1 ± 1.0 vs. 9.8 ± 0.9); viability (32.7 ± 2.0 vs. 32.4 ± 1.9 vs. 32.7 ± 2.0); ROS production (59.9 ± 1.6 vs. 58.1 ± 1.2 vs. 61.1 ± 0.9) and MMP (32.1 ± 1.9 vs. 31.3 ± 1.5 vs. 30.0 ± 1.6). Even though NAC is a potent ROS scavenger, its addition to the freezing medium does not improve equine sperm quality post-thaw. More studies are needed to fully elucidate the effect of NAC addition at different concentrations to equine sperm freezing media.

P 186 | Influence of osmolality of the media for dilution and cryopreservation of turkey toms' sperm on fertilization ability of thawed sperm

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Development of the methods of cryopreservation is very important for preservation and effective use of valuable males' sperm and gene pool preservation. The efficiency of application of cryoconservated sperm in big extent depends on the composition of the synthetic media for dilution, cryoprotector and the method of freezing. Crucial parameter of a medium for sperm dilution is its osmolality, because when using a hypertonic medium, escaping of the water molecules from the sperm cell, shrinking of the intracellular space and lowering of the freezing point will occur. This study aimed to define influence of media osmolality on cryopreservation of turkey toms' sperm at the high speed freezing. Statistical analysis was performed using program Statistica 6.0. Two media B-3 and B-8 were tested with respective osmolality 374 and 415 mOsm for cryopreservation of turkey toms' sperm in pellets. The thawing was done by the method of flowing down, artificial insemination- by the method, developed by RRIFAGB. There were incubated 301 eggs in the experimental group-1 (B-3) 269 eggs in the group-2 (B-8). During the experiment there was found, that the increasing of the ejaculates osmolality up to 415 mOsm, cryotolerance and fertilization ability of the frozen-thawed sperm apparently increases. The eggs fertility in two experimental groups was respectively 74.8% and 84.2%, hatchability 64.18% and 69.6%. The RRIFAGB technology of the turkey toms sperm' freezing, with use of the dilution medium B-8 (osmolality 415 mOsm) proved its efficiency at the freezing by high speed of temperature lowering. (The study was supported by Federal Agency for Scientific Organizations (No. 2018-AAAA-A18-118021590134-3); biomaterial used in the study was provided by the Genetic Collection RRIFAGB.)

P 187 | Reproductive qualities of cows in the dosed feeding of micronized yeast during the transit period

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The transition period for cows is decisive in the formation and maintenance of reproductive abilities, health and productivity. As an

additional source of protein and other biologically active substances, micronized fodder yeast (MFY) was used. Investigations established the content of the mass fraction of crude ash 9.95 ± 0.16%, raw fat 0.7 ± 0.01%, crude protein 45.36 ± 0.23%, crude fiber 2.05 ± 0.05% in MFY. Feeding MFY to cows at a dose of 140 g per head 3 times a week during the transition period, positively influenced the preservation of the fetus. Pregnancy proceeded without pathologies, the yield of the calves was 100%. The indiffferent period in animals in which the diet included MFY was 65.3 ± 1.18 days and 70.6 ± 1.05 days for the study and the control Group, respectively. Calving to conception in the experimental group (G1) and the control Group (G2) was 77.5 ± 1.86 days, and 81.4 ± 1.9 days, respectively. The duration of the calving interval in G1 averaged 367.8 ± 4.15 days and 380.2 ± 4.05 days in G2. Fertility after first insemination in G1 was 76.5% on average, while in G2 it was only 65.3%. The insemination index among cows from G1 decreased by 7.9%. In G1 the insemination index on the average for the group was 1.85 ± 1.13 times, and in G2 2.01 ± 0.83 times. The calves' body weight in the first day of life, obtained from the cows from G1, averaged 38.4 ± 0.62 kg in the group, which is 7.47% or 2.67 kg higher than in G2 35.73 ± 0.39 kg. It was visually noted that calves, obtained from dams of G1, appeared to be more robust and viable, with a well expressed sucking reflex. Dosed inclusion of MFY in the diet of cows during the transition period, improves their reproductive functions, calves obtained from these cows are more healthy and viable.

P 188 | Prediction of parturition in Belgian Blue cattle based on rectal temperature or the lunar phase**

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Elective caesarean sections (C.S.) are widely used in Belgian Blue cattle to manage periparturient problems and avoid neonatal death. To ensure proper surveillance at calving, pre-partum temperature control is a commonly used practice to predict impending calving. Furthermore, amongst a lot of farmers and veterinarians there is a saying that cows are more prone to calve at full moon. In this study, 530 elective C.S. (starting labor) performed in the teaching hospital of Ghent University (Belgium) were investigated. Standardized forms were used to report the findings before, during and after every C.S. The age of the cows in this study varied between 2 and 8 years and their parity ranged from nulli- to pluriparous (5th C.S.). Rectal temperature, measured at 8 a.m. and 8 p.m. in the days before the C.S. was revised to detect a temperature drop. Dates on which the C.S. were performed, were linked to the actual lunar phase. On average, cows showed a temperature drop of 0.6 ± 0.27°C at 26.2 ± 11.05 h before the onset of calving. The bigger the temperature drop was, the more likely a cow started parturition in the next 24 h ($p < 0.001$; Survival analysis SAS 9.4®). With a temperature drop less than 0.4°C, between 0.4°C and 0.6°C, 0.6°C and 0.8°C or >0.8°C, respectively 30.43%, 51.87%, 50.00% and 67.61% of the animals calved within the next 24 h. It was shown that 23.33% of the cows calved at new moon, 25.44% at first quarter, 25.93% at full moon and 26.54% at last quarter ($p = 0.93$; Poisson linear regression SAS 9.4®). Based on the results of this study, we were not able to confirm that calving of Belgian Blue cows is associated with the phases of the moon.

P 189 | In-clinic maternal derived antibodies titration to determine the optimal time for the first core vaccination in puppies**

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Puppies are protected by maternal derived antibodies (MDA) for canine adenovirus (CAV), parvovirus (CPV2) and distemper virus (CDV). According to WSAVA guidelines, core vaccines can prevent these diseases (Day 2016, Small Anim Pract 57:E1-E45), but MDA can interfere with them: poor MDA titers allow puppies to respond to vaccination, while high MDA titers do not (Morein 2002, Vet Immunol Immunopathol 87:207–213). The present study aimed to assess by VacciCheck™ Canine the optimal age to start vaccinations in puppies born from vaccinated bitches. Antibodies for CAV, CPV2 and CDV were tested in 233 puppies (45 different breed litters, 35 to 70 days old), and 25 mothers, 2 weeks before delivery. According to the kit guidelines, dogs were protected with titers ≥1:16 for CAV, ≥1:80 for CPV2, ≥1:32 for CDV. Titers and ranks were grouped by age and sex (ANOVA, $p < 0.05$). All bitches resulted to be protected. Mean CAV, CPV2 and CDV titers of puppies younger (n = 156) and older (n = 77) than 56 days were statistically different (1:16, 1:120, 1:16 vs. 1:8, 1:60, 1:8, respectively). Percentage of positive puppies resulted significantly lower for all diseases in older ones: CAV 53.9% vs. 36.0%; CPV2 70.5% vs. 51.9%; CDV 18.5% vs. 0.0%. A gender effect was not observed. The mean CAV and CPV2 titres were fully protective until 56 days. Mean CDV titer significantly declined from 1:16 to non-protective 1:8, in older puppies. In kit guidelines titers ≥1:8 for CAV, ≥1:40 for CPV2 and ≥1:16 for CDV could be considered protective in adults, although weakly positive. Similar MDA titers in puppies may give weak protection yet interfering with vaccines. Our results suggest that in most puppies passive immunity waned by 56 days of age, allowing active immunization.

P 190 | Effects of acute external stress during parturition on the neonatal adaptation in the horse

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Prolonged labor is often associated with poor neonatal outcome. We hypothesised that an external stressor at foaling increases the duration of labor and affects neonatal adaptation in horses. To apply stress, mares of group stress (n = 6) were moved to a novel and totally empty box directly after rupture of the allantochorion while control mares (n = 5) stayed in their straw-bedded foaling box. Time from rupture of the allantochorion to complete birth of the foal was recorded. In newborn foals salivary cortisol, plasma epinephrine concentration, heart rate (HR) and heart rate variability (HRV) was evaluated. Statistical analysis was made by ANOVA using a general linear model for repeated measures with time as within and group as between-subject factor. In stressed mares, length of stage 2 of labor was longer than in control mares (10.0 ± 1.6 vs. 5.4 ± 1.0 min; $p < 0.05$). Neonatal HR increased during the first 15 min after birth in both groups but thereafter was higher in control foals ($p < 0.05$). HRV did not differ between groups. During the first hour of life, cortisol concentration was higher in control than in stressed foals (60 min after birth 38.9 ± 5.7 vs. 16.6 ± 2.6 ng/ml). Directly after birth, epinephrine concentration was low in control but high in stressed foals (19.6 ± 2.7 vs. 38.8 ± 28.7 pg/ml). In control foals, epinephrine concentration had increased 30 min after birth while in stressed foals epinephrine remained constantly elevated (time $p < 0.001$, time × group $p = 0.001$). In conclusion, an external stressor at foaling did not only prolong stage 2 of labor in mares but also affected neonatal adaptation with pronounced sympathetic activation in foals during and after prolonged labor.

P 191 | Effect of the pH pre-adjustment in the freezing and thawing extender on post-thaw boar sperm quality

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The modification of pH of the freezing extender can improve post-thaw sperm quality. The aim of this study was to determine the effect of modifying the pH of the freezing and thawing extender on the post-thaw semen quality. Semen pools from five boars were frozen in 0.5 ml plastic straws (1×10^9 sperm/ml) with lactose-egg yolk-glycerol extender with pH pre-adjusted to 4, 5, 6, 7, 8 and 9; and to the same pH in BTS thawing extender. Total and progressive motile sperm (%TMS and %PMS) and kinetic parameters were evaluated by CASA, live sperm (%LS) by fluorescence microscopy (SYBR14/propidium iodide) and sperm with normal acrosomal ridge (%NAR) were evaluated by phase contrast microscopy after 90 min post-thawing. Statistical analysis was performed by GLM (SAS 9.0) and the means were compared by Tukey test ($p < 0.05$). The values of %LS, %NAR, %TMS and %PMS increased significantly with the increasing of pH, up to the value of 8 where the sperm showed the highest values for these parameters (%LS: 57.7; %NAR: 53.3; %TMS: 48.7; %PMS: 46; $p < 0.05$). Respect to kinetic parameters the pH 7, 8 and 9 showed better velocity and linearity characteristics, than the rest of pHs tested. In conclusion, the pre-adjustment to pH 8 of the freezing and thawing extender would improve the post-thawing semen quality.

P 192 | Precision supplementation of protein enriched Opuntia cladodes and reproductive outcomes in anestrous goats exposed to the male effect: estrus induction and selected blood metabolites

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The possible effect of protein enriched *Opuntia megacantha Salm-Dyck* cladodes targeted supplementation upon changes in serum concentrations across time of total protein (TP), urea (UR), cholesterol (COL) and glucose (GLU) as related to estrus induction (EI%) in adult anestrous goats exposed to the male effect, was evaluated. Cladodes or prickly pear arise from the stem of opuntia replacing the leaves in the photosynthetic function, having a high content of fiber, water and energy, although a reduced protein content. In early May, anestrous Alpine-Saanen-Nubian × Criollo adult goats (n = 38, 26°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}; 0900–1000 h), yet, PEO was enriched in a fermentation bioreactor (1% of Saccharomyces cereveciae, +1% urea +0.1% of ammonium sulphate). Supplementation included a 10d adaptation period plus 20d of exposition to sexually active males. Neither LW ($p > 0.05$) nor BCS ($p > 0.05$) differed among groups, yet, an increased ($p < 0.05$) EI % occurred in PEO & NEO vs. CONT (100%, 57%, 42%, respectively). However, no differences among treatments occurred neither regarding their general averages

($p > 0.05$; GLU (98.46 ± 8.6 mg dl^{-1}), UR (47.77 ± 2.54 mg dl^{-1}) COL (159.56 ± 8.22 mg dl^{-1}) and TP (6.02 ± 0.41 g dl^{-1}) nor across time (treatment × time). Peri-breeding Opuntia cladodes supplementation increased ($p < 0.01$) EI% without augmentations in GLU, UR, COL and TP across time during the natural anestrous season.

P 193 | Variability of egg parameters in Wrocław Meat Pigeons in the winter period

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The Wrocław Pigeon is a Polish meat breed. The number of eggs laid from May to November averages 14. From November to April birds are not expected to breed due to unfavourable environmental conditions in our climate. Nevertheless, eggs are laid also during this period. The aim of this study was to estimate the variability between the successive eggs laid by a female in winter and the correlations between selected egg parameters. It was carried out on 13 same-age females in the winter period (Jan–Apr 2017). Birds were kept in pairs in cages in changeable environmental conditions (temperature, humidity) at stable 12/12 day/night light cycle. Collecting of eggs and feeding took place twice a day, mornings and evenings and water was supplied ad libitum. Eggs were marked, weighted (0.1 g accuracy), measured (length and width) and their colours were recorded. Then eggs were broken and shell thickness (without membranes) was measured. Spearman correlation between the selected parameters was calculated with IBM Statistics 23 PL software. The total number of 64 eggs was collected, with max 7 / female. Variabilities of the selected parameters between the subsequently laid eggs were estimated at 3.0–5.9% for length, width and colour and at 13.5–18.9 for shell area and thickness and total mass. We found that with each subsequent egg its total mass and shell thickness decreased ($r = -0.335$) together with its area ($r = -0.300$, $p = 0.05$). Egg length tend to increase with its mass ($r = 0.544$, $p = 0.01$), width ($r = 0.543$, $p = 0.01$) and shell thickness ($r = 0.270$, $p = 0.05$). These results are going to be compared with the ones obtained in the summer period and thus will add to our comprehensive knowledge about the traits of the Wrocław Meat Pigeon egg characteristics throughout the year.

P 194 | Identification of stallion epididymal fluid phosphoproteins**

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The aim of this study was to isolate and identify phosphoproteins from stallion epididymal fluid. Epididymes were gathered from 7 warm blood stallions, at the age of four years, after castration. Epididymal segments: caput, corpus and cauda were cut into pieces and centrifuged twice. Obtained fluids were purified by a filtration through a nitrocellulose membrane to remove the epithelial cells. Phosphoproteins were isolated with PHOS-select Iron Affinity Gel (Sigma-Aldrich, USA). Gained phosphoprotein fractions were initially concentrated with TCA/DOC method. Afterwards they were separated on 12% SDS-PAGE gels and stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich, USA). Identification of the phosphoproteins was done using nanoLC-MS/MS system combined from the Proxeon EASY-nLC capillary chromatograph (Thermo Fisher Scientific, Austria) and AmaZon ETD mass spectrometer (Bruker-Daltonics, Germany) equipped with nanoFlow ESI ion source. As a result three groups of proteins within the ranges 136–60, 59–20 and 19–1 kDa were distinguished. Phosphoproteins involved in regulatory (34 proteins), transport (11), motility (7) antioxidant (2), chaperones (2), functions and ubiquitination (7), signal transduction (4), apoptotic (2) processes were identified. Expression of mentioned proteins was various in different segments of epididymis. (Supported by National Science Centre, Poland, Preludium: 2016/21/N/NZ9/02319)

P 195 | Comparative study of changes in intracellular calcium levels and localization in capacitated ram spermatozoa in presence of melatonin**

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Intracellular calcium plays an important role in regulation of sperm capacitation and acrosome reaction. We demonstrated that melatonin modulates sperm functionality. Despite the existence of many fluorescent probes to assess calcium levels by flow cytometry, it is not clear what they exactly label. The aim of this work was to assess changes in intracellular calcium levels and localization during ram sperm capacitation in presence of melatonin using two calcium markers, Rhod-5N-AM (low Ca^{2+} affinity) and Fluo-4-AM (high Ca^{2+} affinity), by fluorescence microscopy and flow cytometry. Swim-up-selected spermatozoa were incubated in capacitating conditions with or without melatonin. Staining with Fluo-4/PI showed an entire head-labelling in all samples analysed by microscopy, but four populations were detected by flow cytometry, changing after in vitro capacitation but without differences between treatments. Rhod-5N labelling did not show distinct populations by cytometry, but evident differences between treatments were observed by microscopy. Data were compared by Chi-squared test. Most sperm in swim-up samples (~44.5%) showed a spot at the midpiece, while in capacitated ones this pattern decreased drastically (~1%) and staining was

mainly in the head, with significant differences between treatments ($p < 0.05$). Melatonin incubated samples also showed a significant increase in acrosome labelling. Our results suggest Rhod-5N as a useful dye to assess calcium movements in spermatozoa, while Fluo-4 is a better and quicker method to analyse calcium levels by cytometry. (Grants: AGL-2014-57863-R, DGA 2016-A26, BES-2015-072034.)

P 196 | Metabolic status of newborn calves with intrauterine growth retardation

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The aim of the study was to perform a comparative analysis of the metabolic status indices of newborn calves with physiologically occurring pregnancy and intrauterine growth retardation (IUGR). 53 red-motley calves were examined within 24 h after birth: 28 – with IUGR in history and 25 – with physiological pregnancy in mothers (control group, CG). The blood concentration of glucose, lactate, pyruvate, serum levels of lipids, cholesterol, total protein, electrolytes (Na, K, Ca, Mg), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, the content of trace elements (Se, Cu, Zn, Fe, Co, Mn) in switch hair were determined in the calves. The blood content of glucose in IUGR-calves was 38.5% lower ($p < 0.001$), and lactate 138.7% ($p < 0.001$) higher in comparison with CG, and indicated the prevalence of anaerobic glycolysis. The catabolic character of IUGR-calves metabolism was also indicated by increased activity of AST and the AST/ALT ratio in serum – by 51.3% ($p < 0.001$) and 91.3% ($p < 0.001$) higher than in CG. The serum content of lipids, cholesterol and total protein in IUGR-calves was reduced by 16.3% ($p < 0.01$), 15.4% ($p < 0.01$), and 18.4% ($p < 0.001$), accordingly in comparison with CG. IUGR-calves showed an increase in the serum level of Na, Mg and a decrease in the Ca/Mg ratio by 15.2% ($p < 0.001$), 7.4% ($p < 0.05$), and 11.2% ($p < 0.01$), accordingly, compared to CG. IUGR-calves having electrolyte imbalance was clinically manifested by muscular dystonia. IUGR-calves showed a decrease in the content of Se, Cu, Zn, Co, Mn in the hair by 26.4%, 28.3%, 10.7%, 36.8%, and 9.4% ($p < 0.001$), respectively, compared with CG. The detected metabolic disorders at IUGR-calves are the background for the neonatal diseases progression.

P 197 | Alterations in blood thyroid levels during the postpartum period are related to the reproductive ability of primiparous dairy cows resumed cycling activity

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To meet energy demands during early lactation, dairy cows undergo metabolic adaptations including improved metabolic efficiency via reduced levels of circulating thyroid hormones. The goal of this research was to relate serum profiles of thyroid hormones in the postpartum (PP) period (parity 1 dairy cows) to resumed cycling activity and to further reproductive performance. Blood samples from primiparous Russian Black Pied cows were collected 2 weeks before and 1–13 weeks after calving. For further study, 26 cycling animals were selected. PP cyclicity was confirmed by rectal palpation, ultrasonography, and serum progesterone levels. Thereafter, the cows were divided into three groups: (1) animals with short open days period (<100 days; SOP, n = 10), (2) animals with long open days period (>100 days; LOP, n = 8), and (3) animals remaining non-pregnant for 1 year after calving (NPR, n = 8). Hormonal levels in the serum were measured by ELISA. The serum content of thyroxine (T4) decreased 1.4–1.8 times ($p < 0.05$) in all cows after calving. Meanwhile, this decrease was observed earlier in the SOP group (1 week PP) than in the NPR group (3 weeks PP) or LOP group (13 weeks PP). In SOP and NPR cows, the level of triiodothyronine (T3) gradually declined 1.6–1.7 fold ($p < 0.05$), reaching its minimum at the 7th week PP. By contrast, in LOP cows, the T3 level rose 1.6 times ($p < 0.05$) between the 2nd week before calving and the 7th week after calving. The T4/T3 ratios fell by the 1st week PP in the SOP and LOP groups, but did not change in the NPR group over the PP period. Thus, SOP cows have a greater ability to implement the energy-saving adaptive mechanism associated with a reduced thyroid activity than LOP or NPR cows. (The study was supported by FASO Russia and RFBR (16-34-00875).)

P 198 | Nodal protein expression in mare's endometrium**

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Nodal is a member of TGFb superfamily, that is strongly regulated, and its smallest variations in signaling are implied in aggressive tumor development. Nodal shares similarities with the signaling pathway of TGFb, such as activation of Smad2 and Smad3. Considering it together with its role in tissue turnover and inflammation, this study aims to evaluate the protein expression of Nodal in mare's endometrium, considering: (i) the estrous cycle phase and (ii) inflammation and fibrosis. Endometria were obtained post-mortem in follicular phase (FP; n = 24) and luteal phase (LP; n = 22), and were classified in Kenney and Doig's categories (cat), as follows: cat I (FP: n = 6; LP: n = 6), cat IIA (FP: n = 4; LP = 6) and Cat IIB/III (FP: n = 14; LP: n = 10). Protein expression was assessed by Western blot, using stain free normalization technique and Image Lab 6.0 software. Data were statistically analyzed by one way

ANOVA. Nodal expression, in cat I endometria, was higher in the LP, compared to the FP ($p < 0.01$), which was also observed in cat IIB/III endometria ($p < 0.05$). However, in cat IIA endometria, there was no difference between both phases of the estrous cycle ($p > 0.05$), which might be related to the increased expression of Nodal in this type of endometrium in the FP and the decreased expression in the LP, compared to cat I endometria ($p < 0.05$). Observing these data, in cat I endometria, Nodal may have a distinct physiological role in the LP from that in the FP. Also, the coincidence of altered protein expression of Nodal, particularly in cat IIA endometria, may be related with the onset of inflammation and fibrosis processes. Nevertheless, further studies should be carried out to elucidate the complex role and mechanisms of Nodal in mare endometrium.

P 199 | Bull semen can be stored overnight in various extenders before colloid centrifugation

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Colloid centrifugation is a useful technique for selecting good quality stallion spermatozoa and could be used as a biomarker of fertility when applied to stored samples (Morrell et al. Theriogenology 2014). To develop this technique for bull semen, transport overnight from the bull station to our laboratory was needed. Aim: to identify an extender that could be used to store bull semen overnight before Single Layer Centrifugation that does not contain material of animal origin. Three ejaculates were collected from each of 3 bulls at VikingGenetics, Skara, Sweden; aliquots were extended at a sperm concentration of 50×10^6/ml in Andromeda (Minitüb International, Tiefenbach, Germany), INRA96 or OptiXcell (IMV Technologies, l'Aigle, France). The extended semen was cooled and transported overnight at 6°C in an insulated box. Single Layer centrifugation (SLC) was performed with 15 ml warm (30°C) extended semen over 15 ml Bovicoll, centrifuging at 300 g for 20 min. Pellets were resuspended in the appropriate extenders. Sperm motility was analysed by SpermVision (Minitüb International, Tiefenbach, Germany) and sperm concentration was measured using a Nucleocounter (ChemoMetic, Denmark) to calculate the yield of motile spermatozoa. Means were compared with ANOVA. Mean yields of motile spermatozoa were not different among treatments: Andromeda 52%, INRA96 38%, OptiXcell 49%. Mean kinematics were also not different, e.g. progressive motility 87%, 86% 79%; VCL 153 µm/s 143 µm/s 138 µm/s; BCF 27 Hz, 25 Hz 27 Hz, respectively. Therefore, it appears that any of these extenders would be suitable for transporting samples to the laboratory overnight for further analysis.

P 200 | Bull semen quality is related to differences in sperm protein abundance and their carbonylation level

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In Breeding and Insemination Centers, significant variation in bull semen quality is often observed. Low quality semen is rejected, generating economic losses. The mechanisms leading to the formation of low quality ejaculates are poorly understood. Therefore, the aim of this study was to investigate the proteomic differences and oxidative modifications of fresh bull semen of low and high quality. Flow cytometry and computer-assisted sperm analysis were used to assess differences in viability, reactive oxygen species (ROS) level, and sperm motility. To analyze changes in protein abundance, 2-dimensional (2D) differential in gel electrophoresis was performed. Western blotting in conjunction with 2D electrophoresis was used to quantify carbonylated proteins. Proteins were identified using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry. High quality semen was characterized by higher motility (by 30.1%), viability (by 20.2%), concentration of seminal plasma proteins (by 61.9%), and lower number of ROS – positive cells (by 32.2%). We identified ten proteins showing differences in abundance and 12 proteins showing differences in carbonylation level. The identified proteins were associated with energetic metabolism, capacitation, fertilization and motility. High quality semen was characterized by a high abundance of sperm surface proteins and low abundance of intracellular proteins. In turn, low quality semen was characterized by a high content of carbonylated proteins that were localized mainly in mitochondria or their surroundings. Our results contribute to research concerning the mechanism by which low and high quality ejaculates are formed and identify sperm proteins that are particularly sensitive to oxidative damage.

P 201 | New echotexture parameters to evaluate the testicular parenchyma in boars

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The aim of this work is to investigate the relationship between semen quality in boars and the following testicular echotexture parameters: EC1 (black pixels), EC2 (white pixels), EC3 (mean gray level of pixels), Density (density of hypoechogenic areas), Diameter (mean diameter of hypoechogenic areas) and Area (total percentage of hypoechogenic area). Three transversal ultrasound scans were performed per testicle, in a total of 112 boars. Ultrasonograms

were done using an EXAGO scanner (ECM, France) connected to a 7.5 MHz linear probe. A semen sample was collected per boar and analyzed by microscopy at 1000X to investigate sperm morpho-anomalies. The percentage and type of sperm abnormalities of each sample were determined (n = 200 spermatozoa). In our analysis, we established a cut-off value of 30% major sperm abnormalities (head, abnormalities in the formation of intermediate pieces and proximal cytoplasmic droplets) to differentiate fertile and subfertile samples. There were no differences between ipsilateral testicles in echotextural parameters ($p > 0.05$). The Kruskal–Wallis test indicates that EC2, EC3, Area, Diameter, and Density were significantly related to the percentage of major sperm abnormalities ($p < 0.001$) and with the percentage of total abnormalities ($p < 0.001$). Logistic regression indicates that the density of hypoechogenic areas in the ultrasonogram of a testicle could predict the fertility of subfertility of a semen sample. With a cut-off value of 80 hypoechogenic areas/cm^2 to detect a subfertile boar, sensitivity was 100% and specificity was 83.5%. (This work was supported by Eureka E!11188 and IDI-20170220)

P 202 | Ovarian follicular dynamics and changes in concentrations of estradiol-17β and progesterone during estrous cycle of Beetal goats

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This study characterizes ovarian follicular dynamics and changes in plasma progesterone and estradiol-17β (E2) concentration in eight Beetal goats. Ovulations were synchronized using two injections of $PGF_{2\alpha}$ 11 days apart. Ovaries were scanned daily using a 7.5 MHz transrectal transducer for two consecutive estrous cycles (n = 14). Data were analyzed by *t*-test, ANOVA, Pearson correlation coefficient and general linear model (SPSS, version 20.0). The mean interovulatory interval in Beetal goats was 21.2 ± 0.3 days. The follicular and luteal phases were 4.9 ± 0.1 and 16.2 ± 0.3 days, respectively. The percentage of a 4-wave follicular pattern compared to 3-waves was 71% vs. 29%. In a 3-wave pattern, follicular waves emerged on days 0 (ovulation), 8 and 14.5 of estrous cycle, while in 4-wave patterns, follicular waves emerged on 0.5, 7.5, 12, and 16 days. The maximum diameter of preovulatory follicles and corpus luteum (CL) was 7.2 ± 0.2 mm and 11.8 ± 0.3 mm, respectively. On an average 1.7 ± 0.16 follicles ovulated per cycle. The luteolysis began on 16.2 ± 0.2 days of the cycle. The peak plasma E2 concentration (11.1 ± 2.9 pg/ml) reached 33.6 ± 9.6 h before ovulation. The P4 reached peak plasma concentration (15.3 ± 0.2 ng/ml) by day 12.2 ± 1 of the cycle and it declined below 2 ng/ml within 2.6 ± 0.3 days after initiation of luteolysis. Plasma P4 concentration and CL correlated throughout the cycle ($r = 0.94$; $p \leq 0.01$). In conclusion, Beetal goats had a predominant 4-wave follicular pattern and had high rate of twin ovulations. (A Murtaza was recipient of "Indigenous PhD Fellowship" and partially funded through SGRP-554 by HEC, Pakistan.)

P 203 | Analysis of fixing methods of reindeer (*Rangifer tarandus*) for semen collection

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The general principles of capturing and immobilizing reindeer for sperm collection should be a minimum negative impact on the animal and ensuring human safety. The aim of the study was to determine the effectiveness of fixing methods of males for semen collection. Erection, efficiency and time of sperm collection (fixation and collecting ejaculate) were taken into account. Reindeers were fixed by anaesthetics and analgesic and without drugs, respectively. We used drugs that are allowed in Russia. A total of 30 samples from 10 adult males were collected by electroejaculation (Minitube®) in Taimyr and at private Zoo in St. Petersburg in autumn 2017. Results are depicted as percentages of totals. The combination of ketamine with detomidine (ketamine 4 mg/kg and medetomidine 1.2 mg/kg), Zoletil-100 (3 mg/kg) and muscle relaxants can be considered the most preferred drugs for reindeer fixing. When not using drugs, males were fixed by rope on horns and legs in a standing position or in lying position. With drugs, ejaculates were collected in 50% of cases, and erection was observed only in 25% of cases, the time of semen collection was 20–35 min. Without drugs, semen was collected in 72% of cases, and the erection was in 76% of cases, and the collection time was only 4–5 min. The effectiveness of semen collecting in males in a standing position was higher (75%) than in a lying position (60%). Thus, the most effective way was the semen collection of the males in standing position without drugs. However, this method is not suitable for aggressive or wild animals. For such animals, it is necessary to use drugs for immobilization when collecting sperm. (Authors acknowledge financial support from Russian Science Foundation, Grant No:17-16-01023.)

P 204 | Follicular size and estrus duration in Tunisian Arab mares: what relationship?

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The study aimed to follow up the follicular growth during the estrus period of mares raised in Northern Tunisia. Forty-nine Arab mares (age ≤15 years: n = 37; age >15 years: n = 12) were used to study the relationship of follicular growth and the estrus duration. The ultrasound monitoring of the follicular activity started on the third day of estrus, and it was performed daily with an ultrasound instrument (Aloka 500®). Ultrasound examination involved locating and measuring of the diameter of the preovulatory follicle. The end of the estrus was determined when the ultrasound examination revealed a corpus luteum. ANOVA was carried to compare variables between the 2 age classes using a software SAS (SAS, Institute, Inc.). Results showed that the estrus duration was higher in the younger mares compared to the older ones (10.8 ± 1.2 vs. 4.2 ± 1.3 days; $p < 0.01$). However, the follicle diameter did not vary in both age classes (38.0 ± 1.5 vs. 37.0 ± 2.7 mm; $p > 0.05$). The latter was weakly correlated with the estrus duration ($r = 0.16$; $p > 0.05$). Besides, 10% of the mares had follicular diameter less than 30 mm (estrus duration = 7.5 ± 3 days), 41% had follicular diameter between 30–40 mm (estrus duration = 8.0 ± 1.2 days) and 49% had follicular diameter greater than 40 mm (estrus duration = 8.5 ± 2 days) ($p > 0.05$). Our results showed that the age of mares affected the estrus duration and there was no relationship between the follicular size and the estrus duration.

P 205 | Automatic detection of cows at risk for ketosis based on moving activity

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Ketosis (Acetonemia) is a metabolic disorder that occurs in cattle when energy demands exceed energy intake and result in a negative energy balance. The course of the disease often starts with a subclinical phase, so early detection is of crucial importance. The aim of this study was to determine whether daily moving activity could be used as a predictor of subclinical ketosis in early lactation. The study was carried out in a 75-cow dairy farm over 6 months. β-hydroxybutyrate concentrations were evaluated in milk samples using a rapid on-site ketosis test. The animals were divided in two groups: group 'Healthy' (H) and group 'Ketosis' (K). Daily milk yield, concentrate intake and movement activity were recorded from a computerized dairy management system with the associated software (DairyPlan C21). Animals of group K showed generally a higher level of milk yield, as well as higher concentrate intake during the whole experiment time. Furthermore, cows of group K had an average daily activity of 30.8% lower than animals of group H. In this study, a statistically reduced movement activity in animals of Group K compared with the mean of herd's diurnal activity was observed on days 6–12 post partum ($p < 0.001$, χ^2 test). The retrospectively determined sensitivity for the detection of ketosis-diseased or -endangered cows by their activity behaviour was 85.0% and the specificity 69.2%. This method may help in future to establish an early warning system for the risk of ketosis in dairy cows. Thus, cows at risk may be identified for further targeted diagnostics. (Supported by Schaumann Foundation.)

P 206 | Preservation of black crested mangabey (*Lophocebus aterrimus*) spermatozoa**

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The black-crested mangabey (*Lophocebus aterrimus*) is an African monkey listed as Near Threatened by the IUCN and its population is decreasing in south of the Congo River area due to habitat loss and hunting pressure. In captivity the population in Europe is limited, and a European Endangered Species Programme (EEP) tries to maintain the ex-situ population, that could help increase breeding rates, maintaining genetic variability and facilitate the study of this species. Spanish zoo Río Safari Elche houses a group formed by one male and two females within breeding age. The male (Pollux, 26 years old) suspected of infertility, was sedated (ketamine and medetomidine), and a seminal sample was obtained by electroejaculation. Fresh semen (F) was initially evaluated and part of the sample was diluted in Refrigeration Medium Test Yolk buffer (Irvine Scientific, USA) and cooled to 15°C for 6 h (C sample). The rest of the sample was cryopreserved by dilution in freezing media (Irvine Scientific) (FT sample). ANOVA was applied to compare sperm parameters in F, C and FT samples. Sperm progressive motility (type a) was 80% in F, 71.8% in C and 51.4% in FT. Motion parameters measured by CASA (VCL, ALH and BCF) were significantly higher in cooled compared to frozen sample ($p < 0.05$). Viability and acrosome status was assessed by staining with propidium iodide and lectins PNA-FITC and measured by flow cytometry. Live spermatozoa with intact acrosome decreased from F: 83.8 ± 0.2% to C: 75.1 ± 1.0% and FT: 69 ± 1.3% ($p < 0.01$). To our knowledge this is the first report on preservation of spermatozoa from black crested mangabey. (Supported by Fundación Séneca 20040/GERM/16)

P 207 | Low flow anesthesia for caesarean section in dogs

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The aim of this study was to assess the safety of the application of the small gas flow method for cesarean section in bitches. As an anesthetic was used isoflurane, the accuracy of the dose was provided by the "MINIVAP-20" evaporator. The dynamics of changes in acid-base indexes at different stages of anesthesia in 30 females with caesarean section up to 1 h was studied. Before the introductory anesthesia, 0.1% atropine solution was injected (0.025 ml/kg). Introduction to anesthesia was carried out using 1% propofol (2 mg/kg) intravenously. After that, intubation of the trachea was performed and the patient was transferred to the main anesthetic with isoflurane (concentration 1.5–2.0%) in an oxygen-air mixture with a gas flow of 1–1.5 l/min. Arterial blood samples were taken at 5, 20, 40 and 60 min, which corresponded to different stages of general anesthesia. The analysis of the samples was carried out using the Micro Astrup's method. The dynamics of the obtained results at the stage of introduction into anesthesia in comparison with the initial data shows the decrease of the pCO^2 to 35.1 ± 3.1 mmHg and the increase of the metabolic component of the BE to +1.0 ± 0.3 mM. Such changes have determined unreliable pH shifts to the alkaline side. This is due to manual hyperventilation of the lungs during the period of introduction of anesthesia. During the period of maintenance of general anesthesia, there were significant shifts of BE towards acidosis (-0.7 ± 0.2 mM before anesthesia and -1.7 ± 0.3 mM maintenance of anesthesia, $p < 0.05$, Student's *t*-test), which did not lead to significant changes in blood pH. The parameters of the acid-base state during the recovery period did not differ significantly from the initial data.

P 208 | Contractile uterus activity of dairy cows in the early postpartum period and its reaction to myotropic medication

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Thirty six cows were studied. The recording of uterine motility was made by internal hysterography. The contractions intensity was assessed by the frequency, amplitude, duration of contraction wave and the value of contraction index (CI), calculated acc. to Döcke. In 24 cows uterine contractions were recorded in 0.5, 3, 6, 12, 24 h after repulsion of the placenta. 12 of them were healthy animals (6 with physiological puerperal period, 6 with subinvolution of the uterus). 12 cows were injected with 50 IU of oxytocin, propranolol 50 mg, cloprostenol 500 μg. In 12 animals with postpartum metritis the recording of uterine contractions was made on the 6^{th}–8^{th} day after birth. The evaluation of its reaction to myotropic medications was made according to the same procedure. The comparative evaluation of the uterine contractions intensity is presented as CI. In cows with a physiological course of the postpartum period, a high level of uterine activity was recorded at 3 h. The CI was 1024-721u. In 6 h the uterus activity decreased 1.75 times, in 12 h 3.3 times and at 24 h 10.4 times. In cows with delayed uterine involution, the contraction intensity within the first 6 h was 1.8–1.2 times lower. In 12–24 h it surpassed healthy animals by 2.7–3.3 times. Injection of oxytocin caused an increase in the uterine contraction by 2.2 times, lasting up to 1.5–2 h, propranolol 2.6–3.1 times. When $PGF_{2\alpha}$ was infused, the amplification of its contractions was recorded after 6 h, reached a maximum by 12 h and was kept at a high level up to 24 h. The CI within these terms exceeded the intact animals 1.3, 3.7 and 2.2 times accordingly. These results should be taken into account when using uterine treatment in the post partum period in practice.

P 209 | Isolation and antibiotic resistance of *S. aureus* strains isolated from cow milk in different regions of Russia

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Staphylococcus aureus is highly pathogenic and is causing clinical and subclinical mastitis. The aim of the research was to study and to analyse the antibiotic resistance of *S. aureus* strains isolated from cow milk in different regions of Russia. Milk samples were taken from the cows in the Central (n = 98), Privolzhsky (n = 88), Northwestern (n = 69), Siberian (n = 84) and Far Eastern (n = 72) Federal Districts of Russia. From 411 cows examined, 167 (40.63%) and from 1644 quarters examined, 256 (15.57%) showed evidence of infection of subclinical mastitis. The isolation of *Staphylococcus* spp. was carried out on the basis of growth on Baird Parker Agar and Azide Blood Agar medium, plasma coagulation and biochemical identification on API 20 STAPH. A total of 85 (20.68%) strains were identified as *S. aureus*. The disc-diffusion method was used to determine the sensitivity of *S. aureus* strains to 10 antibiotics: penicillin, oxacillin, gentamicin, erythromycin, lincomycin, rifampicin, ciprofloxacin, vancomycin, fucidin, novobiocin. The maximum carriage of *S. aureus* was found in Siberian animals (n = 25, 29.76%), minimal – Central (n = 10, 10.20%) FD. The antibiotic resistance has shown that in all regions *S. aureus* strains have manifested maximum sensitivity to vancomycin (100%). Strains resistant to β-lactam antibiotics (38.46 and 38.10%) were isolated in the North-West and Volga FD. The resistance to macrolides and lincosamides was observed in strains from the Siberian (92.00, 96.00%) and Privolzhsky (90.48, 100%), to oxacillin from the Far Eastern (6.25%),

Siberian (8.00%) and Privolzhsky (9.52%) FD. (The study was supported by the Russian Science Foundation, project No.15–16-00020.)

P 210 | Utilizing homologous ram seminal plasma in semen extenders for increasing the spermatozoa viability and motility assets following cryopreservation**

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Homologous seminal plasma (SP) is reported to have preventive and cryodamage reverse effects on cryopreserving ram spermatozoa (CS) (Barrios et al. 2000, Biol Reprod 63:1531–7). The objectives of this research were to detect alterations in CS viability and motility assets following supplementation of SP, and to determine the time of their occurrence. Homogenous quality ejaculates of Ovchepolean Pramenka rams (N = 10) were pooled and allocated in four groups: K1, E1, K2, and E2, each with equal sample size (n = 25). K1, K2 and E2 were extended with control (CE-soybean based), whereas E1 was extended with experimental extender (EE-control+20% SP) prior deep-freezing. Post-thawing, samples were placed at 37°C; K2 and E2 were diluted in 1:1 ratio with CE and EE, respectively, and analyzed by CASA for a sequence of parameters (viable cells-Viab, total motility-tMot, progressive motility-pMot, fast velocity-FVel) in two-time periods of incubation: 0-h and 3-h. Wilks and least significant difference post-hock tests revealed no significant difference between K1 and K2 during incubation ($p > 0.05$). K2 values for Viab and tMot were no different to E2 for 0-h ($p > 0.05$), whereas following 3-h incubation, E2 indicated significantly higher values in all parameters ($p < 0.001$). E1 indicated significantly higher values for Viab and tMot than E2 on 0-h ($p < 0.01$), but following 3-h incubation, no significant differences were found ($p > 0.05$). Results are indicative that SP affects CS viability and motility when it's utilized as additive in extenders, and its effects are occurring with significantly higher extent following incubation for 3 h at 37°C.

P 211 | The effect of different concentrations of sucrose on goat sperm vitrification

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The aim of the present study was to compare the effect of different sucrose concentrations on the goat sperm vitrification. Semen samples from six healthy goats were collected by artificial vagina. After semen collection sperm selection was carried out by swim up and the suspension of selected spermatozoa was divided into four groups. Sperm sample of Group 1 was frozen by conventional methods using Tris-citrate modified solution extender containing 20% egg yolk and glycerol 5%. Next, sperm suspension samples were vitrified after addition of different concentrations of sucrose (0.1 M (Group II), 0.25 M (Group III) and 0.5 M (Group IV)) in proportion 1: 1 v/v with HTF-BSA 1%. Before vitrification and after warming, standard semen analysis, plasma membrane integrity, sperm mitochondrial membrane potential, apoptosis, sperm chromatin integrity and Motile Sperm Organelle Morphology Examination (MSOME) were determined. Results showed a significant decrease of progressive motility (PR) of sperm after addition 0.5 M sucrose solutions, it is 54.6% in control vs. 28.6%, $p \leq 0.01$). Fast addition of sucrose solutions causes a greater decrease in sperm PR than slow addition. After thawing spermatozoa of group II showed the highest plasma membrane integrity (20 ± 0.7%) and total acrosome integrity (70 ± 1.0%) in comparison with 0.1 M ($p \leq 0.05$) and 0.5 M ($p \leq 0.01$). There was no difference in spermatozoa DNA fragmentation rate among the experimental groups. Sucrose increased early and late apoptotic effects. There was no difference between the experimental groups in MSOME results. In conclusion the 0.25 M sucrose solution seems to be the most appropriate concentration for the vitrification of goat sperm.

P 212 | Effects of highly dispersed silica nanoparticles on morphology of lipid droplets in growing or fully grown porcine oocytes

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Lipid droplets (LDs) in the ooplasm are essential for energy production required for maturation. In oocytes LDs form small or large clusters. The presence of LDs in the form of small clusters testifies to the good quality of the oocyte (Gao et al. 2015, Front Cell Dev Biol 3:49). Brilliant cresyl blue (BCB) staining has been used for selection of oocytes. The aim of the study was to determine the effect of highly dispersed silica nanoparticles (HDSn) on morphology of LDs in BCB-(growing) and BCB+ (fully grown) oocytes. Before IVM COCs were incubated in 13 μM BCB (B-5388) solution for 60 min. Then oocytes were divided into BCB- (colorless cytoplasm) and BCB+ (colored cytoplasm). COCs were matured in Sage Media Cleavage (CooperSurgical, USA) with 5% Serum Protein Substitute (CooperSurgical, USA), 10 IU/ml hCG (Sigma-Aldrich, USA) at 38.5°C in a humidified atmosphere of 5% CO_2 for 44 h (control). Medium for maturation in experimental group was added by 0.001% HDSn (Chuiko Institute of Surface Chemistry, Ukraine). After IVM the oocytes were stained by 1 μM Nile red for 5 min. The morphology of LDs was assessed under a fluorescence microscope (Carl Zeiss Ex/Em = 552/636 nm). Morphology of LDs in 139 of BCB+ and 104 of BCB– oocytes (in total 243 oocytes, in 3 replicates, 17–20 oocytes/group) was evaluated after 44 h. We did not find significant differences between the level of

BCB- oocytes with small clusters of LDs independently of culture medium [64% (34/53) vs. 65% (33/51)]. The addition of 0.001% HDSn to culture medium increased the level of BCB+ oocytes with small clusters of LDs [76% (60/79) vs. 90% (54/60), $p < 0.01$, χ^2 test]. The mechanism of the influence of HDSn on other cellular organelles remains to be explored. (Funded by FASO Russia, project #181180215901329.)

P 213 | Improvement of reproductive potential of chicken hens from parent broiler flock by means of the use of supplements based on triterpene spirits

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Poultry breeding is facing the problem of decrease in reproductive potential of chicken hens from parent broiler stock after the peak of productivity. To discover the genetic potential in the age of 310 days are given the organic supplement based on triterpene spirits (1 ml per hen/day) for 30 days. To the hens from the control group no supplements were given. The intensity of egg-laying in both control and experimental groups was 70%. The mass of a hatching egg in the control group was 66.8 g and in the experimental group 70.3 g (normally 67–73 g). Hatching of broiler chicks was 89% in the experimental group, which was 10% higher than in the control group. The mass of a day-long broiler chick was 62.1 g in the experimental group, which was 13.0% higher than in the control group. The livability of broiler chicks from the laying hens at the age of 7 days was 96.2%, which was 3.6% more than in the control group. Improvement of incubation data in the experimental group is caused by the use of the organic supplement based on triterpene spirits: the content of vitamin A in yolk increased by 36%, of vitamin B2 by 12%, calcium in egg shell that provides the hardness of egg shell of hatching eggs, by 9.4%. The incubation wastes regarding blood ring in the experimental group were not identified, and day-long chicks had more intensive pigmentation of floccus. The manure of the hens from the experimental group was more solid which resulted in reduction of wetness of bedding and impurity of hatching eggs. Improvement of reproductive potential of the laying hens from parent broiler flock is caused by lipolytic, antibacterial, hepatoprotective, and anti-inflammatory characteristics of supplements based on triterpene spirits.

P 214 | LH and FSH response after repeated low doses of GnRH analogue (buserelin) in treatment of anovulatory anoestrus in dairy cows

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The aim of this study was to evaluate the influence of repeated low doses of buserelin on LH and FSH secretion in anoestrous cows with ovarian follicles smaller than 5 mm and without a corpus luteum. Five cows from experimental group (EG) received 0.0042 mg (1 ml) of buserelin (Receptal, MSD, Poland) i.m. and 5 control cows (CG) 1 ml of saline (i.m.) 5 times 24 h apart. After every injection blood samples were collected every 20 min for 6 h, centrifuged serum separated and stored in a freezer at -70°C. FSH and LH concentrations were measured by radioimmunoassay. Ultrasound examination of the ovaries was performed every day. After treatment cows were controlled by ultrasonography once a week for 5 weeks. The average FSH concentration in EG cows was 1.66 times higher than in CG cows (0.37 ng/ml (0.18–0.52) vs. 0.23 ng/ml (0.15–0.35)). Also the average LH concentration was 1.7 time higher in EG (0.11 ng/ml (0.06–0.17 ng/ml) than in CG (0.06 ng/ml) cows. The average number of all ovarian follicles in EG was 6.8 (6–11) while in CG 5.0 (1–8) and the average number of follicles ≥ 5 mm was 2.0 and 1.3 in EG and CG, respectively. The average diameter of all follicles was 5.36 mm in EG and 3.94 mm CG. During the next 5 weeks 4/5 cows from EG developed a CL whereas only 1/5 from CG. LH concentration was not significantly different between EG and CG. FSH concentration was higher in EG from day 2 to 5 of treatment ($p < 0.05$; *U* Mann–Whitney test). There was no difference in the average number of follicles ≥ 5 mm ($p > 0.05$). We conclude that repeated low doses of GnRH had not a positive influence on the follicular development in cows with true anoestrus during the period of treatment.

P 215 | Effects of breed and feeding intensity on progesterone profiles in postpartum cows

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The aim of this study was to investigate the effect of two feeding intensities on cycle characteristics in primiparous Holstein (n = 22) and SRB (n = 22) dairy cows. The control group (High energy: HE, n = 23) was fed a diet targeting 35 kg/d energy-corrected milk, ECM.

A lower feeding intensity (Low energy: LE, n = 21) was achieved by giving 50% less concentrate to target 25 kg/d ECM. Diets were implemented 30 days before expected calving and up to 120 days after calving. Milk samples were collected three times per week, between Days 7 and 120 postpartum, and analysed for progesterone (P4). The first two postpartum P4 values above the limit for luteal activity (≥ 3 ng/ml) preceded by a low P4 value and not earlier than Day 10 were used to define commencement of luteal activity (CLA). The length of luteal phase (LP) was measured as the length between two consecutive milk samples ≥ 3 ng/ml. An atypical P4 profile (APP) was defined as no rise of P4 for 45 days or with a rise of P4 that was not followed by regular cyclicity for 45 days or more after calving. The magnitude and severity of energy deficit was greater in Holstein cows than in SRB (-27.3 ± 2.9 vs. -19.2 ± 2.9 MJ; $p < 0.05$). Holstein cows in the HE group tended to have the highest probability for developing an APP, compared with the other three groups (58.3% vs. Holstein LE; 33.3%, SRB HE; 27.3%; SRB LE; 27.3%, $p = 0.08$). APP was correlated with longer CLA (29.0 ± 3.2 vs. 20.4 ± 2.4 days, $p \leq 0.05$) and LP intervals (21.2 ± 2.2 vs. 7.0 ± 1.7 days, $p \leq 0.001$). Body condition losses were unfavourable correlated with first ovulatory oestrus ($r = 0.36$, $p < 0.05$). From the results, feeding intensity had a limited impact on progesterone profiles following calving. (Financed by EU, project "PROLIFIC" (grant number: 311776))

P 216 | Rainbow trout sperm proteins phosphorylation after sperm motility activation at hypoosmotic and isosmotic conditions

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Sperm motility in fish can be initiated by either hypotonic (for freshwater fish) or hypertonic (for marine fish) osmolalities. The initiation and maintenance of motility of spermatozoa involves phosphorylation of sperm proteins, and motility is regulated by a cascade of phosphorylation-dephosphorylation events affecting the activities of protein kinase substrates. The aim of the study was to compare phosphoprotein profile of spermatozoa immobilized and activated with Billard solution (isosmotic) or hatchery water (hypoosmotic) rainbow trout sperm by 2D gel electrophoresis coupled with mass spectrometry. We observed different patterns of phosphorylation of sperm proteins in response to activation with different media. A total of 22 phosphoproteins were changed in response to activation of sperm, 13 phosphoproteins were found to be upregulated after activation of sperm with H2O, activation with Billard resulted in 8 enriched phosphoproteins. The identified proteins belong to structural proteins and those involved in metabolism and ATP binding. Phosphoproteins changed after Billard activation mostly participate in TCA, cilium movement and sperm development, activation with water resulted in changes in phosphoproteins involved in ATP binding, protein folding and also cilium movement. Our results clearly indicate different patterns of protein phosphorylation in relation to osmotic conditions of sperm motility activation. The functional significance of our result is still unknown, but could reflect differential regulation of the phosphorylation signaling network in trout sperm in response to oxidative and osmotic stress. An understanding of gamete characteristics is necessary for experimental manipulations such as artificial fertilization and sperm cryopreservation.

P 217 | Comparative of two sperm DNA damage assays after ultraviolet light pulses

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Antibiotics are added to semen extenders to control bacterial contamination. Finding alternatives would be beneficial to avoid resistance development as long as maintain sperm quality. Sperm DNA integrity is an essential pre-requisite for birth of healthy offspring and a prognostic marker of sperm reproductive potential. The objective of this study was to compare the effect of ultraviolet light (UV) pulses on stallion sperm DNA status by two techniques, Diff-Quick (DQ) and Toluidine Blue staining (TB). Ejaculates were collected from 12 stallions of different ages during breeding season, using a Missouri-model artificial vagina. Sperm samples were centrifuged (1000 *g*/5 min), resuspended in chilled medium (Kenney) and kept at 4°C during 96 h. Before DNA integrity evaluation using DQ and TB, sperm samples were exposed at UV pulses during 5 min. The results showed low percentages of DNA damage (<9%) along experimental study. UV pulses didn't increase sperm DNA damage, however storage time decreased significantly ($p < 0.05$) DNA integrity using DQ as well as TB. We found a positive correlation between both DNA status assays (r^2:=0.69). In conclusion, UV didn't affect DNA integrity and DQ and TB are effective methods for detecting DNA status on stallion sperm. (Supported by DGA and Fondo Social Europeo (IA2).)

P 218 | Does the corpus luteum presence at the beginning of the progesterone plus estradiol protocol affect the dynamic of synchronized follicular wave in ewes?

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This study was designed to evaluate if the presence or absence of corpus luteum at the beginning of the progesterone plus estradiol

protocol affect the synchronized follicular wave dynamic in ewes. In a random day of the estrous cycle (D0), twenty-four Santa Ines ewes received an intravaginal progesterone device (CIDR®) and an injection of 17β-estradiol (350 μg, 500 μg or 1000 μg of E2, Sincrodiol®, n = 8/dose). Ultrasound examinations were performed daily during the CIDR permanence (10 days) using MyLab30Vet equipment (Esaote, Italy) connected to a 7.5 MHz transrectal linear transducer. Follicular wave was defined as a follicle or a group of follicles 2 to 3 mm in diameter that grew to greater or equal 4.5 mm in size before regression or ovulation. Data were analyzed by ANOVA with Turkey's post hoc test (mean ± SEM; $p < 0.05$) using SAS software. Half of the animals had a corpus luteum at the beginning of the protocol while the other half had no corpus luteum. All animals had large follicles (4.00–5.75 mm of diameter) at this moment and a new follicular wave emerged during the protocol. There was no difference ($p > 0.05$) between animals with presence or absence of corpus luteum at the beginning of the protocol for follicular wave emergence day (4.00 ± 0.44 vs. 4.14 ± 0.63) (D0 = CIDR insert), maximum diameter of largest follicle (5.59 ± 0.21 vs. 5.28 ± 0.17 mm), day with maximum diameter (9.33 ± 0.37 vs. 9.00 ± 0.44) and growing period (128.00 ± 8.00 vs. 116.57 ± 9.70 h). We conclude that the presence or absence of the corpus luteum at the beginning of the follicular wave synchronization protocol does not interfere with the follicular wave dynamics in ewes. (Financial support: CNPq and FAPESP.)

P 219 | An application of histometry in semiautomatic uterine biopsy quantification in different endometrial features in mares

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Uterine endometrium has a crucial role in implantation regulation. Its functioning affects embryo survivability in early pregnancy, therefore changes in endothelium may result in disturbances in implantation. The aim of this study was the assessment of the semi-automatic quantitative histometry to evaluate biopsy samples. Biopsies were taken from mares with significantly different endometrial features: in physiological diestrus (PE) and on the day of embryonic death (ED) in order to estimate the differences. Samples were fixed according to HE staining protocol and examined histometrically using semiautomatic quantitative analysis of slides on bright field system for the scanning and analysing (TissueFaxs Plus), with advanced HistoQuest PLUS image analysis software. The measurement (mean% ± SD) area of cytoplasm (MAC) and nuclei (MAN) in epithelium and size of endometrial glands (perimeter (PEG) and area (AEG)) in selected regions was performed. In ED MAC (114.6 ± 36.71 μm^2) and MAN (134.4 ± 46.65 μm^2) differed significantly from PE MAC (71.9 ± 29.11 μm^2; $p = 0.02$) and MAN (354.2 ± 143.31; $p = 0.002$), what may indicate lowered cell activity and swelling in occurrence of ED. In ED, PEG and AEG were 227 ± 42.3 μm and 4228 ± 1590.1 μm^2, respectively; both higher than in PE: PEG (157 ± 15.4 μm; $p = 0.0004$) and AEG (1961 ± 374.5 μm^2; $p = 0.001$). Enlargement of glands indicates increase of secretion during gestation, which has not stopped yet after ED. In ED also folds of epithelium were found, with average length 108.4 ± 35.4 μm. Our findings demonstrate the high usefulness of semiautomatic histometry which may be a fast and specific tool for mare biopsy examination.

P 220 | Generation and in vitro differentiation of porcine bone marrow-derived mesenchymal stem cells (MSC)

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Cervical dysfunction in pigs may lead to contamination of the uterus, impaired fertilization or impeded pregnancy and parturition. We hypothesized that MSC implantation into a dysfunctional cervix may improve its function, as measured by electromyography. We have established a protocol for isolation and rapid expansion of clinically-relevant numbers of bone marrow-derived MSC. Bone marrow was aspirated from the head of the humerus. Following mechanical dispersion, extensive washing and density gradient centrifugation the crude fraction comprising stem cells and hematopoietic cells was collected. These cells were cultured in expansion medium that facilitated the removal of leukocytes and, at the same time, prevented premature differentiation of the MSC. Purity and multipotency of the obtained population was assessed by both flow cytometry and trilineage in vitro differentiation. A set of positive (CD73/90/105) and negative (CD19/34/45) surface markers were analyzed, indicating high purity of cultured MSC. The cells were successfully differentiated into adipogenic, osteogenic and chondrogenic lineages, as demonstrated by histochemical staining with Oil Red O, Alizarin S and Alcian Blue, respectively. During the expansion phase the undifferentiated cells were cultured under hypoxic conditions, which improved overall yield without compromising their multipotency. We were also able to successfully cryopreserve and revive the cells. Eventually, the cells were fluorescently labeled by stable expression of red fluorescent protein and autogenically implanted into porcine cervix. The tissue was harvested several weeks later, the cells derived from MSCs were identified and found viable.

P 221 | Cryopreservation of ram sperm (trehalose vs. glycerol) in soy lecithin and powdered egg yolk based extenders

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To determine the efficacy of trehalose (T) as an alternative to glycerol (G) for ram semen cryopreservation in two different extenders based with soybean lecithin (SL) or powdered egg yolk (PEY), ejaculates from eight males were collected by artificial vagina twice a week (two ejaculates/male/day). Briefly, semen samples with good quality were pooled, centrifuged twice at 600 g for 10 min and diluted in four different Tris-based media: (1) 15% PEY + 5% G, (2) 1% SL + 5% G, (3) 15% PEY + 100 mM T and (4) 1% SL + 100 mM T. All diluted samples were refrigerated for 4 h at 5°C before frozen in liquid nitrogen. After thawing, sperm quality was evaluated by flow cytometry using the following fluorescence probes: SYBR-14 and Propidium Iodide (PI) for viability, Phycoerythrin-Peanut Agglutinin (PE-PNA) for acrosome integrity and Mitotracker deep red for mitochondrial activity. Results (mean±SE; n = 6) showed that despite very low mitochondria function in all SL media, sperm viability differed significantly amongst treatments ($p < 0.05$) being higher in 1% SL + 5% G (59.5 ± 2.1) compared to 15% PEY + 5% G (36.1 ± 2.1),15% PEY + 100 mM T (23.0 ± 1.6) and 1% SL + 100 mM T (8.1 ± 0.8). Also, total acrosome damage in all SL media with glycerol (22.6 ± 1.7) or Trehalose (20.7 ± 2.4) significantly differed ($p < 0.05$) from all PEY media with 5% G (42.7 ± 1.4) and 100 mM T (34.7 ± 4.7). Furthermore, it is important to note that the combination of SL based media with 100 mM of trehalose had the lowest mean value (0.3 ± 0.2) for mitochondria activities. In conclusion, trehalose had a negative effect on sperm viability and mitochondria activities. (Supported by INIA (RZP2014-00001-00-00).)

P 222 | Localization of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the genital tract of fertile boars**

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The granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine with pro-inflammatory immune response, secreted mainly by endothelial cells, macrophages and activated T cells. In porcine, GM-CSF plays an important role in spermatogonial differentiation and renewal (Dirami G, 1999, Biol Reprod, 61:225–230) and its receptors have been evidenced in male germ cells of human, bovine and ovine species (Rodriguez-Gil JE, 2007, Theriogenology, 67:1359–1370). This study aimed to identify the localization of GM-CSF using immunohistochemistry (Anti-GM-CSF antibody, orb6090, BioNova, Madrid, Spain) and immunoblotting in the genital tract of 5 healthy and fertile boars used in artificial insemination programs and slaughtered for genetic replacement. All boars showed a same pattern characterized by a positive staining of spermatogonia, spermatozoa and Leydig cells of testis, epithelial basal cells of epididymis, and epithelial cells of prostate and seminal vesicles. No positive staining was found in bulbourethral glands. The Western Blot analysis identified a strong immunoreactive band (50 KDa) in the positive tissues, which correspond to a GM-CSF glycosylated form. In conclusion, healthy and proven fertility boars expressed GM-CSF in the genital tract. (Supported by MINECO & FEDER (AGL2015-69738-R), Madrid, Spain and SENECA Foundation (19892/GERM/15), Murcia, Spain.)

P 223 | Sexual active rams enhanced the immediate increase in plasma LH concentrations and extends the LH surge of ewes during the male effect

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The effect of a sudden introduction of sexual active rams on LH secretion of ewes was studied. Two rams were sexually-activated by exposing them to 2 months of artificial photoperiod (16 h light/8 h dark) started on 1st of Jan (SAR rams). Another two rams were exposed to the natural photoperiod (control rams; C). Fifteen ewes, synchronized by intravaginal sponges for 12 days, were separated into three groups at sponge withdrawal (20 Mar, hour 0): SAR (n = 5), exposed to SAR; C (n = 5), exposed to C (rams were introduced 24 h after hour 0), and ISO group (n = 5), kept isolated from rams. Groups were housed in different barns. Blood samples were obtained at 6-h intervals from hour 0 until ram introduction, then collected at 4-h intervals until 60 h after ram introduction. Plasma LH concentrations were compared by ANOVA and *t*-student tests. SAR induced a more marked increase of LH plasma concentrations ($p < 0.05$) compared to the ewes in C or ISO groups (SAR: 1.34 ± 0.19 vs. 6.94 ± 2.66; C: 0.96 ± 0.29 vs. 3.60 ± 1.44; ISO: 1.26 ± 0.42 vs. 2.14 ± 1.36 ng/ml, before vs. after ram introduction, respectively). No differences were observed among groups for the proportion of ewes presenting LH surge (SAR: 3/5; C: 2/5; ISO: 2/5), or for the LH surge amplitude (SAR: 86.08 ± 26.98; C: 46.99 ± 9.24; ISO: 32.95 ± 26.39 ng/ml). However, the duration of the LH surge was longer ($p < 0.05$) in the SAR compared to the other two groups (SAR: 13 ± 1; C: 8 ± 0; ISO: 6 ± 2 h, resp.). In conclusion, a sudden introduction of sexual-active rams produced higher increase in LH plasma concentration and extended the duration of the LH surge.

P 224 | Viability, colostrum intake and total proteins in calves born after induced Caesarean section or normal transvaginal delivery

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Calf health is very important for a future milking cow. It is known that calves born from partus induced cows are less healthy. In the bovine teaching clinic of the Department of Farm Animal Health, elective Caesarean sections (CS) for teaching purposes take place in Holstein Friesian cows. Parturition is induced with 0.06 mg/kg dexamethasone when blood progesterone (P4 ng/ml, 3 × weekly) is decreasing. Colostrum quality was estimated with a colostrometer. Only good quality colostrum 50 g IgG/l was used. Calves were bottle fed a minimum of 6 l colostrum within 24 hrs. If the calf did not drink enough itself, colostrum was given by stomach tube. At day 4 after birth total protein in serum (TP, g/l) and PCV was calculated. The aim of the study was to compare viability (TBL = time to attain sternal recumbency after birth (min)), total amount of colostrum and TP and PCV at D4 after birth in CS calves (n = 22) and normal transvaginal delivery (NTD) calves (n = 22). A T-test was used for statistics $p < 0.05$; (Microsoft Excel, USA). There was no significant difference between CS- and NTD calves in total amount of colostrum (8 l), PCV (0.32) and TBL (3.7 min). Average birth weight was 39 kg in CS calves and 43 kg in NTD calves ($p = 0.05$). A significant difference ($p < 0.05$) was found in pregnancy length of CS cows and NTD cows, 276 and 280 days, respectively. Furthermore, a significant difference ($p < 0.05$) in TP in CS calves 73 g/l and NTD calves 81 g/l at D4 of birth was found. In this study CS calves were not less viable and did not drink less than NTD calves. The TP of CS calves at D4 of birth was significantly lower; however, 50 g/l serum TP is considered as sufficient. In conclusion, calves from induced healthy cows were not less viable, nor did they have too low serum TP.

P 225 | Boar seminal plasma induces in vitro expression of transforming growth factor ß1-3 (TGF-ß1-3) by the genital tract of the sow

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TGF-ß1-3 are cytokines with potent immune-modulating action, highly involved in reproductive functions, and apparently modulated by seminal plasma (SP). This study analysed TGF-ß1-3 expression in uterine and oviductal explants in response to boar SP. Endometrial tissue from middle uterine horn (U) and uterotubal junction (UTJ), and mid-oviduct (O) tissue (30–40 mg) from ovariohysterectomised preovulatory sows (n = 3) were individually cultured (M199; 0.1% BSA and 2.2 g/l of $NaHCO_3$) under controlled conditions (5% CO_2; 37°C; 95% humidity) during 16 h in presence of a SP-pool (1:40 v/v) derived from entire ejaculates of 5 fertile boars. The TGF-ß1-3 expression was analysed by a multiplex bead assay kit (TGFB-64K-03; Milliplex MAP, Millipore) in explant supernatants at 0, 24, 36 and 48 h after SP-exposure. All tissue explants expressed TGF-ß1-3 being that of TGF-ß1-2 higher in U and UTJ than in O ($p < 0.05$), while TGFß3 expression was lower without differences among tissues. Culture time influenced TGF-ß1-3 expression in all tissues ($p < 0.05$), particularly between 0 and 24 h. In conclusion, boar SP induces expression of TGF-ß1-3 in sow genital tract, particularly of TGF-ß1-2 in uterus, reaching the peaking peak at 24 h of in vitro culture. (MINECO&FEDER EU-Funds (AGL2015-69738-R) and Murcia Seneca Foundation (19892/GERM/15), Spain; FORSS (grant 745971) and The Swedish Research Council FORMAS (grant 2017-00946), Stockholm, Sweden.)

P 226 | The effect of natural and pharmacological agent reducing mitochondria activity on frozen-thawed canine semen

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The aim of the study was to evaluate the effect of 2 agents limiting mitochondrial activity on post-thaw function of dog spermatozoa when used in cryopreservation media. Semen samples were collected from 6 Slovakian Hound dogs, then pooled and cryopreserved with extender (Tris, citric acid, fructose and 20% egg yolk) and different combinations of a polyphenol: delphinidin (5 μM, 50 μM), and two insulin-sensitive agents: metformin (50 μM, 500 μM) and phenformin (25 μM). Motility was assessed by CASA system; acrosomal and chromatin status, mitochondrial activity, oxidative stress (membrane integrity, lipid peroxidation), apoptosis and membrane lipid disorder were analyzed by flow cytometry. Post-thaw sperm parameters were compared between extenders by Wilcoxon signed-rank test. After thawing, metformin increased motility. The highest percentage of motile (57.0 ± 5.3% vs. 40.6 ± 4.5%) and rapid (34.4 ± 3.3% vs. 21.4 ± 3.9%) sperm were observed in metformin 50 μM group, in comparison to the control ($p < 0.05$). Moreover, the highest percentage of progressive sperm was found in metformin 50 and 500 μM (30.4 ± 2.9% and 29.8 ± 3.7% vs. 19.1 ± 3.2%), in comparison to the control ($p < 0.05$). No significant differences ($p > 0.05$) were found in flow cytometric analysis between tested supplementations and the control, except mitochondrial activity. Metformin 50 μM gave the highest value ($p < 0.05$) for mitochondrial activity, in comparison to the control and delphinidin 50 μM (61.9 ± 8.6% vs. 48.9 ± 6.4% and 50.8 ± 9.9%, respectively). In conclusion, the presence of 50 μM metformin in the cryopreservation media had beneficial effect on canine sperm motility, by improving quality of frozen semen.

P 227 | Sperm-binding to the perivitelline membrane of chicken egg yolk as a functional test for epididymal collected spz evaluation in dogs

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Spermatozoa from the tail of the epididymis are able to fertilize oocytes and produce normal offspring, being a potential source of spermatozoa for cryopreservation. The sperm's fertilizing capacity can be evaluated by functional tests. The aim of this study was to evaluate the efficiency of sperm-binding to the perivitelline membrane of chicken eggs as a functional test for evaluation of canine epididymal collected sperm. For this purpose, six post-thaw samples (100 million sperm/ml) of epididymal collected sperm were used. Samples were previously evaluated for membrane integrity with supravital (eosin-nigrosin; E-N) and fluorescent probes (propidium iodide and hoechst 33342, PI-H) staining and by hyperosmolarity swelling test (HST). An in house apparatus was developed, consisting of an Eppendorf with a lateral hole covered with a fragment of perivitelline membrane. Each apparatus was filled with 500 µl medium + 40 µl sample + 100 µl H + 20 µl PI. After 30 min of incubation (38°C, 5% CO_2 in air, humidified incubator), 200 µl of E-N was added for 1 min in order to stain the membrane. The apparatus was washed, the membrane removed and fixed in a glass slide with a glass coverslits and visualized under fluorescent microscope at 40× magnification. The mean number of sperm attached per unit area of membrane (0.09 mm^2) showed a positive and significant correlation ($p < 0.05$) with the percentage of spermatozoa positive for membrane integrity by PI-H ($r = 0.42$). Although results are promising, studies with more samples are needed. (Funding: UID/CVT/00276/2013; Bolsa CAPES, programa Estágio Senior, Processo 88881119107/2016-01.)

P 228 | Analysis of basic biochemical and gasometric parameters in the blood as marker of a health herd of dairy cattle

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The constant increase of milk yield in Holstein Friesians (HF) cows causes a reduction in fertility, expression of oestrus, pregnancy rates as well as an increase in the occurrence of postpartum clinical problems and metabolic disorders. The aim of the present study was to evaluate selected basic biochemical and gasometric parameters in a herd of dairy cows. The material was collected from clinically healthy HF cows (n = 122), at the age from 2 to 6 years with an average annual milk yield of 11550 kg. Based on the nutrition and level of milk yield, the animals were divided into 5 groups. In groups 1–4 were lactating cows, while in group 5 only dry cows were included. Parameters such as pH, pCO_2 (mmHg), pO_2 (mM), HCT (%), tHb (g/dl), FO_2Hb (%), FCOHb (%), FMetHb (%), FHHb (%), HCO_3-act (mM), BEecf (mM), $ctCO_2$ (mM), Na^+ (mM), K^+ (mM), Ca^{++} (mM), Cl^- (mM), AnGap (anion gap, mM), Glu (mg/dl) were assessed in whole blood, using the Siemens RAPIDPoint 500 analyzer. Data were analyzed with Kruskal-Wallis and Dunn-Bonferroni tests. There were significant ($p < 0.01$) differences for 11 parameters: pH, pCO_2 (mmHg), pO_2 (mM), HCT (%), tHb (g/dl), FO_2Hb (%), FMetHb (%), FHHb (%), HCO_3-act (mM), $ctCO_2$ (mM), Glu (mg/dl). These markers seem to be important in the assessment of the oxidative status of dairy cows. Still, there is a lot to be learned about how oxidative stress can affect the health of dairy cattle, especially during periods of high metabolic activity. After standardization, gasometric and biochemical parameters can be used to detect subclinical diseases and hence, may be used as markers of the oxidative status and general health at a dairy herd.

P 229 | Conservation methods of granulosa cells from mare oocytes**

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Developmental competence of the equine oocyte might be related to DNA damage in granulosa cells. Those cells are commonly stored before DNA assessment; however, the effect of preservation methods on this parameter remains unexplored. The aim of this study was to compare the effect of four granulosa cells conservation techniques on DNA damage. Equine oocytes were recovered from post mortem ovaries of 15 mares. Granulosa cells were washed by centrifugation and stored in cryotubes according to the following protocols: (P1) directly plunged in liquid nitrogen and then stored at -80°C without cryoprotectants; (P2) directly plunged in liquid nitrogen and then stored at -80°C but adding cryoprotectants (7.5% EG + 7.5% DMSO); (P3) stored at -80°C without cryoprotectants; or (P4) stored at -80°C but adding the same cryoprotectants. Granulosa cells samples were processed with the D3-MAX® (Halotech DNA, SL Madrid, Spain) and DNA was visualized under fluorescence microscopy. DNA fragmentation of at least 300 granulosa cells per sample was assessed. Results were compared between conservation techniques by ANOVA and expressed as mean ± SEM. No significant differences ($p > 0.05$) were found between protocols (P1: 6.07 ± 1.04 vs. P2: 4.18 ± 0.14 vs. P3: 3.64 ± 0.21 vs. P4:5.34 ± 1.42). Therefore, the four conservation protocols could be considered as efficient methods of DNA

preservation of granulosa cells from mare oocytes. This is the first study in which four storage methods obtain reasonable DNA fragmentation values, becoming a beneficial alternative when instantaneous evaluation is unfeasible. (This study was supported by project AGL2013-42726-R.)

P 230 | Utilization of extenders of ram semen without glucose and low percentages of egg yolk added with honey bee

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The objective of this experiment was to replace glucose by honey and to lower the percentage of egg yolk in extenders for refrigeration to 5°C in ram semen. This study was conducted in the farm of the Vet. Faculty of the ULPGC in Arucas, Gran Canaria, Spain. Semen from 3 rams of the Canarian breed was collected with an artificial vagina. The base sperm concentration to dilute the ram semen was 400×10^6 sperm/ml. The extender control (C) was with 15% of egg yolk, citric acid, Penicillin G and glucose; the treatment extenders were M1 (5% honey, 10% egg yolk), M2 (5% honey, 5% egg yolk) and M3 (2.5% honey, 7.5% egg yolk). To M1, M2 and M3 was added only citric acid and Penicillin G, without glucose. The parameters evaluated after dilution at 0, 24 and 48 h were linear progressive motility (LPM), plasma membrane integrity (V), HOST (hypo-osmotic swelling test) and acrosome integrity (AI). Once diluted the semen was slowly cooled to 5°C and kept for 48 h to perform the evaluations. Variables were analysed for differences within and among treatments. The results to LPM were at 0 h: C 57.1 ± 5.0%, M1 59.6 ± 6%, M2 49.6 ± 5.5%, M3 55.4 ± 5.6%; 48 h: C 46.3 ± 4.3%, M1 40.8 ± 3.5%, M2 33.3 ± 2.6 and M3 35.8 ± 4.2%. V was at 0 h: C 52.6 ± 5.3, M1 61.4 ± 4.6%, M2 64.4 ± 3.8%, M3 60.1 ± 3.6%; 48 h: C 54.9 ± 3.1%, M1 56.3 ± 1.8% M2 60.3 ± 4.1% and M3 60.2 ± 3.7%. HOST was at 0 h: C 55.3 ± 3%, M1 51.8 ± 3.3%, M2 47.6 ± 3.8, M3 50.3 ± 3.6%; 48 h: C 49.7 ± 4.1%, M1 41.7 ± 5.1%, M2 31.7 ± 2.4 and M3 37.2 ± 5.7%. AI was at 0 h: C 83.8 ± 2.8%, M1 84.3 ± 2.2%, M2 80.1 ± 2.6%, M3 85.8 ± 1.9%; 48 h: C 73.3 ± 2.2%, M1 72.9 ± 3%, M2 68.8 ± 5.2%, M3 74.6 ± 2.6%. Among treatments no significant differences were found for variables evaluated, but over time ($p<0.05$). Reduced percentages of egg yolk and substitution of glucose by honey had a comparable efficacy.

P 231 | Sperm production in three Andalusian autochthonous avian breeds: comparison of two methods to determine sperm concentration

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The aim of the present study was to determine the sperm production values in three Andalusian autochthonous avian breeds and to compare two methods to assess sperm concentration. For this purpose, a total of 15 roosters from the breeds Combatiente Español (CE), Utrerana (U) and Andaluza Azul (AA) were used. A total of 9 ejaculates per roosters were collected, and volume and concentration were assessed. The concentration was evaluated using the hemocytometer (Bürker chamber) as a reference method and it was compared with the absorbance of the samples determined by photometer (Accuread, IMV Technologies, France) to produce a standard curve to calculate the sperm concentration. For the assessment, sperm samples were diluted 1:200 in distilled water. Pearson's correlation test was used to evaluate the association between both methods used. The results of sperm volume and concentration for CE were 0.24 ml and 5.14×10^9 spz/ml, 0.27 ml and 2.96×10^9 spz/ml for U and 0.14 ml and 2.97×10^9 spz/ml for AA. The determination of sperm concentration by photometer showed a moderate correlation with sperm concentration (determined by hemocytometer). It was concluded that photometer is not useful to determinate the concentration of spermatozoa in this species. New studies are needed to determine more practical methods to evaluate rooster sperm concentration.

P 232 | TGF-β1 levels in seminal plasma relates positively to porcine in vivo fertility**

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Boar seminal plasma (SP) is rich in cytokines, particularly of the transforming growth factor-β (TGFβ) family. This study evaluated putative relationships between the concentration of TGFs-β1, -β2 and -β3 in the SP of AI-boars and their fertility (farrowing rate: FR, litter size: LS). Samples of SP (n = 84) of sperm-rich fractions collected from 21 AI-boars used to produce semen doses for the cervical AI of 4524 sows (between 540 and 80 sows/boar) were analyzed for TGFβs using specific multiplex bead assay kits (Luminex's × MAP®). The direct boar effect (raw fertility dataset corrected for parameters related to farm and sow) ranged from −2.79 to +2.28 and from −0.42 to +0.34 for FR and LS, respectively. BayesVarSel package of R was used to determine the relationship between SP-TGF-βs concentrations and fertility; with TGF-β1

alone providing the best fit. TGF-β1 had inclusion probabilities of 0.85 and 0.94 for FR and LS, respectively, whereas those for TGF-β2 and -β3 were lower than 0.20. The results clearly show that SP-TGF-β1 levels in SRF is positively related to in vivo fertility outcomes of boars included in AI-programs. (Supported by MINECO & FEDER EU-Funds (AGL2015-69738-R) Madrid, Spain; Seneca Foundation, Murcia, Spain (19892/GERM/15); FORSS (grant 745971) and FORMAS (grant 2017-00946), Stockholm, Sweden.)

P 233 | Efficiency of using semen extender VNIIGRZH for insemination in chickens

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The effectiveness of artificial insemination in poultry largely depends on the use of semen extenders. The aim of the study was to test the effectiveness of extender VNIIGRZH (patent No. 2566656, RU, 2015) for insemination in chickens of different directions of productivity. Extender VNIIGRZH is used for semen dilution in cocks, turkeys, gander and ducks. Glutamate sodium, glucose, fructose are parts of the extender. We tested this extender in two farms. Semen was diluted 1:1 by extender VNIIGRZH. Laying chickens were on the one farm, broiler chickens were on the second farm. Laying chickens were inseminated by not less than 80 mln motile spermatozoa per one AI once every 5 days, and broiler were inseminated by not less than 100 mln motile spermatozoa per one AI once every 4 days. The age of laying hens was from 23 to 74 weeks and broilers from 26 to 61 weeks. 4.474.500 eggs of laying chickens were collected and incubated. Fertilization rate was 94.9%, chickens rate 83.4%. 25.814.500 eggs of broiler were incubated. Fertilization rate was 93.0% and chickens rate was 81.1%. The high reproduction rates of both laying hens and broilers obtained during the testing extender VNIIGRZH show its effectiveness in artificial insemination in chicken. The results are similar to the results of natural mating in chickens. Authors acknowledge financial support from (The Federal Agency for Scientific Organizations (FASO Russia), project №AAAA-A18-118021590134-3.)

P 234 | Proteomic changes during time-course of capacitation in fresh and frozen-thawed ram sperm**

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Proteomic technologies are relevant in spermatozoa due to its transcriptional and translational silence. It has been shown that cryopreserved sperm requires shorter incubation time than fresh to reach the capacitation status because of cryodamage. However, there is a lack of work investigating the proteomic changes during capacitation at different incubation times, which could help to elucidate the molecular mechanisms involved in this phenomena. The dynamics of capacitation in fresh and frozen-thawed ram sperm was evaluated in the present study using a proteomic approach to shed light on those elements of the proteome that are likely to be of functional relevance. Four ejaculates were mixed and divided into fresh and cryopreserved samples. Both groups were incubated in synthetic oviductal fluid (SOF) with 2% (ESS) for 1 min, 15 min and 240 min at 38.5°C. A negative control without ESS was further used (0 min). After protein extraction by urea and thiourea lysis buffer and tryptic digestion, the peptide mixture was analyzed by reverse phase liquid chromatography coupled to mass spectrometry (RP-LC-MS/MS). A Chi-square test was used to compare the differential protein representation. The statistical analysis revealed that two proteins in fresh samples (M6P/IGF2R and LOC101123268) and three proteins in frozen-thawed sperm (HADHA, CAPZA2 and LOC101123268) underwent significant changes ($p < 0.05$) over time. Those proteins related to sperm-oocyte interaction and protein transport showed important differences at 240 min in fresh semen while proteins involved in metabolic process, sperm-oocyte interaction and motility displayed meaningful variations at 15 min in frozen samples. The results suggest that cryopreserved and fresh spermatozoa have a different capacitation behaviour.

P 235 | Association between milk yield and fertility traits in developing Russian Ayrshire cattle breed

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Russian Ayrshire breed was formed from imported in 1963–1971 Finnish animals. Today, the total breed size is 41630 milking cows and the largest herds are in the Leningrad region, Karelia republic and Krasnodarskiy kray. Russian Ayrshire population has an average milk productivity of 6363 kg with phenotypic trend 1004 kg during the last five years. According to Canadian and Finland productivity reports Russian breeding industry see quite high potential for growing productivity traits. Utilization of genomic selection and modern selection program can increase production level with fast dropping down fertility traits. Understanding of correlations between milk improvement and reproduction traits becomes a vital step for breed developing. Our studies were performed using data from 12 Leningrad region herds with an average size of 700 cows. Phenotypic data included 76890 305-day repeated milk records from 28607 cows with mean (M) and standard deviation (SD) of

6252 and 1529 kg, respectively. Fertility traits were presented by days from calving to next conception interval (CCI) and age of first calving (AC). Cows were divided in three groups according to productivity level: M-3SD to M-1SD, M ± 1SD and M + 1SD to +3SD. Negative effect of growing productivity on CCI was detected using Generalized Linear Model (GLM) in RStudio, solution for CCI was 28.8 ($p < 0.0001$). The smallest AC recorded was observed in group 3 (833 days), the highest (863) in the group with lowest productivity ($p < 0.001$). Conducted studies show the negative impact of growing milk yield on CCI (reduced number of productive inseminations). (Study was funded by the FASO State Assignment AAAA-A18-118021590134-3 and AAAA-A18-118021590138-1.)

P 236 | Ultrasound evaluation of ovarian follicular growth after superstimulation treatment by corifollitropin alfa in rabbit

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Superstimulation protocols in farm animals ensure the maximum number of embryos recovered per donor. Although eCG and pituitary FSH are the most commonly used hormones, recent studies have demonstrated the efficiency of long-acting recombinant FSH (corifollitropin alfa, CTP). CTP, used 60–72 h before ovulation, superstimulates nulliparous rabbit females, without compromising the quantity and quality of embryos. Nevertheless, 20% of the females do not respond to treatment. The aim of this study was to evaluate ultrasound efficacy in order to estimate the superstimulation response after CTP treatment in multiparous and nulliparous rabbits. Ovarian monitoring activity was conducted by transabdominal ultrasonography (n = 12 females) using 18 MHz linear probe when the superstimulation started (D0, 0.75 mg/Kg CTP) and 3 days later (D3). Examination of the ovaries was performed in order to record the number and size of the follicles and ovarian size (length and width for each ovary). Superstimulation response was considered positive when the number of follicles in each ovary was higher than 15. The results showed that ovarian size was not changed by the superstimulation treatment, although differences between nulliparous and multiparous were significant (length 15.6 ± 0.48 vs. 21.2 ± 0.48 mm, $p < 0.01$, and width 5.6 ± 0.21 vs. 6.7 ± 0.21 mm, $p < 0.01$). Nevertheless, D3 follicle size was not different between nulliparous vs. multiparous females (0.85 ± 0.021 vs. 0.90 ± 0.022 mm, respectively). Finally, nulliparous females tended to show the better response to superstimulation treatment (75% vs. 57%, nulliparous vs. multiparous respectively, $p = 0.085$). Ultrasound evaluation could be a non-invasive tool to detect superstimulation response in rabbit. (Study supported byAGL2014-53405-C2-1-P.)

P 237 | The mammary gland tumor Shear Wave Elastography in bitches**

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Each tissue is characterized by its natural flexibility, which can change as a result of aging, inflammation, fibrosis or neoplastic lesions. Tissue elasticity is defined as the proportion of tension (pressure force) exerted on a tissue to a relative change of its volume (elastic deformation) caused during the pressure. Shear Wave Elastography (SWE) is the most advanced technique which enables objective measurement of stiffness of a structure examined together with a numerical value of stiffness. The aim of the study was to evaluate the mammary gland tumor tissue elasticity in bitches regarding to fine-needle aspiration (FNA) biopsy results. The study was performed on 12 bitches with mammary gland tumors. SWE was performed using the Aixplorer® ultrasound system machine (SuperSonic Imagine, Aix en Provence, France). Elasticity results were expressed in kPa. The mean value was calculated from 5 measurements in each patient. Afterwards FNA biopsies of mammary gland tumors were performed and evaluated cytologically. Cytology results showed malignant neoplasms in all cases. The SWE examinations of the mammary gland showed stiffness ranges in low malignancy lesions (n = 7): 32.3 to 79.1 and average 50.3 ± 13.0 and in high malignancy (n = 5): 86.1 to 189.6 and average 134.73 ± 32.0 [kPa]. Comparison of our results with other study results performed in female dogs revealed different range of malignant lesion elasticity values. However our study distinguished malignancy results of examined tumors into low and high malignancy groups (regarding to pleomorphism and mitotic indexes). We concluded that elastography of the mammary gland tumors can be useful diagnostic method. However more studies are required to estimate accurate values ranges for different degrees of malignancy.

P 238 | A novel seminal additive for porcine increases fertility in Iberian sows after artificial insemination

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Artificial insemination with refrigerated semen is routinely performed in the pig industry. Although efficiency has been increasing for years, there is room for improvement, especially in problematic

breeds. We have tested a novel additive (Suinfort™, University of León, Spain), hormone-free, for supplementing the semen dose before artificial insemination (AI). This trial was performed in Iberian sows as a model of a breed with irregular fertility results. Semen doses produced from 62 Duroc boars were used for inseminating 1252 Iberian sows, with a total of 3380 AI. In 908 AI, the additive was added to the semen dose. The additive effects in fertility and prolificacy (total, live, dead and mummified piglets) were analyzed by linear mixed-effect models, including the cycle number, days from the last weaning, month, and male and female as random effects (results as mean±SEM). The additive increased fertility from 95.7% to 97.5% (p = 0.014). Prolificacy parameters were not affected (overall, 8.08 ± 0.04 piglets/sow, 7.78 ± 0.04 alive and 0.19 ± 0.01 dead). However, the number of mummified piglets increased in the additive group from 0.10 ± 0.01 to 0.13 ± 0.02 per sow (p = 0.017). Other significant factors were: Month, affecting fertility and prolificacy (lower in summer); days from weaning, affecting fertility; and the cycle number, affected prolificacy (with extreme numbers 1 and 12, lowering it). This new additive enables a small increment in pregnant sows, which might be relevant in problematic or special interest breeds with a lower fertility.

P 239 | Basic semen evaluation in reindeer (*Rangifer tarandus*)

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The aim of the study was to evaluate basic sperm traits in reindeer. Semen was collected at an experimental farm in Kutoharjo, in Taimyr and at the private Zoo in St. Petersburg in autumn 2017. Males were fixed by rope on the legs and horns in Taimyr and at the Zoo, by anaesthetics (Domosedan, Ketaminol) and analgesic (Metacam) in Kutoharjo. A total of 36 samples from 11 adult males were collected by electroejaculation. First, color, odor and consistency of fresh semen were examined. Then, ejaculate volume and pH were evaluated. Total and progressive motility and sperm concentration were measured by CASA (computer-assisted semen analysis). Results are depicted as ranges, percentages of totals and averages ±SDs. Reindeer semen has a characteristic strong odor. Ejaculate color varied from viscous honey-like to non-viscous milky or watery. Ejaculated volume (0.6 ± 0.11 ml) and sperm concentration (0.61 ± 0.12 billion/ml) were in the same range as earlier reported. pH varied from 6.7 to 7.2. Total and progressive sperm motility had a high variability from 50 to 91% (73.3 ± 11.8%) and from 33 to 79% (63.2 ± 14.2%), respectively. Large variations might be explained by different periods and the intensity of the rut. Further studies are required. (Authors acknowledge financial support from Russian Science Foundation, Grant No:17-16-01023 (sampling in Taimyr and St. Petersburg), and by NordForsk, ReiGN in Russia (sampling in Kutoharjo).)

P 240 | Evaluation of the cocks spermatozoa membranes' damaging during cryopreservation with use of Sperm VitalStain colorant

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Sperm cryopreservation is an important method of gene resources preservation in poultry. During the cryopreservation some decreasing of the sperm fertilization ability is unavoidable due to the damaging of sperm cells. At the freezing and thawing spermatozoa can get damages, mainly because of breaching of the membranes' structure. The goal of the study was to upgrade informativity and quality of evaluation of the frozen-thawed individual ejaculates during formation of cocks' sperm cryobank. The investigations were carried out on the base of Genetic Collection of the Rare and Vanishing Chicken Breeds of the RRIFAGB. There were used Rhode Island Red chicken (♂n = 12; ♀n = 54) at the individual cage keeping. The cocks had ejaculates volume of 0.4–1.4 ml; concentration ≥ 3 bln/cm^3; spermatozoa activity in fresh sperm ≥ 80%. As a cryoprotector dimethylacetamide was used (final concentration 8%). The degree of spermatozoa damaged was evaluated by using colorant Sperm VitalStain («Nidacon International AB»); 50 mcl of sperm / 50 mcl of colorant smeared on a glass slide. For microscopying Axio Imager («Carl Zeiss Microscopy GmbH») was used, 200 cells in each sample (white cells- intact, pink cells- damaged). A large individual diversity was found among cocks concerning integrity of membranes (37.7% to 70.2%) and activity of the thawed sperms (45% to 85%). The activity of spermatozoa after thawing correlated with the degree of damage of the membrane structures, rs = 0.49 (p < 0.01). Fertility of eggs (%) after use of the frozen-thawed sperm depends on the degree of integrity of spermatozoa membranes and varies from 64.3% to 85.7%. (The study was supported by Federal Agency for Scientific Organizations (project № AAAA-A18-118021590134-3).)

P 241 | Single-layer colloid centrifugation as a method to process urine contaminated stallion semen after cryopreservation**

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Urospermia is a major problem affecting stallion fertility. Currently, it is suggested that urine contaminated semen should not be frozen. This study aimed to assess single-layer colloid centrifugation (SLC) to process

urine contaminated semen after thawing. Raw ejaculates (n = 49) from eight stallions were split into three groups: no urine, low (20%) or high (50%) urine contamination. Semen was extended 1:1, cushion centrifuged (1000 g/20 min) and resuspended to 200 million/ml in Botucrio. Resuspended semen was loaded in 0.5 ml straws and cryopreserved in LN_2. Samples were thawed (37°C/30 s) and processed by SLC (400 g/30 min) (Equipure, Nidacon, Sweden). Total motility (%TM) and progressive motility (%PM) were assessed with CASA. Sperm viability (%VIAB) and yield were assessed with a Nucleocounter pre- and post-gradient. Data were analyzed with ANOVA and Tukey's. Motility parameters decreased with increasing urine contamination %TM (Control 35 ± 2 to 51 ± 3.6, Low 33 ± 0.7 to 42 ± 2.2, High 22 ± 1.8 to 25 ± 2.8, respectively pre- and post-gradient) %PM (Control 24 ± 1.8, 40.3 ± 3.2, Low 21 ± 1.14, 31 ± 3.9, High 12 ± 1.5, 14 ± 2, respectively pre- and post-gradient) ($p < 0.05$). Urine contamination marginally reduced %VIAB after cryopreservation and centrifugation (Control 45 ± 0.7 to 54 ± 0.5, Low 27 ± 0.2 to 49 ± 0.7, High 27 ± 0.3 to 38 ± 0.6, respectively pre- and post-gradient) ($p < 0.05$). Recovery rates were not significantly different between groups. In conclusion, urine contamination affects sperm motility parameters in a dose-dependent manner. Post-thaw SLC improved motility and %VIAB in Control and Low groups but only improved viability in the High group.

P 242 | Hair dehydroepiandrosterone in newborn beef calves from birth up to 10 months of age

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Although steroidogenesis in maternal, placental and fetal compartments is interdependent, the maternal and fetal hypothalamic-pituitary-adrenal (HPA) axes represent separate biological systems, with dehydroepiandrosterone (DHEA) recognized as the main fetal steroid. Fetal steroids are likely to influence development and have long-term effects on HPA function. Hair analysis represents a promising methodological approach for the non-invasive measurement of steroids, allowing for a retrospective analysis of the total exposure to steroids over time, and avoiding the influence of acute events or circadian fluctuations (Schury et al. 2017, BMC Psychiatry 17:213). Hair coat DHEA (hcD) concentrations have been investigated in cows (Peric et al. 2017, Livestock Sci 202:39–43), but no studies have been performed on newborn calves. Hair samples of 12 beef calves (7 males, 5 females) were collected by shaving at calving (T0) and monthly up to 10 months of age (T1-T10), only on the re-growth area. Hair DHEA was analyzed by RIA2. Statistical analysis revealed that hcD concentrations in calves were influenced by age, with higher levels at T1 and T2 compared to the other samples ($p < 0.05$). HcD levels were not influenced by newborn gender, birth weight and Apgar score. These data demonstrate that DHEA is quantifiable in the hair coat of newborn calves, and that hcD levels are influenced by the age of calves. The higher accumulation of DHEA was detected from birth to 2 months of age, suggesting that DHEA continues to be secreted by the newborn calf also beyond birth and could be involved in the events occurring during the first months of age.

P 243 | Effects of mutations in MSTN on carcass traits in Holstein Friesian (HF) cattle without antagonistic effects on fertility traits

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It is well established that DNA variants in the MSTN gene are responsible for the improved conformation and muscle mass accrual paraded by double-muscled (DM) animals. However, the condition is often associated with dystocia amongst other calving complications. The objective of this study was to estimate the effects of polymorphisms in MSTN on carcass traits in HF dairy cattle and moreover, to determine whether there are any antagonistic effects on fertility traits. MSTN genotypes (n = 17) on 14,939 dairy cows were obtained from the Irish Cattle Breeding Federation (ICBF) who also provided phenotypes, expressed as predicted transmitting abilities (PTAs). Associations between each SNP and PTA were analyzed in ASReml using a weighted mixed animal model. Association analysis comprised of n = 4414, n = 893, n = 1933 and n = 5111 cows for carcass weight, carcass conformation, carcass fat and cull cow weight respectively, while fertility traits included n = 2467, n = 3237, n = 12688, n = 2157 for calving interval, calving difficulty, gestation length and maternal calving difficulty respectively. Significant associations ($p < 0.05$) were identified between MSTN variants nt267 (−3.89 kg, s.e. 1.77, $p = 0.028$) and nt748-78 (0.82 kg, s.e. 0.37, $p = 0.025$) with carcass weight while nt267 (−0.34 kg, s.e. 0.17, $p = 0.04$) showed an association with carcass conformation. No significant ($p > 0.05$) antagonistic effects were observed on fertility performance. Results suggest that these mutations are associated with carcass traits of economic importance without having negative effects on fertility traits in Irish HF dairy cattle.

P 244 | First description of the seminal microbiome in healthy stallions and donkeys**

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Gastrointestinal tract microbiome has shown to have a major impact on local and distant regions of the body. Thereupon, there

is a growing interest describing the microbiome of several human systems. It has been proven that semen microbial composition varies between fertile and infertile men. Similarly, some bacterial families happen to be more common in genital tract diseases as prostatitis or AIDS. Most of these studies focus on men, while little is known regarding stallion or donkey. Therefore, the aim of our study was to describe the seminal microbiome of fertile stallions and donkeys. Semen samples were collected from four stallions and two donkeys with proven fertility. Cell disruption was performed by high-speed homogenization of samples in grinding media. DNA was isolated using ZymoBIOMICS® DNA Miniprep kit (Zymo Research, CA). Bacterial families were characterized via V3 amplification. Data was directly taken from IonTorrent database. Only families with a higher than 1% presence were taken into consideration. More than 70 bacterial families have been found, but only eight appeared to be common in all samples. *Porphyromonadaceae* (+26.10%), *Prevotellaceae* (+8.94%) and *Corynebacteriaceae* (+12.85%) were the most representative ones, but it was *Firmicutes phylum* which displayed a higher number of families. Samples showed high inter-subject variability and no relevant differences between species was observed. Findings previously described in men notably differ from our findings in equids. To sum up, *Porphyromonadaceae*, *Prevotellaceae* and *Corynebacteriaceae* families were highly represented in the seminal microbiome of stallions and donkeys, but high variation among individuals was also observed.

P 245 | Segregation of a candidate novel lethal recessive gene in Irish Holstein Friesian dairy cattle**

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The LFNG (O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase) gene has previously been observed to be associated with fertility and reproduction in vivo in zebrafish, avian and mice. As a member of the NOTCH signalling pathway, this gene has been shown to play a critical role in embryonic development through the regulation of the formation and patterning of somites in vertebrates. These studies suggest LFNG may be required for successful early bovine embryo development and any polymorphisms affecting the function of LFNG may contribute to embryonic lethality in cattle. A nonsense mutation in LFNG was added to the content of the International Dairy and Beef custom genotyping array. A total of 10,707 Irish Holstein Friesian dairy cow genotypes at this loci and fertility phenotypes (calving interval and calf mortality expressed as predicted transmitting abilities (PTAs)) were obtained from the Irish Cattle Breeding Federation. The nonsense snp in LFNG was segregating in the heterozygous state, albeit at a very low frequency (minor allele frequency (MAF) <0.01), with no cows homozygous for this variant identified. Association analysis, carried out in ASReml using a weighted mixed animal model, revealed a tentative association ($p < 0.1$) between this snp and an increase in calving interval which may represent a role in early embryonic loss in carrier animals. In order to validate the association with this gene and calving interval in Holstein Friesian dairy cattle future work includes repeating this analysis using a larger dataset of cattle, including trios (genotyped carrier animals and their parents) where possible. Further analysis could include in vivo functional studies to investigate the possible mechanism of action of this gene with regards to bovine fertility.

P 246 | Prevalence of EAV among Swedish stallions

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According to Swedish legislation since 1995, all stallions used for artificial insemination (AI) shall be tested for EAV. Before the start of the breeding season, a blood sample is taken from the stallion and analyzed with a serologic method at the National Veterinary Institute (SVA). If the blood sample is serologic positive, a semen sample shall be analyzed with PCR for the occurrence of EAV virus. In addition, if the semen is EAV positive, it is allowed to use for insemination given that the mare owners are informed. In most other countries, stallions with EAV in semen can't be used for breeding. No follow-up on EAV positive stallions has been done since 2005 (Strand et al. 2006, Anim Rep Sci, 94:104–106). In this study, SVA data on EAV analyses from the period January 2007–September 2016 was extracted from the data base in which analyses results are stored. Thereafter, only cases where stallions and serologic result with a titer >1: 15 were included. From the data set it was in many cases not possible to identify the breed of the stallion (information missing), therefore breed had to be excluded. The number of blood samples analyzed per year varied between 252 (2016) and 299 (2008), the mean being 283.1. During the 10 year period, the sero-prevalence varied from 9.1% to 12.7% (NS). The mean number of semen samples per year, analyzed with PCR, was 50.3 (range 25–65). The highest proportion of EAV positive semen was found 2009 (6.8%) and for three years (2013, 2015, 2016) all samples were negative. The prevalence for serologic positive stallions was lower than reported by Strand et al. (2006). During the 10 year period, only one case was diagnosed positive causing a newborn foal to die due to weakness.

P 247 | Development of a new, practical test for the diagnosis of neonatal isoerythrolysis in the newborn foal: a preliminary study**

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Neonatal isoerythrolysis (NI) is a life-threatening disease in the neonate. Three tests are currently available to diagnose NI: the jaundiced foal agglutination test, the hemolytic cross-match and the direct Coombs test, all having delayed results and some no predictive value. Therefore we developed a new practical method to diagnose NI. We investigated two different techniques. In a first study, 18 newborn foals were tested with an immunochromatography strip containing an antibody directed against equine IgG, IgM and C3 component. A second study used a gel agglutination test made of calibrated beads containing antibodies detecting equine IgM, IgG and C3 fraction of the complement on 66 newborn foals. The gel characteristics do not allow antibody-erythrocyte complexes to migrate through it during centrifugation. To investigate the link between anti-Ca immunization and the occurrence of NI, Ca typing has been tested using an immunochromatography strip containing a monoclonal antibody specific for the Ca factor. The strip detected negative cases, while one out of two NI was not detected. The gel test produced some false positives (PPV = 20%). Of the 14 mares typed for Ca, one was Ca-positive with a positive gel test, one was Ca-negative with a positive gel test, and 12 were Ca-positive with a negative gel test. No Ca-negative mare with a negative gel test was reported. Because the gel test has a high sensitivity and the strip a high specificity, both tests would lead to more reliable diagnostic results. It has been impossible to establish a conclusive link between Ca blood type and the risk of NI because not enough mares were available for testing. Testing the mare's anti-Ca immunization instead of their Ca status may be a better way to investigate this hypothesis.

P 248 | *Coxiella burnetii* phase-specific serological response and status of offspring in dairy cows in Latvia – preliminary results**

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Coxiella burnetii (*C. burnetii*) – an ethiological agent of zoonosis Q fever can be present in two phases of antigen (phase1 (Ph1) and phase2 (Ph2), respectively). Phase-specific serological response demonstrates chronic (Ph1) or acute (Ph2) *C. burnetii* infection. Outcomes of *C. burnetii* infection in cows can be abortion, stillbirth, weak or normal offspring. The aim of this study was to detect *C. burnetii* phase-specific serological response and outcome of offspring alive in dairy cows. This is the first study to detect this relation in dairy cows in Latvia. In this study sera samples from 44 randomly selected animals belonging to 5 herds with previous history of *C. burnetii* infection were collected from different parishes in Latvia, in 2017. Samples were tested by "VetLine Coxiella Phase1 and Phase2 ELISA" (NOVATEC). Data of status of the last parturition/offspring were collected from Agricultural Data Centre Republic of Latvia. Results showed that in 6 cows with a positive serological response to Ph1 (Ph1+), status of offspring was 2 abortions, 1 stillbirth, 2 died, 1 alive. In 3 cows with a questionable serological response to Ph1 (Ph1+/–), status of offspring was 3 alive. In 35 cows without any serological response, status of offspring was 1 abortion, 6 stillbirth, 6 died, 22 alive. None of the cows demonstrated a serological response to Ph2. Outcome of offspring alive was significantly ($p < 0.05$) lower in cows demonstrating Ph1+ compared to those without any serological response, but there were no significant ($p > 0.05$) differences comparing cows demonstrating Ph+/– and those serologically negative. A conclusion of this study is that a positive serological response to Ph1 is associated with outcome of offspring alive. The study will be continued.

P 249 | The effect of melatonin applications on offspring in experimentally induced uterine torsion in pregnant rats

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The aim of this study was to investigate the effect of melatonin application on rat fetus survival after experimentally induced uterine torsion. With this aim, uterine torsion was experimentally generated in 35 rats between day 18 and 19 of pregnancy. The animals were randomly divided into five groups, and melatonin was administered prior to torsion, at the time of torsion, and during detorsion (10 mg/kg IP); in this way, we could determine the best time for melatonin administration to promote foetal survival. The offspring born out of these mothers were followed subsequently, and the 1-month-old male rats and female rats that had reached puberty were decapitated. Tissue samples, including the organs of the offspring, were evaluated by histopathology and brain tissue apoptosis was investigated by TUNEL. In the application group, 28.6% of offspring in the 4th group were aborted, and 70.6% of the offspring in the 5th group died; these outcomes were not statistically significantly different from the outcomes in the controls. No congenital anomalies were observed in the offspring of any group, no microscopic lesions could be detected in the organs, and no apoptosis was found in the brains. As a result, melatonin administration did not have any effect on offspring born from rats with uterine torsion. (This study was supported by the TUBITAK-115O381.)

P 250 | Effect of the addition of essential oils upon the presence of reproductive and metabolic diseases in multiparous Holstein cows in the transition period

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The effect of the addition of essential oils (EO) in the diet on reproductive and metabolic outcome in Holstein cows in transition period was assessed. Sixty-one multiparous Holstein cows passing through the transition period were used. The cows were fed with a completely mixed diet (RTM, 60% forage, 40% grain) and randomly assigned in two treatments: LU (n = 30; 100 g/cow), to which EO were supplied and TE (n = 31) to which 100 g of wheat bran instead of EO were given. Treatments lasted for 21 days before and 21 days after calving. In both groups, the following factors were evaluated: retention of fetal membranes (RFM), considering positive those cows with more than 12 h of RFM; metritis, considering positive cows with purulent and fetid vaginal discharge 6 h after calving; ketosis, using qualitative detection strips (PortaBHB™); and lameness problems, scale from 0 to 5, considering lame those cows with a minimum classification of 3. Data was analyzed by a chi-square test, (SYSTAT 10, Evenston, ILL, USA). Of nine cows registered with RFM, a lower proportion ($p < 0.05$) was observed in the LU group (1/9), compared to those in the TE group (8/9); the record of cows with metritis (10) showed a lower proportion ($p < 0.05$) in the cows of the LU group than in those of the TE group (2/10 vs. 8/10, respectively). None of the groups showed cows with ketosis, while there was no statistical difference for the case of lameness (4 cases: 2/4 in LU group, 2/4 in TE group). Although RFM is dependent on multiple factors, the results allow us to infer that by adding EO in the diet of multiparous cows in the transition period, the frequency of metritis and placental retention might decrease; further studies including larger groups are warranted.

P 251 | Functional Sertoli cell tumour associated with paraprostatic abscess and spermatic cord torsion in a pseudohermaphrodite abdominal cryptorchid male dog: A case report

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Testicular tumours (TT) are common in dogs. Feminizing syndrome (FS) and cryptorchidism have been associated with Sertoli cells tumours (SCT) and prostatic abscess (Ródenas et al. 2017, Reprod Dom Anim 52:95). We report a case of a 7-year-old Golden Retriever with recurrent haematuria, FS and unilateral abdominal cryptoquidia. Hematological analysis revealed non-regenerative anemia. Leukocytosis and bacteria were observed in urinalysis. Abdominal ultrasonography examination revealed a mixed echogenicity with anechogenic areas mass in the caudal abdomen, compatible with TT in cryptorquid right teste. Moreover, one cystic structure was seen in each prostatic lobe consistent with cysts or abscesses paraprostatic. On abdominal Computed Tomography findings, consistent with TT, spermatic cord torsion and bilateral paraprostatic cyst were observed. After conventional orchiectomy, neoplastic teste was removed by laparotomy. A structure compatible with uterine horns was removed by hysterectomy. Bilateral paraprostatic abscesses were drained, omentalized and treated with antibiotics. Evolution was desfavorable after surgery. Histopathological study revealed SCT, prostatic squamous metaplasia and presence of uterus. Association of cryptorchidism, SCT, FS and spermatic cord torsion has been published in the literature (Quartuccio et al. 2012, J Vet Sci 13:207–9). However, pathologies described above with pseudohermaphroditism and presence of bilateral paraprostatic abscess have not been reported previously in the dog.

P 252 | Influence of the age of Nellore and Caracu bulls on measures of accessory sex glands

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The aim was to define if the ultrasound measurements of the accessory sex glands differ between Nelore (*Bos taurus indicus*) and Caracu (*Bos taurus taurus*) bulls and their age classes: (1) ≤ 18 months and (2) > 18 months. 282 bulls were used, Nelore (class 1, n = 137; class 2, n = 66) and Caracu (class 1, n = 42; class 2, n = 37). B-mode ultrasound examinations with 7.5 MHz transrectal transducer was used to obtain the mean of three vertical dimensions of the vesicular glands (VG), disseminated prostate (DP) and ampullae of the vas deferens (AD), and dimensions skull-caudal and ventral-dorsum of the body of the prostate (BP) and bulbourethral glands (BG). For the paired organs the mean was calculated. The data were analyzed by PROC MIXED of SAS ($p < 0.05$). Age class and breed were considered fixed effects and their interaction was included. The biometrics of the VG (1.48 ± 0.02 vs. 1.15 ± 0.01 cm), DP (1.17 ± 0.02 vs. 1.08 ± 0.01 cm) and skull-caudal measurement of the BG (1.67 ± 0.03 vs. 1.48 ± 0.02 cm) were higher in Caracu bulls. Effect of age class for observed for VG (1.19 ± 0.02 vs. 1.45 ± 0.02 cm), BP ventral dorsum (0.75 ± 0.01 vs. 0.91 ± 0.01 cm), BP skull-caudal (0.81 ± 0.02 vs. 0.72 ± 0.02 cm) and DP (1.08 ± 0.02 vs. 1.16 ± 0.02 cm), for class 1 and 2, respectively. Interaction between breeds and age classes was detected for AD (0.40 ± 0.01^{c}, 0.59 ± 0.01^{b} cm and 0.66 ± 0.01^{a}, 0.70 ± 0.01^{a} cm) and ventral-dorsum of the BG (1.13 ± 0.01^{b}, 1.12 ± 0.02^{b} cm and 1.44 ± 0.03^{a}; 1.19 ± 0.03^{b} cm), for Nelore class 1 and 2 and Caracu class 1 and 2, respectively. In conclusion, the biometric characteristics of the accessory sex glands are influenced by the genetic group and the animal age class.

P 253 | Preliminary results of quantitative ARFI elastography of the uteroplacental structure during the last gestational week of bitches

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ARFI (acoustic radiation force impulse) is a safe and noninvasive technique that provides quantitative measurements of tissues. The aim of the study was the evaluation of the uteroplacental structure in the last gestational week of bitches by means of the quantitative ARFI, predicting the most reliable time for delivery and establishing normality standards for these evaluations. Three healthy adult brachycephalic bitches (6 placental units) weighing 10–15 kg, were submitted to elastography, using the ultrasound equipment ACUSON S2000/SIEMENS and 9.0 MHz transducer. The evaluation of the uteroplacental structure was performed every 12 h, from day 56 of gestation until parturition, a minimum of five samples of the imaging technique were obtained in each portion evaluated (dorsal, ventral and lateral) to obtain the mean of the shear wave rate (SWV). The experimental design was based on a comparison of the SWV placental between the hours before parturition (HBP): −120 to −24 h by ANOVA test and Tukey test and correlated this variable with the Pearson test ($p < 0.05$). The SWV mean was higher in the dorsal region (CI = 2.55 ± 0.36 m/s) ($p < 0.01$) than in the ventral (CI = 1.57 ± 0.21 m/s) and lateral (CI = 1.68 ± 0.09 m/s) regions. There was no statistical difference among the SWV of the different regions in HBP ($p > 0.05$), although the SWV of the dorsal region correlated positively with the moment of delivery ($r = 0.640$; $p = 0.014$). In conclusion, these results are important and should be confirmed in a larger population set to add information concerning the evaluation of fetal maturity.

P 254 | Evaluation of entire or divided progestogen impregnated sponges upon estrus and ovarian response in Dorper ewes

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The possible effect of the use of entire (WS) or divided (HS) intravaginal sponges impregnated with medroxyprogesterone (MAP) or fluorogestone acetate (FGA) upon estrus and ovarian response in Dorper sheep, was evaluated. The trial was performed during April in Northern Mexico (26°N). Dorper anovulatory ewes (n = 40) with an average body weight (BW) of 54.3 ± 2.2 and body condition score (BCS) of 3.5 ± 0.2, were randomly divided into 4 groups (n = 10 each), homogeneous regarding BW and BCS, and randomly assigned to the following treatments: (1) Entire sponge + MAP (65 mg); (2) Half sponge + MAP (32.5 mg); (3) Entire sponge + FGA (40 mg) and (4) Half sponge + FGA (20 mg). At the moment of sponge removal (day 6), all ewes received an i.m. dose of 300 UI of eCG to stimulate follicular growth. Estrus activity was registered twice daily (08.00 and 17.00 h) for the following 5 days after eCG administration. Ovulations were determined by trans-rectal ultrasonographic scanning (ALOKA SDD-500) 10 days post-eCG administration. Data were analyzed by a chi-square test, (SYSTAT 10, Evenston, ILL, USA). Estrus and ovarian activity were similar ($p > 0.05$) for both progestogens (MAP 100% and 82% vs. FGA 90% and 81%, respectively). The same was true regarding the use of entire and half sponges: (WS 95% and 81% vs. HS 83% and 81% for estrus and ovarian activity, respectively). Results of this study could not demonstrate a difference between both progestogens and no difference was detected in inducing estrus and ovarian response of adult anovulatory Dorper sheep. Such results should be interesting from an economic and pharmacologic view point.

P 255 | Optimal timing for canine artificial insemination with frozen semen: a retrospective study**

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Although artificial insemination (AI) has been used for nearly 40 years in dogs, it remains unclear what is the optimal time to inseminate in order to improve fertility and prolificity. Data of all bitches inseminated with frozen semen in our clinical department between January 2007 and December 2015 were analyzed. Information about the number and date of AIs, the estimation of ovulation date (progesterone plasma concentration, Elecsys®, Roche diagnostics, Germany and ovarian ultrasonography), the quality of semen after thawing, the pregnancy diagnosis (around 25 days post-ovulation) and the litters born were analyzed. AIs were performed by trans-cervical endoscopy. Pregnancy rate was 61.7% (92/149) and prolificity 4.6 ± 3.0 puppies per litter. The average time between ovulation and the 1st AI was of 2.9 ± 0.6 days post-ovulation. Due to incorrect implementation of the database, the number of AIs per bitch was precisely known for 138/149 bitches. 37% (51/138) were inseminated once (average progesteronemia 29 ± 8.3 ng/ml) and 62.3% (86/138) had a second AI in an average of 3.5 ± 0.6 days post-ovulation. 1/138 bitch had 3 AIs. The decision not to perform the 1st AI before 3 days post-ovulation was based on the study by Reynaud et al. (2005, Reproduction 130:193–201) which showed, that, in vivo, canine oocytes resume their meiosis only around 90 h post-ovulation. Our results are in accordance with one of the most recent studies on a large scale

(pregnancy rate 68%) (Mason 2017, Reprod Dom Anim 52, Suppl 2:275–280), although in our protocol the timing for AIs was completely different. In conclusion, AIs with frozen semen should be performed after 3 days post-ovulation.

P 256 | Pregnant rate using artificial insemination after three control of sexual cycle treatments in Majorera goats

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Eighty two Majorera goats were used in a farm on the island of Gran Canaria (28° North latitude and 15°35′ West longitude), Spain. The objective of the study was to determine the pregnancy rate of three cycle control treatments in autumn. In the first treatment, an intravaginal sponge impregnated with Flugestone acetate (Chronogest 20 mg) was left for 11 days, and a dose of Equine Serum Gonadotropin (250 UI) (Foligon 5000 UI) as well as a dose of D-cloprostenol (1 ml) (Luteosyl 0.075 mg/ml) was applied intramuscularly at the time of extraction (n = 27). The second treatment consisted of the application of two doses of D-cloprostenol (1 ml) (Luteosyl 0.075 mg/ml) at an 11 day interval (n = 35). The third treatment was the same as the second with an administration of Gonadorelin intramuscularly (2 ml) (Gonasyl 50 μg/ml) at the time of insemination (n = 20). 48 h after the end of the treatment without heat detection, the goats were inseminated with fresh semen diluted with skimmed milk obtained from males from the same farm. A minimum dose of 400×10^6 sperm was used. Pregnancy diagnosis was made 35 days after insemination by ultrasound examination. The pregnancy rate was 40.74% in the first group (11/27), 31.43% in the second (11/35) and 15% in the third one (3/20). Significant differences were observed ($p < 0.025$) among treatments. Comparing the first and the second treatment, significant differences were also observed ($p < 0.001$). Therefore, the preferred cycle control treatment using artificial insemination in goats of a commercial herd (in the Canary Islands) in autumn is the first one.

P 257 | Cystic endometrial hyperplasia (CEH) of the uterine mucosa in a pregnant queen – a case report**

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CEH is characterized by endometrial hyperplasia with cyst formation (Sapierzyński 2009, Veterinarni Medicina, 52:345–350). Cysts may occur in nulliparous Queens and in older queens without relationship to the number of parturitions. Infertility is the most common clinical sign of CEH. A 5-year-old Maine coon breeding cat was unsuccessfully covered. In the ultrasound examination, thickening of the uterine wall, lack of fluid in the lumen of the uterus and lack of characteristic signs of pregnancy were observed. At the age of 1.5 she had had a healthy litter of kittens. Due to the strong plasmocytic Gingivitis in the next year, she was treated with steroids and her cycle was blocked with medroxyprogesterone once a week. After drug discontinuation, she was no more attractive to males. A decision of castration was made. During surgery, one thickening in each corner of the uterus was seen, resembling to pregnancy ampullae. Diameter of the left horn ampulla was 22 mm and of the right horn 32 mm. After the intersection, a cystic endometrium without a liquid content in the middle was observed. In histopathological examination cystic hyperplasia of the endometrium, with focal, light inflammatory infiltration with mononuclear cells were observed. In the endometrium, trophoblast fragments were locally present, as well as haemorrhages and edema. In conclusion, cats with CEH not always show clinical signs, but the disease can be associated with failure of implantation and subsequent smaller litters as well as infertility or early embryonic death (Agudelo CF 2005, Vet Quart 27:173). To assess the possibility of developing and maintaining pregnancy in cats with CEH more tests are needed

P 258 | AGP concentrations at 7 days post partum as a predictor for uterine disease in dairy cows**

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Dairy cows who fail to resolve uterine inflammation by 21 days post-partum (DPP) may present with purulent vaginal discharge (PVD) or subclinical/cytological (CYTO) symptoms. Although PVD and CYTO are both considered to be independent manifestations of uterine disease, they are both known to delay the onset of ovulation in the post-partum period in the dairy cow. Identification of these cows before diagnosis would facilitate early therapeutic intervention, improving reproductive outcomes. In this study, we propose that the concentration of the acute phase protein alpha-1-acid glycoprotein (AGP) at 7 DPP may identify cows at risk of developing uterine disease. In Study 1, 60 mixed-parity Holstein-Friesian (HF) cows were diagnosed at 21 DPP by vaginal mucus score (VMS) and percentage of uterine polymorphonuclear (PMN) cells and grouped as healthy (VMS = 0, <18% PMN, n = 19), PVD (VMS ≥2 and <18% PMN, n = 14), CE (VMS ≥2, ≥18% PMN, n = 19) or cytological endometritis (CYTO: VMS ≤1, ≥18% PMN, n = 19). Plasma was obtained at 7 DPP. In Study 2, 84 mixed-parity HF

cows were diagnosed by VMS on 21 DPP; healthy (VMS = 0–1, n = 54) or PVD (VMS = 2–3, n = 30). Serum samples were obtained at 7 DPP. In SAS 9.4 group differences were determined by PROC MIXED with a Bonferroni adjustment. In Study 1, AGP concentrations were higher in CE (1.05 ± 0.16 mg/ml) vs. healthy cows (0.52 ± 0.16 mg/ml; p = 0.03) at 7 DPP. The healthy, CYTO and PVD groups had similar AGP concentrations between each other (p > 0.05). In Study 2, greater concentrations of AGP at 7 DPP were detected in the PVD (1.35 ± 0.1 mg/ml) than in the control group (1.05 ± 0.1 mg/ml; p = 0.03). Results indicate AGP has potential as an early prognostic biomarker for CE and PVD. Future work will assess AGP's specificity for uterine disease.

P 259 | Implementation of new mineral additives for stud bulls

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The new mineral additive "Aleksanat-Zoo" contains mineral elements (Ca, Mg, Fe, Zn, I, Si, Cu) in micellar form, which significantly improves their assimilability. The aim of our work was to study the indices of homeostasis and indicators of sperm production of Holstein stud bulls. Studies were carried out with two groups of bulls of 6 heads each. The animals of the experimental group received 300 ml of supplement daily for 90 days. We recorded an increase in experimental bulls' serum of Ca content from 1.78 ± 0.08 mM to 2.15 ± 0.14 mM (by 20.78%) and a decrease in magnesium level to reference values from 1.46 ± 0.07 to 0.98 ± 0.03 mM (by 32.87%). The hormonal status of animals had changed: the level of free triiodothyronine increased (from 13.47 ± 2.31 pM to 18.34 ± 3.14 pM) and free thyroxine (from 19.35 ± 0.58 mM 20, 53 ± 0.92 mM) by 36.15% and 6.10%, respectively. The sum of these hormones in the serum of bulls receiving the supplement increased by 18.3% (32.87 ± 4.32 vs. 38.84 ± 4.95 pM). Testosterone level increased from 13.98 ± 2.89 nM to 30.49 ± 7.50 nM (more than 2 times) and the estradiol level from 0.43 ± 0.06 nM to 0.51 ± 0.06 nM (by 18.6%). Estradiol-testosterone ratio decreased from 0.03 ± 0.06 nM to 0.01 ± 0.02 nM. The volume of bulls' ejaculate from the experimental group increased from 4.06 ± 0.20 to 5.52 ± 0.17 ml (by 35.96%). The concentration of spermatozoa in ejaculate increased from 0.99 ± 0.70 to 1.44 ± 0.52 billion/ml (by 45.4%). We obtained 46.7% more doses of frozen semen from bulls than we received from them prior to the experiment (1050.50 ± 132.04 vs. 1541.01 ± 117.07 pcs). In conclusion, the use of the new mineral additive "Aleksanat-Zoo" has a positive effect on homeostasis and sperm quality of stud bulls. *p < 0.01

P 260 | Effect of temperature and sperm concentration in Tris-glucose-BSA liquid storage on the sperm motility rate in Murciano-Granadina male goats

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Five Murciano-Granadina male goats were used. Pools of ejaculates of different males were used. Seminal plasma was removed (washing) or not by centrifugation. Sperm samples were diluted at different concentrations (250×10^6 or 50×10^6 sperms/ml) and stored in Tris-citric-glucose-BSA medium at different temperatures (5°C or 17°C). Sperm were evaluated at 0 and 48 h after collection. Total and progressive motility rate were evaluated by CASA-mot system. Parameters were analyzed by a GLM model included washing, sperm concentration, temperature and time of storage and double interactions. After 48 h in Tris medium, total motility dropped significantly as it was expected (55 vs. 38% for 0 and 48 h, respectively); however, progressive motility was similar. Washing procedure improved total motility, but not progressive motility. Regarding sperm concentration, a higher sperm concentration in semen storage showed greater total sperm motility than a lower sperm concentration (49 vs. 43% for 250 or 50×10^6 sperm/ml, respectively; p < 0.05). Spermatozoa showed a higher sperm motility, both total and progressive, at 17°C in comparison with 5°C (32 vs. 60% for 5°C and 17°C, respectively; p < 0.05). No interaction between temperature and sperm concentration was found. Tris medium is a medium with a less variable composition than milk- or egg yolk-based extenders, however, it does not have lipoproteins helping to avoid cold shock. For this reason, sperm motility at 17°C was possibly less impaired by a cold shock because, in general, milk extenders present higher sperm motilities at 5°C after 48 h. (This work was supported by INIA (RTA2013-00107-C03-03).)

P 261 | The expression of potentially regulatory proteins in porcine cervix regeneration process after MSC transplantation

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Mesenchymal stem cells (MSC) are plastic, adherent, colony forming cell populations that survive after transplantation into porcine

uterine cervix. They have shown high proliferative potential and probably act as stimulator of the endogenous cells. The aim was to establish the paracrine signaling pathway through the expression of basic Fibroblast Growth Factor (bFGF), Hepatocyte Growth Factor (HGF) and Insulin-like Growth Factor (IGF1). Polish Landrace sows (n = 9) underwent 3 surgeries to enable bone marrow collection, MSC transplant (3 weeks later) and cervix collection (4 weeks later). MSC were isolated from red bone marrow, cultivated with PKH26 and DID markers and transplanted into the muscle layer of the cervix. After hysterectomy, 10-μm thick slices were labeled with primary (anti-bFGF, anti-HGF, anti-IGF1) and secondary antibodies, and then quantified using scanning cytometer. Both cells populations (MSC and endogenous cells) showed bFGF, HGF and IGF1 expression in cytoplasm. The bFGF expression was higher (p = 0.03) in cells close to MSC (8.1% ± 3.58 : 6.9% ± 0.94) than in MSC (22.4% ± 4.88 : 21.1% ± 3.24) and control (7.8% ± 2.20 : 7.8% ± 2.20) both in PKH26:DID marked MSC with no differences (p < 0.0001) between PKH26:DID. The HGF expression was higher (p < 0.0001) in MSC (3.1% ± 0.40 : 2.4% ± 0.56) and close to MSC (3.5% ± 0.21 : 2.7% ± 0.37) in comparison to control (0.4% ± 0.09 : 0.4% ± 0.09) with no differences (p < 0.0001) between MSC markers. No differences in IGF1 expression (p > 0.05) was found between groups. bFGF and HGF may be potential regulatory proteins in tissue regeneration process, bFGF in endogenous and HGF in xenogenous paracrine signaling pathway.

P 262 | Stable nitrogen oxide metabolites and S-nitrosothiols in blood plasma of cows with reproductive organs pathology

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The concentration of stable nitrogen oxide metabolites (NO•) and S-nitrosothiols (RSNO) in blood plasma of cows (n = 58) was studied during physiological and pathological course of reproductive processes. In case of late pregnancy toxemia (n = 9), the amount of NO• exceeded the levels of healthy animals (n = 9) by 38.1% (83.0 ± 7.87 vs. 60.1 ± 8.02 μM/l). In case of a physiological course of the postpartum period (n = 9), the level of NO in the blood plasma was 41.9 ± 1.08 μM/l. In case of delayed uterine involution (n = 4), not accompanied by signs of inflammation, it was 117.8 ± 4.26 μM/l. In case of postpartum metritis it was (n = 9) 138.7 ± 7.14 μM/l. The animals suffering from metritis had 12.4% lower RSNO content than healthy ones (2709 ± 42.5 vs. 3046 ± 139.2 M/ml). High NO• production exhibit a muscle relaxant effect, depress the contractile activity of the uterus and cause a dysfunction in the physiological course of the postpartum involutionary processes. On the contrary, a low level of plasma concentration as stable NO• metabolites is typical for cows with ovarian dysfunction (n = 9), manifested by acyclism (20.6 ± 2.21 vs. 52.9 ± 2.46 μM/l among cycling animals (n = 9)) and RSNO (2258.0 ± 34.3 vs. 3215.0 ± 101.0 M/ml). Since nitrogen oxide is involved in the control of the secretion of gonadotropin-releasing hormone and luteinizing hormone, it can be argued that the follicular-steroidogenesis disorder in cows with ovarian disfunction is associated with abnormal NO synthesis. The strategy for pharmacological use, affecting NO production, may be a promising approach for animals with obstetric and gynecological pathologies.

P 263 | Effects of epidural versus intramuscular administration of GnRH analogue on the Ovsynch-56 protocol

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The aim of this study was to investigate the effects of epidural vs. intramuscular administration of GnRH analogue on the Ovsynch-56 (GnRH-7d- $PGF^{2\alpha}$-56h-GnRH-16h-timed AI) protocol. In the study, we included 135 Holstein cows dichotomized as control (i.m.; n = 74) and study (epidural; n = 61) with similar lactation number (1.49 ± 0.08 vs. 1.67 ± 0.11; p = 0.163), parity (1.41 ± 0.06 vs. 1.46 ± 0.06; p = 0.535), age (33.93 ± 1.14 vs. 36.71 ± 1.55; p = 0.114) and DIM (62.69 ± 0.25 vs. 62.74 ± 0.17; p = 0.877). During the synchronization protocol, control and study groups were treated with 10 μg buserelin acetate (Receptal, MSD) via i.m. and epidural (sacrococcygeal intervertebral (S5-Co1) space) route, respectively. Ultrasonographic examinations were performed to determine dominant follicle (DF) diameter and pregnancy status on d 0 (the day of GnRH1), d 1 (24 h after GnRH1), d 2 (48 h after GnRH1), d 7 (the day of $PGF_{2\alpha}$), d 9 (the day of GnRH2) and day of timed AI, and at d 30 and 60 after timed AI. DF diameters were higher in the study group than in the control on d 1 (1.44 ± 0.03 vs. 1.29 ± 0.03; p < 0.001), d 2 (1.33 ± 0.04 vs. 1.21 ± 0.03; p = 0.03), d 7 (1.24 ± 0.02 vs. 1.14 ± 0.02; p < 0.001), d 9 (1.34 ± 0.03 vs. 1.25 ± 0.02; p = 0.008) and day of timed AI (1.41 ± 0.02 vs. 1.35 ± 0.02; p = 0.044). Also, pregnancy rates were numerically higher in the study group than in the control group on d 30 (44.26% vs. 36.49%) and 60 (32.79% vs. 28.38%), but no significant difference could be detected (p > 0.05). In conclusion, administration of GnRH treatment of the Ovsynch protocol via epidural route – properly done – tended to be better compared to intramuscular application. The usage of lipophilic GnRH analogue could be favorable and is being investigated.

P 264 | Extended equilibration time affects motility and some kinematic parameters in thawed semen in Holstein and Asturiana de los Valles bull breeds

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Semen cryopreservation is routinely performed in domestic cattle (Bos taurus) with great efficiency. However, extended semen must be stored for a longer time due to working procedures or when farms are at a distance from the centres where semen is cryopreserved. Whereas some studies have determined that extended equilibration times could be even positive, reports are controversial, and breed could affect results. We cryopreserved bull semen from the Holstein and Asturiana de los Valles breeds (4 males each), using a 4-h and a 24-h equilibration period at 5°C (BioXCell, IMV; 2 ejaculates/male). Thawed doses were evaluated by CASA, and linear mixed-effect models to analyze data (mean±SEM). No interactions breed×equilibration were found. Total and progressive motility were higher for Holstein than for Asturiana (72.3 ± 2.0 vs. 54.2 ± 5.7%, p = 0.030 and 44.9 ± 2.9 vs. 31.9 ± 4.4%, p = 0.049). The 24-h extended equilibration yielded similar values for total motility (62.6 ± 2.8% overall), but they were lower for progressive motility (41.2% ± 1.1 vs. 34.8% ± 1.1) and for kinematic variables VAP (128.0 ± 1.9 vs. 115.1 ± 1.9 μm/s), VSL (107.6 ± 2.2 vs. 88.9 ± 2.2 μm/s), LIN (53.6 ± 0.8 vs. 48.6 ± 0.8%), STR (87.6 ± 0.5 vs. 83.0 ± 0.5%), BCF (20.8 ± 0.4 vs. 18.0 ± 0.4 Hz) and WOB (62.8 ± 0.5 vs. 60.7 ± 0.5), with p < 0.001 except for WOB with p = 0.002. The 24-h equilibration time could be advantageous in the practice, but the decrease in some motility and kinematic parameters, especially progressive motility, must be considered. Field fertility should be checked to determine the pros/cons balance. (Acknowledgements: We thank the breeder associations ASCOL and ASEAVA.)

P 265 | Both close and far phylogenetic distance between epigenomically modulated nuclear donor cell and host ooplasm is not an obstacle for generation of inter-species nuclear-transferred (NT) blastocysts

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The current research was aimed to examine whether intra-family and inter-genus (caprine-bovine; C/B) or inter-family and inter-genus (porcine-bovine; P/B) NT embryos can be developmentally competent to progress to blastocyst (B) stage under in vitro culture conditions. To obtain inter-species (C/B or P/B) cloned embryos, ex vivo-matured bovine oocytes underwent enucleation, followed by subzonal microinjection and subsequent electrofusion either with adult goat peripheral blood-derived fibroblast-like cells (AGPB-FLCs) that had been epigenomically modulated by 24-h treatment with 3 mM sodium valproate (SV; Group I/GI) or with adult pig bone marrow-derived mesenchymal stem cells (APBM-MSCs) that had been treated with 50 nM trichostatin A (TSA) for 24 h (Group II/GII). Among 153 cultured C/B NT embryos allotted to GI, 121 (79.1%)A were able to divide. The frequencies of embryos that reached morula (M) and B stages were 64/153 (41.8%) A and 36/153 (23.5%)A, respectively. In GII, out of 138 P/B NT embryos, 90 (65.2%)B exhibited cleavage activity, but 39 (28.3%) B and 14 (10.1%)B developed to M and B stages, respectively (A,B p < 0.001; Chi-square test). In conclusion, not only intra-family and inter-genus (C/B) NT embryos, but also inter-family and inter-genus (P/B) NT embryos displayed capacity to complete their extracorporeal development to B stage. Furthermore, due to presumptive improvement of donor cell nuclear reprogrammability, the attempts of either SV-dependent epigenomic modulation of AGPB-FLCs or TSA-dependent epigenomic modulation of APBM-MSCs brought about both retaining relatively high M formation rates by C/B or P/B NT embryos and acquiring developmental capabilities to reach B stage. (This work was supported by grant number BIOSTRATEG2/297267/14/NCBR/2016.)

P 266 | The effect of maternal hyperthermia on oocyte quality in sheep**

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Maternal hyperthermia is known to compromise reproductive performance through the impairment of oocyte developmental competence, decreasing in vitro blastocyst rate and quality during the warm season in sheep (Mara et al. 2014, Zygote 22:366–71). In that aspect, most of the studies regarding the oocyte are focused on the process of maturation onwards. In an attempt to determine the extent to which an elevated temperature commonly seen during summer has on the quality of oocytes prior to maturation, we isolated 132 cumulus-denuded oocytes and assessed the effect of heat stress by means of altered gene expression. Genes related to oxidative stress, apoptosis, mitochondrial DNA (mtDNA), growth factors and oocyte maturation were compared in oocytes collected during the warm (WS; August-October) and cold seasons (CS; February-March). The expression levels of antioxidant- (MnSOD and GPX1), oxidative stress-activated (NRF1, SHC1 and TP53) and pro-apoptotic genes (AKR1B1 and ITM2B) were upregulated (p < 0.05) in WS group. Likewise, anti-apoptotic- (BCL2), mtDNA transcription factor- (POLG2), growth factor- and

oocyte maturation-related genes (IGF2R and FDF8) were also significantly higher ($p < 0.05$) in the same experimental group. This indicates that a) an elevated environmental temperature directly alters the quality of sheep oocytes associated with the presence of oxidative stress, and b) heat stress could affect the process of maturation and early embryo development to a greater extent. (This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2017-89017-R).)

P 267 | Determination of the correlation between α, β and γ – globulin in dogs with pyometra

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The aim of the study was to determine the correlation between an α, β and γ – globulin values in the dogs' blood samples suffering from pyometra (n1 = 16) with blood samples from healthy females (n2 = 15). Pyometra was diagnosed on the basis of clinical signs: polydipsia, anorexia, vomiting and general depression. Purulent discharge from the vagina was observed. At palpation, the enlarged ligaments of the uterus were noticed, which was confirmed by ultrasound examination. Dogs were of different breeds: poodle, French bulldog, boxer, kurzhaar, pomeranian, sharpei, German shepherd and mestizo (from 2.5 to 11 years old). Blood samples were examined on a biochemical analyzer "CLIMAMC-15". The average values of α-globulin were 19.24 ± 1.1% in dogs with a pyometra and 19.03 ± 0.9% in healthy dogs, of β-globulins 12.48 ± 0.9% in pyometra dogs (12.12 ± 3.7% in healthy ones). Significant differences ($p \leq 0.05$) of γ-globulin values were found in pyometra dogs in comparison to healthy dogs: 39.78 ± 0.8% vs. 20.96 ± 0.5%. Correlation analysis showed a high positive correlation between α- and β-globulins (+0.68) in females with pyometra. Correlation bonds between α- and γ-globulins, and between β- and γ-globulins were weak. In healthy bitches, the correlation between α- and β-globulins was negative and of medium strength (−0.52). All of the sick females underwent ovariohysterectomy. On day 15 after surgery, correlation between α- and β-globulins approximated the value of healthy dogs (−0.45). Acute inflammatory process with pyometra leads to disruption of homeostasis, which is associated with a quantitative change in the parameters of α, β, and especially γ – globulins.

P 268 | Interferon-tau and progesterone in the blood of cows in the period of early embryogenesis

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In the cow, Progesterone (P4) and trophoblastic interferon-tau (IFNT) are key molecules for embryo formation, development and survival during early pregnancy. In an experiment on 18 cows, P4 and IFNT dynamics at the physiological embryo formation and its death were studied. ELISA method using Bovine Interferon-Tau ELISA Kit and ImmunoPa-PG test systems were used on days 7, 14, 21 and 35 after insemination. The sensitivity of the IFNT assay is less than 2.9 pg/ml and of the PG it is 0.4 nM/l. The presence/absence of the embryo in the uterus was detected by P4 concentration on days 21 and 35 as well as by ultrasound on days 35 and 50. When the embryo was physiologically formed (n = 15), the IFNT concentration increased from 925 ± 28.6 pg/ml by day 14 up to 1140 ± 54.2 pg/ml or by 23.2% ($p < 0.05$), on day 21, decreased to 984 ± 27.5 and by day 35 to 800 ± 33.4 pg/ml or by 30.8%. P4 concentration increased over this period from 11.8 ± 1.09 nM/l to 37.3 ± 1.67 nM/l or 3.2 times ($p < 0.001$). In cows with late embryo death (n = 3), the IFNT content increased by day 14 up to 1052 ± 36.1 pg/ml, which was 7.7% lower than in healthy animals and P4 up to 21.5 ± 1.27 nM/l by day 21, which was 26.5% lower than in healthy ones ($p < 0.01$). Although IFNT production by day 21 increased up to 1297 ± 48.9 pg/ml and exceeded its concentration in healthy animals at this time by 1.32 times ($p < 0.01$), the INFT increase did not maintain the CL function or continuation of pregnancy. By the time of the embryonic death registration (day 35), the level of IFNT decreased to 679 ± 31.4 pg/ml and P4 to 4.0 ± 0.21 nM/l. Pharmacological control of P4 and interferon status of inseminated animals will contribute to the status of pregnancy.

P 269 | Histone acetylation in matured and aged bovine oocytes exposed to progesterone or prolactin during the second phase of IVM

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Processes of histone acetylation/deacetylation are related to mammalian oocyte maturation and aging. The present work was aimed to study effects of progesterone (P4) and prolactin (PRL) during the second phase of IVM on the acetylation state of histones in bovine oocytes reaching the M-II stage, as well as after their aging. Cumulus-enclosed oocytes (CEOs) were cultured for 12 or 24 h in TCM 199 supplemented with 10% fetal calf serum (FCS), 10 μg/ml FSH, and 10 μg/ml LH. After the 12 h-culture, CEOs were transferred to fresh TCM

199 containing 10% FCS and cultured for 12 h in the absence (Control) or presence of P4 (50 ng/ml) or PRL (50 ng/ml). A part of matured CEOs were cultured for further 24 h in the aging medium (TCM 199 containing 10% FCS). Following culture, levels of acetylation of histone H4 at lysine 12 (acH4K12) and histone H3 at lysine 14 (acH3K14) in M-II oocytes were determined by immunostaining with specific antibodies. The fluorescence signal was evaluated using ZEN 2 Pro software (Carl Zeiss) and assigned to one of four grades (intense, moderate, weak, absent). No effects of P4 or PRL on the acetylation state of H4K12 in matured oocytes and H3K14 in aged oocytes were found. As compared to oocytes matured without transfer, P4 increased the rate of M-II oocytes with the intense fluorescence signal of acH3K14 (from 42.7 ± 4.2 to 61.8 ± 0.3%, $p < 0.05$). By contrast, after 24 h-aging, the rate of oocytes with the intense signal of acH4K12 in the PRL-treated group was 1.2–1.3 times lower than in other groups ($p < 0.01$). Thus, during the second phase of IVM, P4 elevates levels of H3K14 acetylation in matured oocytes, whereas PRL can enhance the resistance of these latter to age-related acetylation of H4K12. (Supported by Russian Science Foundation, grant 16-16-10069.)

P 270 | Dynamic changes in the proportion of binucleate cells in the ovine placenta

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The actions of binucleate cells (BNCs) are vital in supporting placental function during ruminant pregnancy to ensure successful fetal development. The BNC proportion in the trophectoderm is reported as 15–20% throughout pregnancy, but factors affecting BNC proportion (%BNC) as the sheep pregnancy proceeds to its 3rd term are unknown. Pregnant sheep uteri were collected at a local abattoir for veterinary teaching, following removal and measurement of the fetuses, two placentomes were fully excised from the middle or the tip of each uterine horn for each separate pregnancy, and processed for subsequent H&E analysis under x400 magnification. The curved crown rump length (CRL) was used to stage the pregnancies between 9–12 (term 2) and 13–18 weeks (term 3). Binuclear and mononuclear trophoblast cell numbers were counted in 5× views per placentome to determine the average %BNC. Data were checked for normality and subsequent linear mixed model analyses determined the effect of fetal gender, stage of gestation and placentome location as predictive factors. Overall, in this study gender did not affect the %BNC in placentomes. A significant interaction between stage of gestation and placentome location revealed that %BNC was lower ($p < 0.01$) in placentomes obtained from the mid uterine horn region during term 2 of gestation relative to the tip of the uterine horn or any region during later gestation. This study has demonstrated dynamic temporal and spatial changes in %BNC in the ovine placenta, from mid/late gestation. This possibly supports the accelerated fetal growth occurring in term 3. We also found previously unreported effects of placentome location within the uterine horn on %BNC, which are significant for further studies of BNC physiology and placental maturation.

P 271 | Endocrine profile of Holstein stud bulls depending on their age

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One of the underresearched scientific issues is endocrine regulation in bulls of high genetic merit and methods of increasing sexual potency, providing long-term use of stud bulls of high genetic potential. The present study investigated the endocrine profile of Holstein bulls depending on their age. The objects of the study were 36 Holstein stud bulls with an average weight of 970 kg. The animals were divided into two age groups. The first group included young bulls aged 17.6 ± 0.6 m and the second group consisted of animals aged 30.5 ± 1.8 m. The testosterone level in bulls' blood fluctuated from 7.03 to 17.56 nM. Average hormone content was 11.05 nM. Such hormonal profiles indicate high potency of bulls. Concentration of triiodothyronine was 5.79 ± 0.54 pM, of free thyroxine -22.29 ± 3.78 pM, and total value of thyroid hormones –29.52 ± 2.97 pM. Free triiodothyronine in bulls' blood serum is 3.8 times less than free thyroxine. However, the level of free fraction of thyroxin and the total amount of hormones is high enough to confirm an intensive metabolism in the animals' body. Differences were observed in the proportion of thyroid hormones (CT3/CT4) and the level of testosterone. Importantly, the level of free triiodothyronine among all stud bulls was low (5.02–6.12 pM) and did not surpass the content of free thyroxine. Positive correlation between the free thyroxine content and total value of free fractions of thyroid hormones ($k = 0.99$), negative correlation between CT3/CT4 and free thyroxine ($k = -0.64$), between CT3/CT4 and total CT3+ CT4 ($k = -0.58$), CT3 and the age of stud bulls ($k = -0.51$) were observed. Thus, our research illuminated aspects of endocrine profiles of highly valued stud bulls and found differences depending on their age.

P 272 | Anesthesia concerns for MRI, functional MRI and CT of the prostatic carcinoma with local metastasis to urinary system of the dog – a case report

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Imaging of the reproductive and urinary tract in dogs relies on ultrasound (US) as the first-line imaging modality; however, MRI including functional MRI with CT has an invaluable role to play. MRI is the technique of choice for imaging tumors when it cannot be satisfactorily delineated by US, but MRI usually takes 30–45 min and therefore generally requires a safe general anesthetic carefully tailored to the patient problem list. A 7 years old, male, French bulldog, ASA III (acute renal failure, neoplastic disease) with worsening clinical status was presented for emergency, advanced diagnostic imaging of reproductive and urinary system before further, immediate surgery with history of oncological treatment including chemotherapy and multiple cytoreductive surgeries of prostate gland and urinary bladder. Main concern of fast, short anesthetic plan to maximize patient management success for future surgery was to maintain normotension, isovolemia and adequate cardiac output sufficient to maintain renal perfusion. The general inhalational anesthesia (Sevoflurane, Fa. Baxter, Poland) with endotracheal intubation following intravenous premedication (midanium 0.1 mg/kg; Midanium 5 mg/ml, Polfa, Poland) and induction (etomidate 1 mg/kg; Etomidate-Lipuro 2 mg/ml, B Braun, Germany) was performed and polyionic balanced crystalloid (Solutio Ringeri Lactate, Fresenius Kabi, Poland) at dosage 20 ml/kg/h was given to both hydration and alkalization to avoid postcontrast (Ultravist 370, Bayer Pharma; Prohance, Bracco Imaging, Germany) dialysis. No general side effects were observed. The procedure allows for optimal safe management of the MRI, functional MRI, CT necessary for surgical planning. The goals on anesthetic management should focus on using a balanced, multimodal approach.

P 273 | Case report: primary unilateral low-grade paratesticular sarcoma in a two years old dog

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A two years old dog was brought to the clinic with complains of testicular enlargement. The tissue was diffusely affected as confirmed by ultrasonographic examination, being the right testicle atrophied and the right epididymis enlarged, with loss of echotexture and presence of several anechogenic areas. Inguinal lymph nodes were ultrasonographically normal, with no evidence of metastasis. Abdominal and thoracic radiographs were also made, with no gross abnormalities detected. The situation required the excision of the referred testicle and epididymis. The tissue was sent to histopathological analysis, presenting an extensive destruction of seminiferous tubules with a certain degree of intertubular connective tissue proliferation. Spermatogenic cells showed evident signs of degeneration, ranging from cellular swelling to apoptosis phenomena. Immunohistochemical study of cell proliferation showed positivity to Actin, Ki67 (5–10%) and Vimentin, and negativity to Desmin, CD34 and AE1/AE3, which indicates a low-grade Sarcoma with muscle differentiation. Scarce bibliography is found on this matter, with several cases reported on human, and none in dog. Although recurrence and metastases of paratesticular sarcoma is common in humans, no evidence of either cases was detected on the follow-up. This case report is therefore important in the area of small animal oncology directly related to the reproductive tissue.

P 274 | Structure of the vaginal *Escherichia coli* population among healthy bitches in estrus

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Pyometra is a common diestrous disease of bitches. *Escherichia coli* is isolated from the uterus of up to 90% of bitches with pyometra, being mainly assigned to phylogenetic group (PG) B2, and characterized by a high number of uropathogenic *E. coli* virulence factor (VF) genes (Mateus et al. 2013, Vet Microbiol 166:590–594). As *E. coli* gain access to the uterus during proestrous and estrous, the main objective of this study was to characterize vaginal *E. coli* population for clonal identity and phylogenetic background in female dog in estrus. Vaginal swabs were collected from 30 bitches in estrus, from which only 11 had positive growth. Up to 10 colonies per sample of suspected *E. coli* were randomly picked, and confirmed by PCR screening for the presence of *E. coli* 16S rRNA. A total of 99 *E. coli* vaginal isolates were obtained and analyzed. Phylogenetic group and clonal relationships among *E. coli* isolates were assessed by PCR and REP-PCR, respectively as described by (Silva et al. 2009, J Dairy Sci 92:6000–6010). Results were analyzed by *Z* test. The 99 isolates were discriminated in 16 *E. coli* clones, which were assigned to PG B2 (69%), B1 (19%), and D (12%). Eight from the 11 dominant clones identified were from PG B2 ($p < 0.05$). The majority (88%) of B2 dominant clones were β-hemolytic ($p < 0.05$). The majority of PG B2 clones (8/9) were associated with pauciclonal samples (with one or two different clones). Besides host susceptibility, the PG structure of the vaginal *E. coli* population may play an important role as a trigger of *E. coli* infection and the development of pyometra. (Funding: UID/CVT/00276/2013)

P 275 | Ultrasonographic evaluation of locoregional lymph nodes in bitches with mammary neoplasms

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The aim of this study was to evaluate B-mode ultrasonography as a diagnostic method for the identification of metastasis in locoregional

lymph nodes of bitches with mammary neoplasms. The echogenicity, echotexture, width, length and the short-axis ratio of 96 axillary and 100 inguinal lymph nodes of bitches with multiple neoplasms of a total of 241 mammary lesions was measured. After ultrasonography, unilateral radical mastectomy and surgical excision of both types of lymph nodes were performed. Tissues removed were referred for histopathology and the lymph nodes classified as free, reactive or metastatic. The qualitative variables were compared between histopathological classification by the Chi-square test, and the quantitative variables by the ANOVA test. The parameters that revealed significant differences ($p < 0.05$) were subsequently submitted to discriminative power analysis through ROC curves, calculating cutoff value, sensitivity, specificity and area under the curve. Only the width of the inguinal lymph node and height of the axillary lymph node were significantly increased in metastatic lymph nodes. Considering that an inguinal lymph node with a width > 1.53 cm was indicative of metastasis with a sensitivity of 84% and specificity of 53%, and lymph nodes with a height > 0.93 cm were indicative of metastasis, with a sensitivity of 67% and specificity of 46%. We conclude that B-mode ultrasonography of the regional lymph nodes is a limited diagnostic tool for the identification of lymph nodes affected by metastasis. However, it can be used additionally to other diagnostic techniques such as cytology of fine needle punctures to allow a better diagnostic accuracy, prognosis and an appropriate treatment.

P 276 | Comparison of three non-penetrating cryoprotectants in the freezing of Black Belly ram sperm

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Egg yolk, a common component in semen extenders, acts at the level of the cell membrane protecting the sperm against cold shock and freezing-thawing process. Because the heterogeneous composition between lots of eggs together with the potential risk of bacterial contamination, the possibility of substitute the fresh egg yolk is highly desirable. Therefore, the aim was to assess the effect of the type of non-penetrating cryoprotectant used in the preservation media on the quality of sperm ram after freezing-thawing. Briefly, fresh ejaculates of four Black Belly rams (three years old) were collected by artificial vagina and immediately mixed in equal quantities. Pooled semen was split into three equal aliquots and re-suspended in a one step in an extender containing different non-penetrating cryoprotectant FEY: 15% (v/v) fresh egg yolk or PEY: 15% (v/v) powdered egg yolk, or SL: 1% (v/v) soy lecithin, supplemented with 5% glycerol in Tris-based media. No differences were found in post-thaw sperm viability determined by eosine-nigrosin staining (mean±SE, n = 12), between non-penetrating cryoprotectants FEY (72.5 ± 3.3%), PEY (69.1 ± 3.1%) and SL (64.8 ± 2.9%). Likewise, no differences were between extenders in the Host test, FEY (69.6 ± 3.0%), PEY (69.1 ± 2.3%) and SL (64.3 ± 2.7%). These preliminary results suggest that powdered egg yolk is effective in the cryopreservation of sheep semen and can replace the fresh egg yolk. However, in the progressive motility, analyzed visually with optical microscopy, were different ($p < 0.05$) between non-penetrating cryoprotectants PEY (75.8 ± 2.5%), FEY (73.7 ± 1.9%) and SL (65.1 ± 3.5%), suggesting that more analysis should be done. (Supported by PRODEP DSA/103.5/16/11031.)

P 277 | Graphene oxide drives capacitation dependent membrane remodeling in mammalian spermatozoa**

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Graphene has been attracting a lot of interest since it's discovery with the promise of a myriad of applications. From drug delivery systems, to scaffolds and biosensors, this material shows very promising perspectives in medicine. However, safety issues are still debated amongst scientists and little evidence has been provided in terms of reproductive security. Previous studies show that graphene oxide interacts with the sperm membrane in a way that mimics capacitation and is capable of increasing fertilization rates. Understanding the mechanisms of this interaction and its effect can give important clues to the process of capacitation, and in particular to the membrane remodeling that occurs during sperm activation. Furthermore, once safety concerns are cleared, graphene oxide can become an important tool in reproductive biotechnologies. We have tested the effect of adding different concentrations (0.5/1/1.5/2.5/5 μg/ml) of graphene oxide to a capacitating media. We have proven that membrane's fluidity capacitation related increase is less pronounced in higher concentrations of graphene. Moreover, chromatographic techniques show a decrease in cholesterol levels in relation to the increase of graphene oxide used in capacitation. Our studies indicate that graphene is depleting cholesterol from the spermatozoa membrane, which in turn causes the changes in membrane fluidity and enhances fertilization potential. The mechanism driving this process still needs further analysis, however it appears as if graphene oxide is acting similarly to other cholesterol depleting molecules like BSA or methyl-β-cyclodextrin.

P 278 | The mRNA expression of PPARs in the bovine blastocysts correlate with embryo quality markers expression

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Prostacyclin I2 (PGI2) is a member of biologically active lipids, essential for blastocyst formation, hatching, implantation and decidualization, acting through nuclear peroxisome proliferator-activated receptors (PPARs). Our previous data show, that bovine embryos are the target of the PGI2 action on all of the stages of early development, mainly by the expression of PPARδ and PPARγ. The aim of this study was to investigate whether PGI2 signaling in in-vitro cultured bovine embryos reflects their quality. The bovine embryos (n = 486) were divided into early- and late-cleaved groups. After 7 days of embryo culture, the mRNA levels of PPARδ, PPARγ and embryo quality markers (OCT4, SOX2, PLAC8, IGF1R, IGF2R) were examined in the bovine blastocysts on different developmental stages (early, early with cavity, expanded and hatched), according to their quality (according to IETS). The transcript levels of PPARs were correlated with mRNA of the quality markers. The mRNA abundances were measured by Real-time PCR method. Statistical analyses were performed using two-way ANOVA and Pearson's correlation tests. The results confirm presence of examined embryo quality markers in blastocysts at different stages of development. The expression of OCT4, SOX2, PLAC8, IGF1R, IGF2R is dependent on the time of the first cleavage. The mRNA expression of quality markers in bovine blastocysts positively correlates with the PPARs. Stronger correlations were documented for early cleaved embryos. The number of correlations were higher for PPARδ than PPARγ. Summarizing, the obtained results suggest that PGI2 signaling may reflect bovine embryos quality and their developmental competence. (Supported by Grants-in-Aid for Scientific Research from the Polish National Science Centre: 2015/17/B/NZ9/01688)

P 279 | Effect of epidermal growth factor during prolonged culture on bovine oocytes matured in vitro

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The goal of the present work was to study the effects of epidermal growth factor (EGF) on the quality and developmental competence of bovine oocytes after their aging in vitro. Bovine cumulus-enclosed oocytes (CEOs) were matured in vitro for 20 h in TCM 199 containing 10% fetal calf serum (FCS), 10 μg/ml FSH, and 10 μg/ml LH. After IVM oocytes were transferred to TCM 199 supplemented with 10% FCS (aging medium) and cultured for an additional 12 or 24 h in the absence (Control) and in the presence of EGF (10 and 50 ng/ml). After prolonged culture for 12 h, oocytes (n = 308) were activated by treatment with ionomycin and 6-dimethylaminopurine. At Days 2 and 7 after activation, the cleavage and blastocyst rates were determined. Oocytes aged for 24 h were used for apoptosis detection (n = 146) and chromosome status was evaluated (n = 181). The data were analyzed by ANOVA. At the end of prolonged culture the rate of apoptotic oocytes in the Control group was 47.4 ± 8.5%. EGF at a concentration of 10 ng/ml reduced this rate to 18.5 ± 2.1% ($p < 0.05$). At the same time, the rate of M-II oocytes with destructive changes to chromosomes (decondensation, adherence, clumping) did not differ between groups, varying from 54.8 to 64.2%. The addition of EGF at a concentration of 10 ng/ml to the aging medium also led to an increase in the yield of blastocysts from 12.9 ± 2.0 (Control) to 23.3 ± 2.4% ($p < 0.05$), but did not affect the cleavage rate and the number of cells in embryos. Thus, EGF was able to maintain the apoptosis resistance and competence for parthenogenetic development of bovine oocytes during their prolonged in vitro culture. (The research was supported by FASO Russia.)

P 280 | Computed tomography (CT) imaging in an atypical case of Canine Ovarian Remnant Syndrome (CORS)

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Canine ovarian remnant syndrome (CORS) is defined by ovarian tissue present in an ovariectomized bitch. Typical clinical signs of CORS include changes which are consistent with proestrus and estrus, less common polyuria and polydipsia, weight loss and alopecia. A 6 year old mixed breed bitch with clinical signs of pain, emaciation and episodes of vomiting was referred to the clinic two years after ovariohysterectomy surgery. The blood test showed leukocytosis. The outcome of the ultrasound examination showed bilateral focal lesions with hyperechoic components found at the region of ovarian pedicles without the presence of ovarian tissue. CT examination was performed subsequently and revealed two bilateral focal masses in the region of ovarian pedicles. The irregular mass on the right side was measuring 34.3 mm – 44.2 mm diameter and was infiltrating into the right kidney, into the mesentery and the pancreas at the cranial pancreaticoduodenal artery. The mass on the left side was 32 mm in diameter and had regular round shape. There was a slight enhancement of the described structures after intravenous iodinated contrast agent injection. The average density of each mass was measured and it was similar for the lesions on the left and right side with values of 57.7

Hounsfield Units (HU) and 58.2 HU respectively. Those features were suggestive of an inflammatory granuloma and less likely of a neoplasm. Both lesions were surgically resected and nephrectomy of the inflamed right kidney as well as partial pancreatectomy were performed. It was confirmed there were inflammatory granulomas in response to retained suture material and residual ovarian tissue that aggravated the patient's condition. Clinical signs resolved after surgery.

P 281 | Inter-genus taxonomic incompatibility between nuclear donor cells and recipient ooplasts brings about differences in development of caprine-bovine cloned (C/B-CL) and bovine cloned (B-CL) embryos

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The current study was undertaken to comparatively assess the extracorporeal developmental outcomes of inter-species C/B-CL embryos (Group I; GI) and intra-species B-CL embryos (Group II; GII). In GI, to create inter-species clonal cytoplasmic hybrids (cybrids), enucleated ex vivo-matured heifer/cow oocytes (ooplasts) were reconstituted with the cell nuclei of adult goat peripheral blood-retrieved fibroblast-like cells (AGPB-FLCs) that had undergone contact inhibition. In GII, to produce intra-species clonal cybrids, metaphase II-stage heifer/cow ooplasts were reconstituted with genomic DNA of contact-inhibited bovine nuclear donor cells. The inter- or intra-species clonal cybrids that had been successfully electrofused and then were subjected to chemical activation were classified for in vitro culture. In GI, from among 176 cultured C/B-CL embryos, 135 (76.7%)a were cleaved. The proportions of embryos that completed their development to morula (M) and blastocyst (B) stages were 61/176 (34.7%)A and 38/176 (21.6%)A, respectively. In GII, out of 184 B-CL embryos, 147 (79.9%)a exhibited ability to divide (a,a $p \geq 0.05$), but 108 (58.7%)B and 65 (35.3%)B reached the M and B stages, respectively (A,B $p < 0.001$; Chi-square test). To summarize, the ex vivo developmental capacities of C/B-CL embryos to progress to the M and B stages diminished remarkably as compared to those indicated for B-CL embryos. This reduction seemed to stem from inter-genus (Capra-Bos) and inter-species (*Capra aegagrus hircus-Bos primigenius taurus*) taxonomic discordance between donor nuclei and host ooplasm, which cannot be compensated even by intra-family (Bovidae) phylogenetic concordance in this model of generating cloned embryos. (This work was supported by grant number BIOSTRATEG2/297267/14/NCBR/2016).

P 282 | Biochemical characteristics of postpartum hepatic functioning in primiparous dairy cows with different levels of ovarian activity

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In dairy cows, suppression of ovarian function can be caused by metabolic disorders during early lactation. In the present study, biochemical indicators of hepatic function over the postpartum period were compared in primiparous animals with different levels of ovarian activity. Blood samples from 47 Russian Black Pied cows were taken 2 weeks before and 1–13 weeks after calving. Animals studied were divided into three groups: (1) normally cycling cows (CY, n = 26), (2) cows with a low ovarian activity (ovaries without corpus luteum and large follicles; LA, n = 11), and (3) cows with inactive ovaries (without corpus luteum and large and medium follicles; IO, n = 10). The diagnosis was confirmed by rectal palpation, ultrasonography, and blood progesterone levels. Serum components were measured using a biochemical analyzer and hormonal levels were determined by ELISA. Bilirubin concentrations and aspartate transaminase activities increased in all groups at the 1^{st} week after calving, gradually decreasing by the 5^{th}–7^{th} week (at least $p < 0.05$). Meanwhile, this increase was 1.3–1.4 times higher in IO cows than in CY cows ($p < 0.01$). Serum concentrations of albumin, total cholesterol (TCH), and phospholipids (PHL) were lower in the IO group than in the CY group over the whole postpartum period ($p < 0.05$–0.001). From the 5^{th} to the 13^{th} week after calving, lower levels of TCH and PHL were found in the serum of LA cows as compared to CY cows. Furthermore, at the 7^{th} week postpartum the serum activity of alanine transaminase in the IO group was 1.4 times less than in CY group ($p < 0.05$). The data indicate that hepatic disfunction during early lactation is related to suppression of the ovarian activity in primiparous dairy cows. (The study was supported by FASO Russia and RFBR (18-016-00227).)

P 283 | Effect of different oxygen levels and medium protein components on morphokinetics of in vitro cultured preimplantation rabbit embryos

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Preimplantation embryo development is the time of metabolic activity that supports essential morphological changes during early cleavages and blastocyst formation. Culture conditions are factors affecting optimal embryo growth. There is also a well-documented correlation between morphokinetic markers and embryo development, but this kind of analysis has not yet been performed in rabbit

embryos. This study reports time-lapse imaging of in vitro cultured preimplantation rabbit embryos, from zygote to hatching. During continuous in vitro culture we studied preimplantation rabbit embryo development kinetics by measuring time at which embryos reached the 2-cell, 4-cell, compaction, morula, cavitation, early blastocyst and hatching blastocyst stages. Times and duration were assessed under different conditions: (1) oxygen levels (5% and 21%) and (2) addition of protein components (BSA – bovine serum albumin, KSR – knock-out serum replacement) to evaluate their influence on embryo development. Embryos cultured in 5% oxygen displayed a shorter time for reaching the compacted morula stage (5%: 71.6(±1.1) hpc; 21%: 76.3(±1.3), errors are SEM, $p < 0.01$) and for achieving the first cleavage (5%: 25.4(±0.5); 21%: 27.5(±0.7), $p < 0.05$, Scheirer-Ray-Hare test) than with 21% oxygen. Time to reach the hatching stage was increased following addition of the KSR protein component (RDH 98.5(±2.6), BSA 99.1(±2.8), KSR 122.0(±4.1), $p < 0.005$). Combining noninvasive assessment of rabbit preimplantation embryo quality with different culture conditions can provide information on embryonic survival under in vitro conditions and may be used as a marker to select embryos before transfer if information obtained in vitro is related to subsequent implantation rate. (Supported by KNOW)

P 284 | Livability of chicken embryos, obtained after insemination by frozen/thawed sperm, depending on storage duration of incubation eggs

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It is well known, that after using frozen-thawed sperm decreases not only the percentage of fertility, but also livability of chicken embryos. Embryo mortality can reach 8.3–16.7%. Probably, the impact of sperm cryopreservation is most influential on the early stages of embryo development. The goal was to investigate livability of the embryos obtained after insemination by individual frozen/thawed ejaculates until 5 days of incubation depending on the duration of hatching eggs storage. The study has been carried out on the base of Genetic Collection of the Rare and Vanishing Chicken Breeds of the RRIFAGB. Rhode Island Red chickens of 50 weeks of age were kept in individual cages. Eggs were stored for 6 days at 15°C and 70% humidity. At candling there were 253 live embryos. Early embryo mortality was detected by egg breaking. The data were analyzed according to each day of egg storage. The duration of pre-incubation egg storage influenced livability of the embryos. In case of egg storage within 1–4 days, the percentage of early stage death (early embryo mortality, blood ring) was 9–15%; if storage was 5–6 days and longer – this percentage increased up to 27–41% (to compare: in case of use of native sperm early embryo mortality is 3.5–5.5%). On the basis of literature data one can assume, that the spermatozoa in frozen-thawed ejaculates can get damaging of their nuclear structures, including DNA. This results in the increasing rate of early embryonic death. Thus, when frozen-thawed rooster sperm is used, the duration of hatching eggs storage should not exceed 4 days. (The study was supported by Federal Agency for Scientific Organizations (project No. AAAA-A18-118021590134-3).)

P 285 | Glucose metabolism and expansion of cumulus cells differs in pubertal and prepubertal cows

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The role of cumulus cells is essential. They are necessary to prepare oocyte to maturation, fertilization and embryo development. Cumulus cells also participate in acquisition the developmental competition and are the source of nutrients for the oocyte. In the literature there is the model of good and poor oocyte quality. Due to it, oocytes obtained from mature cows are considered to be of good quality and oocytes obtained from immature calves are considered to be of poor quality. Cumulus cells were separated after in vitro fertilization of oocytes from pubertal and prepubertal animals. mRNA was isolated and the expression of genes controlling cumulus cells expansion (AREG, EREG, BTC, ADAM10, ADAM17 EGFR, PTX3, TNFAIP6, HAS2, PTGS2), genes responsible for glucose transport and metabolism (GLUT1, GLUT4, GFPT1, GFPT2, PFKP, LDHA) and oocyte quality markers (CTSS, CTSZ, CTSB, CTSK) were measured using Real-Time PCR. Statistical analyses were performed using Student's *t*-test for independent pairs. The expression of oocyte quality markers in cumulus cells obtained from prepubertal, in comparison to pubertal animals was significantly higher but all expression of genes controlling cumulus cells expansion, was lower. There were no significant differences, as in the expression of genes responsible for glucose transport and metabolism in cumulus cells obtained from prepubertal, in comparison to pubertal cows. To conclude, the developmental potential of oocytes obtained from calves is inferior to the potential of oocytes obtained from cows because of the impaired in Expansion of cumulus cells and the interrupted transport of nutrients to oocytes. (Supported by Leading National Research Centre Scientific Consortium 'Healthy Animal – Safe Food' UMO-KNOW2016/IRZiBŻ/PRO1/01/4)

P 286 | Maternal parity and size of the ovarian reserve in dairy cattle offspring

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The number of follicles and oocytes in ovaries of mammals (ovarian reserve) is positively associated with fertility, yet the causes of

its inherently high variation are unknown. We hypothesized that the maternal parity (primiparous vs. pluriparous) during pregnancy in dairy cattle may have a negative impact on the size of the ovarian reserve in their daughters. Twenty-three pubertal heifers (Holstein-Fresian, aged 15 to 18 months) born to primiparous, non-lactating (n = 12) and pluriparous, lactating cows (n = 11) were enrolled in this study. On a random day of the oestrous cycle, the total number of ovarian follicles ≥3 mm in diameter (antral follicle count, AFC) was assessed by ovarian ultrasonography and a single blood sample was collected to measure serum concentrations of Anti-Müllerian hormone (AMH) with a commercial ELISA kit (Ansh Labs, USA) (Mossa et al. 2017 Reproduction 154 (1): R1-R11). Statistical analyses included correlations and ANOVA tests. AMH tended to be positively correlated with AFC (r = 0.38; p = 0.08) and was negatively correlated with age at blood sampling (r = −0.68; p = 0.02). Heifers sampled at 15–16 months of age had higher AMH peripheral concentrations compared to those sampled at 17–18 months, irrespective of maternal parity (born to primiparous 548 ± 282 vs. 271 ± 182, p < 0.01; born to pluriparous cows 526 ± 238 vs. 206 ± 111; p = 0.01). The AFC was not influenced by maternal parity or age at sampling. These results do not provide evidence for a correlation between maternal parity during gestation and the size of the ovarian reserve of their daughters.

P 287 | Effect of gonadotropin (hCG, eCG, or p-FSH) in induction of synchronized estrus on the corpora lutea characteristics and conception rate of dairy goats

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One hundred and eleven Toggenburg goats were subjected to synchronization of estrus with 60 mg of medroxyprogesterone acetate sponge for six days, associated with 30 µg of d-cloprostenol (latero-vulvar) and hCG or FSH or eCG (300, 20 and 200 UI im, respectively), both administrated 24 h before sponge removal. The diameter, volume, and area of the corpora lutea (CLs) were measured by B-mode ultrasonography (M5, Mindray, China) and the vascularization of CLs (number of colored pixels) was evaluated by the Color Doppler mode ultrasonography and then analyzed by the Image J® software. The hCG group was characterized by greater number of CLs (1.9 ± 0.04 vs. 1.6 ± 0.03), but CLs with smaller diameter (8.6 ± 0.21 vs. 10.1 ± 0.19 mm) and volume (6.7 ± 0.17 vs. 8.0 ± 0.15 cm^3), when compared with CLs from eCG group (p < 0.05). The FSH group had 1.35 ± 0.04 ovulations and CLs with similar size to the eCG group. The area and vascularization of CLs (1.19 ± 0.26 cm^2; 4136 ± 420 pixels, respectively) did not differ between the treatments (p > 0.05). The conception rate was significantly higher in the eCG group (72.0%) and FSH group (78.5%) than in the hCG group (53.5%). In conclusion, the number of ovulations and morphology of CLs, with the exception of vascularization, is influenced by the gonadotropin type. The quality of the CLs of goats treated with pFSH and eCG is superior than that after the use of hCG, which corresponded to a better conception rate.

P 288 | Effect of cutting off the velvet antlers on the efficiency of semen collection in reindeer

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Velvet antlers at an early stage of growth are often used for the preparation of medicines. They contain: 17 amino acids, taurine, hormones (testosterone, progesterone, estradiol, cortisol, follicle-stimulating hormone) and insulin. The antlers often are cut off in the beginning of the rut. The aim of the work was to study the effect of cutting off the antlers on the efficiency of semen collection in reindeer. We take into account the efficiency of semen collection, erection, volume of the ejaculate. Seventeen reindeers were caught in the rut 2017 in Taimyr. Males were fixed by rope on the legs and horns. Semen was collected by electroejaculation (Minitube®). The reindeers were divided into 2 groups. First group – 10 males with antlers (G1), second group – 7 males without antlers – cut before rut (G2). Results are depicted as percentages of totals and ranges. In the G1 group, sperm was collected in 90% of the cases (9/10) vs. 57.1% (4/7) in the G2 group. In G1 70% of the males had an erection, but no one had erection in G2. The volume of the ejaculate varied from 0.2 ml to 2 ml in G1, and the volume of the ejaculate varied from 0.1 to 0.5 ml in G2. Thus, cutting off the antlers before rut had a negative effect on the efficiency of sperm collection in reindeer. (Authors acknowledge financial support from The Federal Agency for Scientific Organizations (FASO Russia), project No. AAAA-A18-118021990006-9.)

P 289 | Differences of reproductive traits between domestic and exotic cattle in dairy farms in Ankara

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Fertility is considered to be the most important yield parameter in the livestock industry. The main goal of enterprises is to provide

maximum yield production with minimum inputs from each animal and to obtain a calf in a year. Exotic breeding animals were introduced to improve the herd capacity by selection and adaptation studies using reproductive biotechnological techniques. Domestic animals, however, are suitable for the climatic and environmental conditions. In this study, the first calving age, service period, calving interval and insemination index were evaluated as fertility parameters from domestically produced (n = 2258) and exotic (n = 1481) cattle. Domestic cattle were born in Turkey while exotic were imported. The exotic and domestic cows; Holstein, Mont-Beliarde, Brown Swiss and Simmental were distributed together in the farms. Descriptive statistics for each variable were calculated. Prior to hypothesis testing, data were examined with Shapiro Wilk test for normality and Levene test for homogeneity of variances. Student's *t*-test was used to evaluate the differences in reproductive parameters (exotic vs. domestic). All statistical analyses were calculated using SPSS 14.01 software. The first calving age of exotic and domestic cattle was 28.24 ± 5.57, 29.81 ± 10.66 months; service period was 184.61 ± 137.37, 216.26 ± 190.63 days ($p < 0.001$), respectively; and it was determined that insemination index of exotic cattle was lower with 2.21 ± 1.84, than that of domestic cattle with the value of 2.04 ± 1.45 ($p < 0.05$). The exotic cows in Turkey performed better than the domestic breeds in fertility parameters, suggesting that exotic cows adapted to local climate, care, and feeding conditions. Besides, all reproductive parameters examined were above universal values.

P 290 | Endometritis impairs milk production and fertility in dairy cows

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The aim of the study was to determine the effects of subclinical and clinical endometritis on milk production and fertility. Holstein Friesian cows (n = 201) were examined between 21 and 28 days postpartum from March to November, 2017. Vaginoscopy and uterine cytology were performed to assign cows to four groups: E0- clear mucus, E1- mucus containing <50% of pus, E2- mucus containing >50% of pus, E0SE- clear mucus and subclinical endometritis (>5% of PMNs). The overall number of animals in each group was: E0 – 67 (33.2%), E1 – 45 (22.3%), E2 – 53 (26.4%) and E0SE – 36 (17.9%). Diameter of the cervix and uterine horns was measured using sonography (DRAMIŃSKI 4VET, Poland). Mean daily milk yield per cow in the first 60 days of lactation (DIM60) and days open for each cow were obtained from the VMS milking robot software (ALPRO DELAVAL, Poland). For statistical analysis one-way analysis of variance and Kruskal–Wallis test was performed using IBM SPSS Statistics 24. The average number of PMNs (mean ± SD) in particular groups was E0 (1.6 ± 1.1), E1 (41.1 ± 19.6), E2 (62.5 ± 22.7) and E0SE (22.5 ± 18.2). DIM60 in E0SE (37.9 ± 7.3) and E0 (37.6 ± 6.6) cows was statistically higher ($p < 0.05$) than E1 (33.6 ± 8.3) and E2 (22.5 ± 18.2). Diameter of the cervix, left and right uterine horn (38.1 ± 4.4, 34.5 ± 5.4 and 34.0 ± 7.4 mm) only in E2 group were statistically larger ($p < 0.05$) compared to other groups. Cows from E0 group conceived quicker than the others ($p < 0.05$): E0- 174 days, E1- 214 days, E2- 213 days and E0SE-199 days. In conclusion, subclinical endometritis did not influence milk production but significantly affected fertility, whereas clinical endometritis impaired milk yield.

P 291 | Transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) to collect semen in a black crested mangabey (*Lophocebus aterrimus*) – a case report

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This study examines the effectiveness of transrectal, ultrasound-guided massage of the accessory sex glands (TUMASG) combined with electroejaculation for obtaining samples in a black-crested mangabey (*Lophocebus aterrimus*), an African monkey listed as Near Threatened by the IUCN. On February 7th, this animal was anaesthetised by a projectile dart that delivered 115 mg intramuscular ketamine hydrochloride plus 0.8 mg medetomidine. The penis was manually made to protrude; it was maintained protruded by holding it with the help of gauze just caudal to the glans. The protruded penis was then cleaned with a sperm-washing solution. TUMASG was performed with the ultrasonographic probe placed on the ampulla of the vas deferens. Electrical stimuli (2–5 V lasting 5 s) were provided using an electroejaculator, with intermittent breaks for TUMASG. Ultrasound examination of the prostate, the seminal vesicles and the ampulla of the vas deferens was performed using real-time transrectal ultrasonography employing a 7.5 MHz linear array probe. The size of both the prostate and the seminal vesicles were 0.54 cm^2 and 0.55 cm^2, respectively. Mean testicular diameter was 1.73 cm. Four semen samples were collected (50–750 μl, total: 1350 μl). Sperm morphometry (sperm head – CASA-moph: 16.60 ± 1.58 μm^2) and plasma testosterone concentrations (2.3 ng/ml, i.e. basal levels) were also assessed. This work describes for the first time the accessory sex glands size by ultrasound and sperm morphometry in the black-crested mangabey. The results show that the TUMASG method combined with electroejaculation is a useful way of obtaining sperm samples in a near-threatened species.

P 292 | Metastatic testicular seminoma in a stallion: a case report

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In this report we describe a rare case of a metastatic seminoma in a 20-year old Selle Français stallion. In the andrological exam the ultrasound examination revealed the presence of a testicular mass, compatible with a neoplasia. A left hemi-orchiectomy was performed and the histological analysis identified a diffuse malignant seminoma, with metastatic invasion of the lymphatic ducts. One and an half year following the surgery, the horse began to show anorexia, weight loss, prostration, peaks of fever and edematous swellings in peripheral areas. The ultrasound examination of the right testicle showed lesions compatible with an orchitis. The hemogram presented a mild leukocytosis with a left shift cytotoxic neutrophilia and lymphopenia, and the proteinogram showed an increase of the α2 and β proteins, but no other change of the biochemical parameters. No abnormalities were detected at the abdominal radiographic and thoracic ultrasound examinations. During 6 months the stallion presented periodic episodes similar to the above described, with limited response to antibiotic, anti-inflammatory and diuretic treatments, and a chronic weight loss was noticed. At this time, a mass in the upper left quadrant of the abdomen was detected, which increased over time. Fine-needle aspiration cytology showed the presence of neoplasic cells and euthanasia was proposed. The necropsy revealed a wide spread of the tumor, with metastasis in the remaining testicle, several chains of lymph nodes, both in the pelvis and abdomen, and in several organs. The histological exam confirmed the presence of the seminoma with a pattern of spread by the lymphatic route.

P 293 | The influence of high-hydrostatic pressure (HHP) on fresh boar semen before liquid preservation

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The purpose of the study was to evaluate the effect of HHP treatment (Applied Cell Technology, Hungary) on fresh boar semen before liquid preservation. The semen from 4 boars (n = 16) with ≥80% total motile sperm (TM%) was used in the experiment. After dilution in Biosolwens Plus, semen was split to control (C) and treatment group with 20 (I), 30 (II) and 40 MPa (III) for 90 min at 21°C. After pressurization the semen was stored at 17°C until sperm motility decreased to 30% (Day A). The quality of fresh semen (Day 0) after HHP treatment and extended semen on Day A was verified based on TM% and % of apoptotic sperm with intact membrane (YO-PRO-1+/PI−) (Molecular Probes Inc., USA) using fluorescence microscope. Data were analyzed by Duncan's test ($p < 0.05$). The mean survival time of semen (Day A) was 8.9; 9.5; 9.1 and 9.2 days, respectively for group C, I, II and III ($p > 0.05$). A significant increase in % of YO-PRO-1+/PI− sperm was observed in all analyzed group by comparing Day 0 with Day A (C: 2.7 ± 1.2 vs. 16.3 ± 3.4; I: 2.8 ± 1.5 vs. 14.4 ± 3.2; II: 2.8 ± 1.1 vs. 7.8 ± 1.7; III: 2.8 ± 1.0 vs. 19.6 ± 3.6). When fresh semen was treated with 30 MPa the lowest increase of % of YO-PRO-1+/PI− sperm was observed during storage. Treating fresh semen with 30 MPa does not extend storage time, but reduces the percentage of apoptotic sperm during storage. (The study was supported financially by BIOSTRATEG 2 No. 297267/14/2016.)

P 294 | Dependence of the reproductive performance on the level of inbreeding in Ayrshire first calving cows

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A group of Ayrshire first calving cows of the Leningrad region (n = 2026) was studied to determine the influence of inbreeding on their reproductive performance. Reproductive performances were determined according to the fertility index of J. Doha (FI), which includes the 1st calving age and calving interval. Animals were grouped according to the method of breeding (cross lines – GC and intralinear – GL) and the level of inbreeding (close 5.8 ± 0.3% for GC (GCc) and 5.6 ± 0.5% for GL (GLc), moderate −2.0 ± 0.3% for GC (GCm) and 2.1 ± 0.1% for GL (GLm), distant −0.8 ± 0.0% for GC (GCd) and 0.7 ± 0.0% for GL (GLd). FI in GCm was less than in GCc (1.6 $p \leq 0.001$), in CLm was less than in GLc (4.2 $p \leq 0.001$), in CLd was less than in GLc (2.8 $p \leq 0.001$) and in CLm was less than in GLcd (1.4 $p \leq 0.001$). It was found that the FI in GCc (46.1 ± 0.2) was higher than in GLc (45.6 ± 0.4). FI in GCm (45.9 ± 0.8) was lower than in GCd (46.2 ± 0.2) and in CLm (44.4 ± 0.8) was lower than in GCd (45.8 ± 0.5). This may be a consequence of inbreeding depression. The obtained results indicate a negative effect of increased inbreeding on the reproductive performance of Ayrshire first calving cows. (Authors acknowledge financial support from The Federal Agency for Scientific Organizations (FASO Russia), project No. AAAA-A18-118021590134-3).

P 295 | The effect of administration of rocuronium and sugammadex on progesterone levels in pregnant rabbits under general anesthesia

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The present study aims to determine the effect of administration of rocuronium and sugammadex on progesterone levels in pregnant rabbits under general anesthesia. All the pregnant New Zealand rabbits in the first trimester and second trimester in all groups were sedated with 0.5 mg/kg Midazolam (Zolamid® 5 mg/5 ml, Turkey) IV, 6 mg/kg Propofol (Propofol-Lipuro 10 mg/ml 20 ml, Germany) IV, and Sevoflurane (Sevorane®, UK) and oxygen were administered for a general anesthesia. Rabbits in Group I (GI, n = 7) received 0.6 mg/kg Rocuronium (Esmeron®, 50 mg/5 ml, Germany) IV at 1 min after general anesthesia. Rabbits in Group II (GII, n = 7) were administered IV 2 mg/kg Sugammadex (Bridion®, 200 mg/2 ml, Netherlands) for 60 min unlike the first group. Rabbits in the control group (C, n = 7) underwent only general anesthesia. Blood was collected from all the rabbits at 0, 5, 30, 60 and 90 min. In the first trimester, Progesterone (P4) levels in GI, GII and C were not statistically different among groups at 0 and 5 min. The P4 levels at 30, 60 and 90 min in GI (1.71 ± 0.08 ng/ml, 1.67 ± 0.1 ng/ml, 1.72 ± 0.08 ng/ml) and GII (1.69 ± 0.09 ng/ml, 1.72 ± 0.09 ng/ml, 1.71 ± 0.09 ng/ml) were found to increase significantly compared to Group C (1.41 ± 0.02 ng/ml, 1.40 ± 0.05 ng/ml and 1.42 ± 0.06 ng/ml) ($p < 0.05$). In the second trimester, P4 levels at 0 and 5 min were not significantly different in GI, GII and C. At 30 min, the level of P4 in Group C (5.76 ± 0.38 ng/ml) was found to be lower than GI (6.71 ± 0.32 ng/ml) and GII (6.59 ± 0.29 ng/ml) ($p < 0.05$). As a result, it was determined that administration of rocuronium and sugammadex increase progesterone levels in pregnant rabbits under general anesthesia.

P 296 | Effect of enriching bypass lysine and methionine content of low quality protein diet on fertility in dairy cows

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Two hundred multiparous Holstein dairy cows housed in the same farm were assigned to randomly control (high quality protein diet (HQ); soybean meal, canola meal, corn gluten meal, n = 100) and experiment (low quality protein diet (LQ); sunflower meal, cottonseed meal, n = 100) groups to determine whether increasing bypass lysine (Lysigem, Kemin) and methionine (Smartamine, Adisseo) content of LQ improve the milk yield and fertility parameters. Both diets had the same concentration of lysine (7.2%) and methionine (2.3%). Data were evaluated by independent T-test (MedCalc Statistical Software). Daily average milk yield (LQ, 35.36 ± 1.42 l/head; HQ, 34.72 ± 1.25 l/head) and peak milk yield (LQ, 48.94 ± 1.92 l; HQ, 49.71 ± 1.76 l) did not differ between groups, whereas total lactation milk yield was higher ($p < 0.005$) in HQ (12.566.80 ± 52.71 l) than those detected in LQ (10861.13 ± 35.37). The day (d) of first standing heat (LQ, 40.2 ± 2.4; HQ, 49.6 ± 3.2; $p < 0.001$), number of artificial insemination per pregnancy (LQ, 1.75 ± 0.14; HQ, 2.92 ± 0.57; $p < 0.001$), calving interval (d) (LQ; 415.67 ± 8.29; HQ, 375.72 ± 7.65; $p < 0.001$) and duration of lactation (d) (LQ, 313.25 ± 6.21; HQ, 356.78 ± 5.18; $p < 0.05$) displayed significant differences, as compared to HQ group. The increased total lactation milk yield of HQ cows might be related to an extended lactation period. Moreover, LQ cows dried off much earlier than HQ cows. In conclusion, bypass lysine and methionine can be of better bioavailability than high protein quality feedstuff and this feeding style can be more cost effective. Further studies are needed to understand the mechanism of lysine and methionine bioavailability in dairy cows.

P 297 | Effects of different concentrations of *Mentha piperita L.* extraction on quality of Moghani ram semen following the freeze-thaw process

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Oxidative stress due to production of reactive oxygen species (ROS) during the freezing-thawing process is one of the main causes for the decline in fertility of sperm. The use of antioxidants eliminates free radicals from the sperm diluents. *Mentha piperita L.* has antioxidant properties due to phenolic compounds. The aim of current study was to evaluate the effect of *Mentha piperita* extract as a natural antioxidant on quality of post-thawed ram sperm. In this study, four Moghani ram were used for semen collection twice a week by an artificial vagina and ejaculates with same condition were pooled. Different levels of ethanol extract of *Mentha piperita L.* (0, 2, 4, 8, 12 and 16 ml in dL diluents solution) were added to Tris based diluents. Following cooling and freezing of semen samples, they were stored in liquid nitrogen until evaluation. After freezing-thawing, the sperm motility, viability and plasma membrane integrity parameters were evaluated. The results showed that the addition of 4 and 8 ml/dl extracts resulted in higher ($p < 0.05$) percentages of total motility (53.31 ± 3.54%; and 56.15 ± 3.32%, respectively). The percentage of progressive motility was higher ($p < 0.05$) in the extender containing

4 ml/dl extract (41.28 ± 2.94%) compared to the control group (30.44 ± 2.53%). Plasma membrane integrity of sperms in 4 and 8 ml/dl extract groups was greater than the control group ($p < 0.05$). Addition of 4 and 8 ml/dl extract improved the percentages of viability compared to the control group as well ($p < 0.05$). Also, addition 16 ml/dl extract had a significantly negative effect on all evaluated traits ($p < 0.05$). In conclusion, the addition of *Mentha piperita L.* extract to the extender protected sperm against the harmful effects of ROS and improved post-thaw sperm quality.

P 298 | Effect of the body condition score at the beginning of lactation on productivity and reproductive performance in first-calves cows

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The aim of the study was to determine the effect of body condition score (BCS) in the dry period and in the first month of lactation on the productivity and reproductive performance of dairy cows. A BCS assessment of black-and-white cows (n = 440) from a commercial dairy farm in the Leningrad Region was carried out using a five-point scale with 0.25 incremental steps. Cows were divided into 3 groups based on BCS loss between precalving and 1 month in lactation: G1: loss of 0 till 0.5 points; G2: a BCS loss between 0.5 and 0.75; G3: BCS loss of 0.75 or higher. Phenotypic correlation were measured between BCS in the first month of lactation and the service period and the number of inseminations. The reliability of the difference in the service period and the number of inseminations between groups was calculated. Results indicate that animals in G3 have a higher 305d milk yield (8601 kg) than animals in G1 (8103 kg). First parity animals in G3 have the longest service period (149 days) and highest number of services (2.34). The difference between G1 and G3 was significant ($p < 0.01$). As a result, cows with low BCS in the early stage of lactation may have a prolonged postpartum anestrus. A significantly negative correlation between BCS in the first month of lactation and the service period (-0.288, $p < 0.001$) and the number of inseminations (-0.186, $p < 0.01$) was found. Loss of weight in cows during the first period of lactation is directly related to the increase in milk yield and a decrease of the reproductive function. (Authors acknowledge financial support from The Federal Agency for Scientific Organizations (FASO Russia), project No. AAAA-A18-118021590134-3)

P 299 | Successful multiple ovulation and embryo transfer programs for importing White Suffolk (WS) sheep breed into the European Union (EU)

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Assisted reproductive techniques (ART) improve sheep breeding: with the help of ART we are able to overtake the disadvantages of seasonal sheep reproduction and shorten the genetic intervals. White Suffolk (WS) is one of Australia's most important and productive sheep breed. It is giving outstanding results in the dry, arid, pastoral zones; all types of agricultural areas and also in higher rainfall districts. Arrangements in connection with importing WS embryos started in 2015. The first flock, which is unique in the EU, has been established in Hungary, owned by a private farmer. 116 excellent or good quality WS embryos have been thawed and transferred in two programs (Dec. 2016 and Apr. 2017). Program 1: 70 embryos were transferred and 52% of the recipients became pregnant. The transferred embryo/lambs born ratio was 47%. Program 2: 46 embryos were transferred, 60% of the recipients became pregnant, and the transferred embryo/lambs born ratio was 51%. The project was performed by the Interinstitutional Small Ruminant Biotechnology Research Group. Naturalization of the breed has already been completed (pedigrees of the lambs has been accepted by the Hungarian Sheep and Goat Breeders' Association), production data collection (lambs' weight, average daily weight gain, eye-muscle depth, and fat depth) is in progress.

P 300 | Effect of Leukemia Inhibitory Factor on bovine oocyte vitrification

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Leukemia Inhibitory Factor (LIF) is a 20 kDa glycoprotein and a member of the interleukin-6 family of cytokines. LIF is expressed in the ovary; its concentration increases prior to ovulation and coordinates follicular growth, promotes oocyte maturation and developmental competence. In the uterus, LIF is required for a successful blastocyst implantation and pregnancy. In the present study the effect of LIF on bovine oocyte vitrification/warming and early embryo development in vitro was examined. Oocytes were distributed in: Control (oocytes in vitro matured), LIF (matured with 25 ng/ml LIF), Vit (vitrified/warmed at 20 h of IVM) and LV (LIF and vitrified/warmed). Analysis of the collected data was performed through a logistic model followed

by a Tukey test ($p < 0.05$). At 24 h of IVM, oocytes were in vitro fertilized and in vitro cultured up to day 8 post-insemination (pi). Cleavage rate (CR) and blastocyst yield (BY) were higher for non-vitrified (Control: CR: 87.00 ± 1.48; BY: 35.91 ± 1.26; LIF: CR: 80.47 ± 1.64; BY: 29.74 ± 0.96) when compared to vitrified groups (Vit: CR: 72.50 ± 1.49; BY: 16.50 ± 0.63; LV: CR: 71.30 ± 0.66; BY: 21.08 ± 0.74), regardless of the LIF treatment ($p < .05$). Blastocysts derived from the fresh oocytes treated with LIF showed a higher hatching capacity at day 8 pi (LIF: 56.86 ± 0.73) when compared to the other groups (Control: 47.41 ± 0.97; Vit: 42.42 ± 1.01; LV: 38.30 ± 0.62) ($p < .05$). Despite LIF did not improve blastocyst yield in the vitrified group, further research is required to elucidate the mechanism by which LIF promotes blastocyst hatching in fresh oocytes. (Study supported by the Spanish Ministry of Science and Innovation (Project AGL2016-79802-P).)

P 301 | Macroscopic evaluation of the placenta of the alpaca (*Vicugna pacos*)

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Macroscopic evaluation of the placenta is an essential post-partum examination and can be of special interest in case of an abortion, a premature- or stillbirth. Since there are not many reference values regarding macroscopic properties of normal alpaca placentae, a small descriptive study was conducted. Only placentae from normally foaling alpaca mares, giving birth to healthy crias, after a full-term uneventful gestation (±350 days; range 335–360 days) were taken into account (N = 12). The weight, umbilical cord length, length of the (non–)pregnant uterine horn and corpus, the surface area and the distance between the umbilical cord and the corpus were measured. The volume was measured by placing the fetal membranes in water, collecting and weighing the overflowing water. Crias weighted ± 7.75 kg (range: 5.5–10 kg), while the mean weight (±SD) of the full-term placentae was 0.8 ± 0.21 kg, i.e. 10% of the bodyweight of the crias. The weight of the allantoamnion and chorion was 0.2 ± 0.07 kg and 0.5 ± 0.15 kg, respectively. The umbilical cord length was 8.9 ± 2.72 cm and the length of the pregnant and non-pregnant uterine horns were 71.5 ± 14.13 cm and 54.5 ± 6.53 cm, respectively. The length of the corpus was 14.5 ± 4.69 cm and the distance from the umbilicus to the corpus was 18.3 ± 6.14 cm. The total volume of the allantoamnion was 0.14 ± 0.079 l and the chorionic volume was 0.39 ± 0.089 l. The surface area of the allantoamnion and the chorion were 42.1 ± 14.93 dm^2 and 71.7 ± 9.06 dm^2, respectively. All placentae had small calcifications ('amnion plaques') either only around the umbilical cord, or around the umbilicus and the blood vessels of the pregnant uterine horn. These measurements could be used to evaluate alpaca placentae, although more research is needed.

P 302 | Isolation of the goose spermatogonia

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Spermatogonia are testicular stem cells, the precursors of male sex cells. The aim of our research was to optimize the individual stages culturing goose spermatogonia. Histological examinations of the spermatogenic cells (spermatogonia, spermatocytes, spermatids, spermatozoa) composition in seminiferous tubules from 1 wk to 8 months was studied. Disaggregation of the testis tissue for isolate spermatogonia cells was carried out by consecutive enzymatic treatment in 0.25% trypsin and 0.1% collagenase solution. Purification of spermatogonia from other types of spermatogenic cells was conducted by separation of the cells by adhesion. Spermatogenic cells were cultured for 24 h, after which the supernatant containing unattached cells (spermatogonia) was transferred into a new culture dish for further cultivation. The duration and conditions of cultivation of spermatogenic cells were selected experimentally. For identification of spermatogonia SSEA-1 antibodies were used. The maximum number of spermatogonia in seminiferous tubules of goose occurred up to 5 wk. The percentage of spermatogonia from the total number of spermatogenic cells in the seminiferous tubule reached 85%. In view of this, spermatogonia were isolated from the testes of 3-week-old goose. Maximum homogeneity of the cell population was detected by dividing the cells by 3-fold transfer of the cell supernatant at interval of 24 h. The number of spermatogonia in the suspension reached 88%. The formation of spermatogonia colonies was observed on day 6 to 9 of cultures, depending on the feeder layer. We concluded that the age for retrieving spermatogonia cells in goose was no later than 5 wk and the optimal feeder layer for the cultivation of spermatogonia were their own Sertoli cells. (Supported by RSF (16-16-04104).)

P 303 | Litter size components of three selected maternal lines founded on different criteria

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Litter size is a very important trait in synthetic lines of polytocous species. The aim of the study was to evaluate the effect of foundation criteria from three maternal lines on the litter size components. Line A was originated in 1980 from NZW rabbits reared by farmers (47 generations), Line V was stablished from 4 specialized maternal lines in 1984 (43 generations) and Line LP was founded by selecting females from commercial farms that showed an extremely long productive life associated with prolificacy (12 generations). A total of 207 laparoscopies were carried out between 3rd and 5th parity, and ovulation rate (total number of corpora lutea) and implantation rate (number of embryos implanted at day 12) was recorded. In addition,

implantation losses (percentage of potential embryos produced [number of corpora lutea] that did not reach the implantation), foetal losses (percentage of implanted embryos related to litter size at birth) and perinatal losses (ratio between live born and litter size at birth) were calculated. Ovulation rate, implantation, foetal and perinatal losses rates were analyzed by a GLM model with line and lactation state as fixed factors. Results showed that synthetic lines differed in ovulation rate (15.4 ± 0.50 vs. 14.4 ± 0.44 and 14.0 ± 0.47 from line V, LP and A, respectively, $p < 0.05$). Regardless of the criteria for the foundation of the lines, the percentage of implantation (12.8 ± 2.50%), foetal (14.0 ± 2.33%) and perinatal losses (7.2 ± 2.10%) and litter size at birth (11.4 ± 0.41) were not significantly different. In conclusion, only the ovulation rate currently differentiates among these maternal lines. (Study supported by AGL2014-53405-C2-1-P.)

P 304 | Influence of day length on duration of estrus, size of preovulatory follicle and progesterone levels in mares

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The aim of this study was to determine of the length of estrus, size of the preovulatory follicle, progesterone level in the time of ovulation in mares, with the consideration to the seasonal influence on mares estrous cycle. Mares (n = 34) of warmblood and coldblood breeds in different age (4–18 years) were housed in outside boxes inseminated at Equine Clinic of University of Veterinary Medicine and Pharmacy in Košice from March to August. Mares were evaluated every 6 h from the first sign of estrus. Preovulatory follicle size was detected ultrasonographically (SonoScape S6) with 5-Mhz linear-array transducer. Blood was collected from jugular vein in time of insemination. Real time of ovulation was estimated with accuracy ±6 h. Data about the day length – 15th day in each month were obtained from Slovak Hydrometeorological Institute. Statistical analysis was performed using parametric Pearson's correlation test. Shortest estrus was recorded in June (4.9 days) with duration of day length 16 h 03 min and there was significant correlation between duration of estrus and day length ($r = -0.747$, $p < 0.0001$). No statistically significant correlation was found between day length and preovulatory follicle size ($r = 0.0147$, $p > 0.05$) nor between day length and progesterone levels ($r = 0.078$, $p > 0.05$). The influence of the season and day length on mare reproductive cycle was again confirmed in this study. (This study was supported by the Ministry of Education of Slovak Republic VEGA no. 1/0382/18.)

P 305 | Survey on cesarean section in the buiatric practice: indications, clinical surveys and subsequent fertility

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Retrospective study involving 58 farms, in the area of Cuneo and Torino, performed from October 2014 to November 2015 with the main objective to analyze subsequent fertility on caesarean section (CS) on 154/174 (88.5%) and the incidence of CS consequences on 169/174 (97%). The correlations between the continuing variables were evaluated using the ANOVA-Multivariate method, and between categorical variables the chi-square test was used. Data represent main outputs of a questionnaire of anamnestic information: breed (Piedmontese 71%), housing (freestall 79%), nutrition, (unifeed 61%), category for CS (heifers 41.4% – cows 58.6%), indications (macrosomia 51.7%). Standing left paralumbar celiotomy was performed in 95.4%, (4.6% recumbent celiotomy). During the surgery different systemic antibiotic treatment (ampicillin 58.1%, oxytetracycline 34.5%, kanamycin 6.3%) were administered, while in all cases intrauterine oxytetracycline was used. Principal post-surgical complications were: metritis 19.8%, infected wound 16.8%, retained fetal membranes (RFM) 13%. Beef cows had an earlier resurgence of ovarian cyclicity earlier (57 ± 22 days on average) compared to dairy cows (79 ± 18 days) ($p = 0.006$). The same was observed in the calving to conception interval that was 97 ± 30 days in beef cows and reached 157 ± 69 days in Holstein cows ($p < 0.001$). The average number of artificial inseminations (AI) to obtain a pregnancy in beef cows after CS was 1.5 and 2.3 in dairy cows. The main effect on fertility is influenced by the antibiotic therapy: when oxytetracycline was used for systemic and intrauterine treatment the animals showed resumption of ovarian cyclicity within 57 ± 17 days ($p = 0.03$), kanamycin 97 ± 19 days and ampicillin 71 ± 31 days.

P 306 | Equine alpha-fetoprotein levels in the intra- and peripartal period

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Alpha-fetoprotein (AFP) values are associated with fetal anomalies and liver tumors in humans and pregnancy loss and placentitis (Sorensen 1991, Eq Vet Sci 10:417–21) in horses. Furthermore, elevated AFP levels have been found in the serum of the pregnancy loss group (152 ± 36.48 pg/ml) compared to the healthy pregnant group of

mares (72.93 ± 49.25 pg/ml) previously (Vincze 2015, Theriogenology 84:1581–6). The aim of this present study was to provide basic data regarding AFP levels in mares and foals in the peri- and intrapartal period. A total of 139 samples (107 maternal blood, 8 umbilical cord blood, 15 neonatal foal blood and 9 amniotic fluid) from 19 asymptomatic Lipizzaner mares were collected and analyzed. An enzyme-linked immunosorbent assay kit for alpha-fetoprotein (Mybiosource Ltd., San Diego, USA) was used. The effects of various variables on AFP level were estimated by linear models and generalized additive models. The AFP levels measured were negatively correlated with the mares' age ($p < 0.001$). AFP levels decreased in the last 10 days of pregnancy followed by another decline after foaling ($p = 0.0117$). A significant seasonal effect could be detected with lower values in May, and a significant ($p = 0.0378$) elevated AFP during the hot summer months (June–August). The lowest AFP values could be detected in the amniotic fluid and umbilical blood samples, whilst the greatest variability was among maternal AFP levels. A strong individual effect could be noticed in case of AFP levels in all mare-foal pairs. Although studies have shown health and well-being-related changes in AFP levels in horses, the possible diagnostic role of this glycoprotein still needs further evaluation.

P 307 | Genetic transformation of chicken primordial germ cells in vitro

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Primordial germ cells (PGC) are precursors for both male and female germ cells, and considered as promising target cells for delivery of recombinant DNA in order to create genetically modified domestic birds. We have studied the effectiveness of PGC transformation in vitro using various gene delivery systems – lipophilic transfection, retroviral transduction and electroporation. The PGC cells used in the study were isolated from the gonads of 6-days-old chick embryos. Lentiviral particles (Tu/ml = 1.7×10^7) carrying ZsGreen gene coding a fluorescent protein were applied to transduce the PGC cells. A series of experiments were conducted to determine the optimal MOI (multiplicity of infection) for effective transduction of the PGC. The plasmid pZsGreen1-N1 (Addgene, #54702) coding the ZsGreen gene under the CMV promoter was used for lipophilic transfection and electroporation. The conditions of electroporation were optimised by voltage (170, 350, 500 V) and pulse time (40–100 μs). The effectiveness was evaluated on the BD Acuri C6 cytometer. Transduction effectiveness of the PGC with the lentivirus in volumes corresponding to MOI = 0.5; 1; 2.5; 5 were 12%, 46%, 75% and 42%, respectively. The most effective volume was MOI = 2.5. The increased volume of the virus led to cell death caused by toxicity that resulted in decrease of transformed cells. The effectiveness of transfection of the PGC with the lipophilic agent TurboFect (Thermo Fisher) did not exceed 1%. Optimization by different amounts of DNA and the transfection agent did not result in any improvement. Electroporation was more effective: the percentage of the transformed cells varied from 0.5% to 11% and the optimal parameters were 350 V, 100 μs. (Supported by RSF (16-16-10059).)

P 308 | Persistently infection of BVDV in neonatal calves of the Ural region of Russia

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Bovine viral diarrhea virus (BVDV) is a causative agent of bovine viral diarrhea-mucosal disease (BVD-MD). It may cross the placental barrier and infect the fetus in the first half of pregnancy, causing abortion, congenital defects or the birth of persistently infected (PI) calves. This research aimed to study the structure, clinical and biochemical features of neonatal pathology of calves persistently infected with BVDV. The research was conducted within the farms of the Ural region of Russia. The analysis of serum, collected from newborn colostrum-free calves (n = 228), employing ELISA pointed to the presence of BVDV antigens in 20 calves, which also lacked the BVDV antibodies. Thus, 8.8% of calves were classified as PI with BVDV. Clinical and biochemical studies of PI calves pointed to a high prevalence of multiple organ pathology with a temporary predominance of symptoms of different organs failure (e.g. pulmonary, cardiac, renal, failure). The proportion of dead and slaughtered one month old PI calves accounted for 35%. Histological examination of parenchymal organs and lymph nodes from dead and slaughtered calves revealed signs of glomerulonephritis and nephrosonephritis, changes in the cardiac muscle fibers, symptoms of liver cirrhosis, granular degeneration of hepatocytes and underdevelopment of the lymphatic system. While conducting immunological surveys of calves born from BVDV-infected cows, disorders of the immune system with a prevalence of structural immunodeficiency were registered. The syndrome of multiple organ defects was accompanied by endogenous intoxication and distinct membrane destructive processes.

P 309 | Recipient factors affecting pregnancy rate in an equine embryo transfer program

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Equine embryo transfer (ET) has become common practice in horse breeding. To maximize the success of this procedure it is essential to know the factors that affect pregnancy rates. The aim of this study was to analyze how the different factors related to

the recipient mare affect pregnancy rates in a commercial equine embryo transfer. The retrospective analysis comprises data obtained during breeding seasons 2016 and 2017, from 422 donor mares, 918 recipients, and 1745 transferred embryos at stud farm Lewitz, Neustadt-Glewe, Germany. Oestrous donor mares with follicles ≥35 mm in diameter and endometrial edema were treated with 2500 IU hCG IV to induce ovulation. Donors were inseminated with chilled or frozen semen. Embryos were flushed days 7–10 after ovulation by transcervical approach. Synchrony of recipients ranged from 4 to 9 days after ovulation. The embryo was transferred transcervically. Pregnancy checks were performed by ultrasound at 16 and 45 days after donor ovulation. A chi-Square test was used to evaluate effects of recipient age, days after recipient ovulation, presence of foal, number of previous unsuccessful embryo transfers and previous abortions in pregnancy rate. p-values <0.05 were considered significant. Of 1745 embryos, 1256 (72%) and 1152 (66%) resulted in a pregnancy at 16 and 45 days, respectively. There were no statistical differences in pregnancy rates among the recipient ages, days after ovulation and previous abortions ($p > 0.05$), but the presence of foal and previous unsuccessful embryo transfers were statistically significant ($p < 0.05$). In conclusion, the presence of foal and recipients used for first time in embryo transfer increased the pregnancy rate.

P 310 | Supplementation of post-thaw semen with fractionated seminal plasma improves cryosurvival of boar spermatozoa

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This study investigated the effect of the supplementation of fractionated seminal plasma (SP) to post-thaw semen on boar cryosurvival. Post-thaw semen of seven boars was split into three aliquots and incubated with chromatographically fractionated SP (proteins >40 kDa, SP 1; proteins <40 kDa, SP 2), and with BTS extender. The Newman-Keuls post hoc test showed that CASA-analyzed motility (MOT), mitochondrial function (MF), plasma membrane integrity (PMI), viability and acrosome integrity were higher ($p < 0.05$) in the frozen-thawed (FT) samples treated with SP, regardless to the incubation time. After 1 h post-thaw sperm MOT of samples supplemented with SP 1, SP 2 and BTS extender averaged 31.0 ± 1.9%, 34.2 ± 2.4% and 21.9 ± 1.5%, respectively. Post-thaw sperm MF averaged 47.6 ± 0.8%, 48.8 ± 1.0% and 39.7 ± 1.0% for samples supplemented with SP 1, SP 2 and BTS extender, respectively. Furthermore, PMI of SP 1, SP 2 and BTS extender in FT spermatozoa averaged 49.1 ± 0.9%, 50.1 ± 1.0% and 39.6 ± 1.2%, respectively. The viability of post-thaw spermatozoa (YO-PRO-1/PI) supplemented with SP 1, SP 2 and BTS extender was 45.1 ± 1.2%, 43.6 ± 1.5 and 37.1 ± 1.0%, respectively. Acrosome integrity (FITC-PNA/PI) in FT spermatozoa differed among the treatment groups, being significantly higher ($p < 0.05$) in the SP-supplemented samples. Results of this study indicated that the supplementation of fractionated SP to post-thaw boar semen significantly sperm cryo-survival. (Supported by a NCN project, Poland (2016/21/N/NZ9/02289).)

P 311 | Trichostatin A (TSA)-dependent epigenetic transformation impacts the quantitative profiles of Galα(1,3)Gal epitopes in adult dermal fibroblast cells (ADFCs) derived from bi- and tri-transgenic pigs

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The current study prompted us to investigate the effect of TSA treatment on Galα(1,3)Gal epitope (EPTP) expression in in vitro cultured ADFCs originating from 2 × Tg (hFUT2 × hGLA; n = 3) and 3 × Tg (hFUT2 × hGLA×HLA-E; n = 3) pigs generated for the purposes of xenotransplantation. The ADFC lines were exposed to 50 nM TSA for 24 h and the total protein was then extracted. The expression profiles of Galα(1,3)Gal EPTP were determined by lectin blotting with HRP-labelled GS-IB4 lectin. The ADFCs derived from non-transgenic (nTg) pigs were served as negative (TSA–) and positive (TSA+) control groups (n = 6). All cell cultures were independently replicated three times. A much lower expression of Galα(1,3)Gal EPTPs has been shown in TSA– 2 × Tg and 3 × Tg cells (0.290 ± 0.015* vs. 0.279 ± 0.014*) as compared to TSA– nTg group (1.135 ± 0.057*). Interestingly, the levels of Galα(1,3)Gal expression in TSA+ 2 × Tg, 3 × Tg and nTg ADFCs (0.610 ± 0.031* vs. 0.585 ± 0.029* vs. 2.098 ± 0.105*) were significantly higher than those noticed for their TSA– counterparts (*$p < 0.01$; ANOVA and Tukey's HSD post hoc test). Cumulatively, the extents of Galα(1,3)Gal expression decreased remarkably in both TSA+ and TSA– multi-transgenic ADFCs, confirming proper transcriptional/translational activity of incorporated human genes/transcripts. But, increased levels of Galα(1,3)Gal EPTP in TSA+ 2 × Tg, 3 × Tg and nTg ADFCs may suggest that their epigenomic modulation leads to enhancement in GGTA1 gene expression. This gene encodes α1,3-GT enzyme involved in the formation of Galα(1,3)Gal EPTP. Finally, further detailed research is required to focus on the use of such proteomically profiled 2 × Tg or 3 × Tg ADFCs for somatic cell cloning in pigs. (Funded by grant No. INNOMED/I/17/NCBR/2014 and statutory activity No. 01-19-04-21.)

P 312 | Trichostatin A (TSA) treatment enhances the expression of recombinant human α1,2-FT, α-Gal A and HLA-E proteins in adult dermal fibroblast cells (ADFCs) derived from triple transgenic pigs

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The present study sought to determine the effect of TSA-mediated epigenomic modulation (TSA-EM) on abundance profiles (APs) of α1,2-rhFT, α-rhGal A and HLA-E proteins in ex vivo expanded ADFC lines originating from 3 × Tg (hFUT2 × hGLA × HLA-E; n = 3) pigs. These 3 × Tg pigs are intended to be used for xenotransplantation research. The ADFC lines underwent 24-h EM using 50 nM TSA, followed by isolation of the total protein. The expression levels of α1,2-FT, α-Gal A and HLA-E proteins were estimated by Western blot. The ADFCs derived from non-transgenic (nTg; n = 3) pigs provided TSA+ and TSA− control groups. All cell cultures were independently triplicated. In TSA+ 3 × Tg cells, APs of α1,2-FT and α-Gal A increased by 46.81%^{A} and 115.53%^{A} as compared to APs of TSA− 3 × Tg cells^{B}. Unexpectedly, in TSA+ nTg ADFCs, enhancements in α1,2-FT and α-Gal A expression were maintained at the levels of 170.82%^{a} and 129.93%^{a} as compared to those observed in TSA− nTg cells^{b}. In turn, the impact of TSA-EM on AP of HLA-E in nTg ADFCs was greater (increase by 67.35%^{A,B}) than that noticed for 3× Tg cells (increase by 5.52%) [A,B $p < 0.01$; a,b $p < 0.05$; ANOVA and Tukey's HSD post hoc test]. Altogether, TSA can be successfully used for EM of transcriptional/translational activities of hFUT2, hGLA and HLA-E transgenes integrated with host genome of porcine ADFCs. However, it must be borne in mind that TSA-EM may non-specifically increase the expression of intrinsic pFUT2 and SLA-1 genes, which has been confirmed by elevated levels of α1,2-pFT and SLA-1 proteins in nTg ADFCs. These latter appear to result from relatively high extents of protein sequence homology between α1,2-pFT and α1,2-rhFT and between SLA-1 and HLA-E. (Funded by grant No. INNOMED/I/17/NCBR/2014 and statutory activity No. 01-19-04-21.)

P 313 | First report of transplacental transmission of *Dirofilaria repens* in dogs**

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A 7-years-old chocolate female Labrador retriever was presented to a veterinary cabinet for abortion. A blood test performed before the surgical procedure did not reveal any abnormalities, apart the presence of microfilariae in the blood smear. The aim of this study was to investigate the possibility of transplacental *Dirofilaria repens* transmission to foetuses in the microfilaremic dog. After removal of the uterus, necropsy of the foetuses was performed. The amniotic fluid and blood samples from all foetuses' hearts were collected. Furthermore, tissue samples from heart, lungs, stomach, liver, spleen, kidney and intestines were collected for histopathological examination and PCR testing. Microfilariae were detected in the amniotic fluid and in 1 out of 7 blood samples from the foetuses by smear test. PCR performed on DNA isolated from all blood and organ samples were negative. The investigation of organ's histopathological slides did not reveal the presence of microfilariae. The results of our examination confirm that the vertical transmission of *D. repens* microfilariae is possible in dogs. It should be taken into consideration that transplacental transfer of stage L1 *D. repens* could result in development of immunotolerance in puppies born to infected dogs, which would consequently lead to an increase of susceptibility to the infection in their adult life. (Acknowledgment: The authors are grateful to the Leading National Research Center (KNOW – Krajowy Naukowy Osrodek Wiodacy) for its financial support.)

P 314 | A comparison of subsequent reproductive performance and lifetime performance between nurse sows and non-nurse sows at different parities in Spanish pig herds**

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The objectives of this study were (1) to characterize nurse sows, and (2) to compare subsequent reproductive performance and lifetime performance of nurse sows and non-nurse sows. We analyzed 457,102 parity records and 90,188 lifetime records of sows served between 2011 and 2016 in 69 herds. Nurse sows were defined as sows that had weaned 2 or 3 litters in the same lactation period in any parity. Mixed-effects models were applied to the data. Mean lactation length and number of pigs weaned (±SEM) by nurse sows were 31.0 ± 0.11 days and 21.9 ± 0.04 pigs, respectively, and 7.2% of all sows nursed at least once in their lifetime. Also, 10.0% of first nurse sows had a second nursing at a later parity. There were no differences between nurse sows and non-nurse sows for proportions of sows with weaning-to-first-mating interval (WMI) 0–6 days or subsequent pigs born alive (PBA) at parity 1 ($p > 0.36$). However, at parity 2 or higher, nurse sows had 3.3–3.5% lower proportions with WMI 0–6 days than non-nurse sows ($p < 0.05$). Meanwhile, nurse sows had 0.1–0.3 more subsequent PBA than non-nurse sows at these parities ($p < 0.05$). In addition, there were no differences in farrowing rates of nurse sows and non-nurse

sows in any parity group ($p > 0.18$). Nurse sows had 1.5 higher parity at removal and 1.5 more annualized lifetime PBA than non-nurse sows ($p < 0.05$). In conclusion, there were lower proportions of nurse sows at parity 2 or higher with WMI 0–6 days, but no other negative effects. In addition, they did have better lifetime performance than non-nurse sows.

P 315 | Effect of dietary fish oil supplementation on ketotic dairy cows' reproductive performance

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It was aimed to determine the effect of rumen-protected dietary fish oil (FO) supplementation on metabolic and fertility parameters in ketotic Holstein dairy cows. All cows were housed in the same farm and fed a total mixed ration (TMR) (control, n = 47) or TMR plus FO supplementation (experiment, n = 43) during lactation. On 7 days in milk (DIM), the cows were checked for clinical ketosis (CK), (≥3.0 mM beta-hydroxybutyrate (BHBA), subclinical ketosis (SCK), (≥1.2–2.9 mM BHBA) and healthy (H), (<1.1 mM BHBA). Metabolic profile on 7 and 100 DIM, lactation and fertility parameters were compared using ANOVA. On 7 DIM, blood alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), low-density lipoprotein (LDL) and non-esterified fatty acids (NEFA) concentrations were higher ($p < 0.001$) in ketotic cows than those detected in H cows. Ketotic cows displayed lower aspartate aminotransferase (AST) ($p < 0.001$), triglyceride (TRIG) ($p < 0.005$), total cholesterol (CHOL) ($p < 0.005$) and high-density lipoprotein (HDL) ($p < 0.001$) as compared to H cows in experiment group. On 100 DIM, ALT and GGT remained higher for ketotic cows ($p < 0.001$). FO increased the TRIG level in ketotic cows ($p < 0.001$), while CHOL and HDL increased in H cows ($p < 0.001$). Higher ALP was observed in SCK cows in experiment group ($p < 0.001$). FO decreased NEFA in SCK cows ($p < 0.001$) and increased glucose in SCK and H cows ($p < 0.005$). FO increased daily milk yield ($p < 0.01$) and decreased period until first standing heat ($p < 0.05$) in SCK cows, however peak milk yield, duration of lactation, number of AI per pregnancy, open days and calving interval did not differ. In conclusion, FO does not improve lactation and reproductive performances in CK cows.

P 316 | Conception rate in lactating and dry sheep after melatonin treatment, estrus synchronization and artificial insemination during non-breeding season

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This study reported the effect of preliminary melatonin treatment on the conception rate in lactating and dry sheep after estrus synchronization and artificial insemination during non-breeding season. The experiment was carried out with 243 lactating (group I) and 309 dry (group II) Assaf sheep, housed in a private farm (43°25′N and 24°37′E). Each group was separated in two subgroups. The control sheep (lactating-LC, n = 111 and dry-DC, n = 182) were submitted to estrus synchronization by intravaginal sponges (FGA 30 mg) for 12 days, PMSG treatment (500 IU) immediately after their removal and single artificial insemination between 56–60 h after that. The experimental subgroups (lactating-LE, n = 132 and dry-DE, n = 127) received a subcutaneous implant of melatonin (18 mg) 42 days (23–26 March) before the treatment with aforementioned synchronization protocol. Ultrasound pregnancy check was performed on day 35 after insemination. The conception rate (CR) for each subgroup and a total for control and experimental animals was calculated and the results were statistically processed. Significantly higher conception rate was determined in LE than LC sheep (73.5% vs. 46%; $p < 0.01$) while the values for DE and DC subgroups were close (80.3% vs. 76.9%; $p = 0.53$). Additional analysis showed significant ($p < 0.01$) increase of CR in dry compared to lactating control sheep and lack of significant difference among the melatonin treated subgroups. The total value in melatonin treated sheep was higher than in control animals (76.8% vs. 65.2%; $p < 0.05$). In conclusion, preliminary melatonin treatment increases conception rate in lactating sheep after estrus synchronization and artificial insemination during the non-breeding season and improves the reproductive performance of the flock.

P 317 | Intrauterine growth retardation in pigs as important problem in livestock production

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Intrauterine growth retardation (IUGR) is defined as a neonate born at term but with low birth mass (lower than 1.1 kg), and characteristic changes of facial part of the head. IUGR neonates manifest high early postnatal mortality thereby reducing farmer's benefits. Eight pairs of 7 day-old IUGRs and normal birth body weight (NBW) Landrace × Pietrain piglets were euthanatized. Brain and internal

organs were measured and liver and small intestines were examined using morphology, immunofluorescence, scanning electron microscopy (SEM), Western-blot and mass spectrometry analyses to find the causes of high mortality in IUGR as compared to their non-IUGR littermates. Average number of litter was 13 piglets. Spontaneous IUGR syndrome was observed with 8 6% frequency. The average birth weight of NBW piglets was 1.52 ± 0.17 kg and in IUGRs 0.75 ± 0.08 kg. The mortality of IUGRs in the first week of life was 57% of all IUGR piglets. The main causes of IUGRs losses were crushes, lack of suckling and diarrhea. Organometry analysis showed reduction of liver and intestine absolute weight as well as relative brain weight. Histometry studies showed reduced height of villi and muscularis thickness in the intestines, delayed removal of fetal type of enterocytes, decreased thickness of enterocyte brush border, and in the enterocyte lack of characteristic apiculo-canicullar system. Proteomic and immunohistochemistry studies showed reduced expression of proteins linked with carbohydrate metabolism in the intestinal mucosa and liver. Structural and molecular changes in small intestines may be a cause of decreased ability to absorb and digest nutrients which may have an impact on enhanced mortality of IUGRs and poor body weight gain.

P 318 | Effectiveness of follicular cysts puncture in dairy cattle compares to hormonal treatment alone: preliminary results

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Many treatment methods for cysts have been used, though still numbers of animals fail to respond. Most frequent is hormonal treatment: cyst puncture is also used, however due to technical problems it is less popular. The aim of the study was to evaluate effectiveness and safety of ovarian puncture in connection with hormones. The study included 67 Holstein-Friesian cows from 3 herds in North-Eastern Poland. Follicular cysts were diagnosed by ultrasound rectal examination starting from 50 days post-partum. Group PH (n = 23) cows were punctured through the wall of vagina close to cervical orifice using a covered needle on a stiff catheter and they received a GnRH analogue IM injection at the same time, followed by a $PG_{2\alpha}$ analogue 7 days later. Group H (n = 25) received only the hormonal treatment as group PH and the control group C (n = 19) remained untreated. All groups were examined by ultrasound 37 days after treatment. Number of cows without finding cysts in groups PH, H, C were 15 (65.2%), 11 (44%) and 6 (31.6%), respectively. Animals after puncture were checked also for endometritis, vaginitis or other complications and none of these were diagnosed. Cows that recovered were inseminated in the following estrus and those who did not recover were treated and inseminated later. Statistical analysis was performed by ANOVA summary with Tukey's multiple comparisons test. Mean number of calving-to-conception interval in cows that recovered after first treatment, were as follows in groups PH, H, and C: 101; 119; 224 days, respectively. There was no statistical difference among experimental groups ($p > 0.05$). Among animals that recovered after the first treatment, there was a trend for shorter calving-to-conception interval in puncture together with hormonal therapy group (PH).

P 319 | Photostimulated males are not able to induce an adequate sexual response in 7 or 10 months old female goat kids exposed to male effect, if they have low body weight

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Sexually active males during the seasonal anoestrus induce a very high reproductive response in 7 or 10 months old female goat kids when they are around the 60% of their adult body weight (BW). The objective of this experiment was to determine the ability of these males to induce a reproductive response in prepubertal goat kids selected by their reduced BW (<60% of their adult BW). During natural anoestrus, we selected 7 months old goat kids (n = 18, 7 months group) or 10 months old (n = 17, 10 months group). To induce the male effect in April, half of each group was exposed to males rendered sexually active by exposure to 3 months of long days from November 1st (PHOTO males), or males under natural photoperiod (CONTROL males). Four groups were conformed (2 ages × 2 kind of males). Oestrous activity was checked daily. Ovulation was confirmed via the plasma progesterone concentrations, measured twice per week during 32 days after male introduction. The BW was determined weekly. The BW of the 7 months group (20.4 ± 0.4 kg) at male introduction was lower than the BW of the 10 months group (23.7 ± 0.3 kg, $p < 0.001$). Even though that the oldest goat kids showed higher BW than the youngest ones ($p < 0.01$) during the whole experiment, no differences were observed at the time of showing ovulation or oestrus ($p > 0.05$); the mean BW of the females showing ovulation or oestrus in both groups was 23.3 ± 0.6 kg. Only the percentage of females showing ovulation was higher in the oldest group (65% vs. 28% for 10 and 7 months group, respectively, $p < 0.05$). During the seasonal anoestrus, the use of photostimulated males are not able to improve the reproductive response of 7 or 10 months old goat kids when they show a low BW below of 23 kg. (Funded by Grant AGL2016-75848-R from MINECO (Spain).)

P 320 | Research of cows' reproductive ability with the feeding micronized pumpkin oil meal during the transit period

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The aim of this work was to study the effect of feeding micronized pumpkin oil meal (MPOM) to cows during the transition period on their reproductive function. Research was carried out on cows in pregnant and fresh cows of black-and-white breed. Two groups of animals were formed: cows of the experimental group (G1, n = 20), 2 months before calving and 10 days after calving MPOM was added to the main diet at the rate of 1 g per 1 kg of live weight (3 days of feeding, 4 days – break). The animals of the 2nd group were control (G2, n = 20). The anestrus period in the G1 group was reliably reduced by 17.98%, the duration of the intercalving period was 369.6 ± 5.79 days, or 4.62% lower than in the G2 group. In the G1 group the reduction of the duration of the service period was noted 6.88%, the insemination index by 17.39% compared to the cows of the G2 group. Cows of the G1 group noted an increase in the fertilization rate from the 1st insemination to 80.3%, compared to the cows of the G2 group with 67.4%. The number of pathological calving decreased in G1 (2.3 times or 57.14%), as well as postpartum complications (3.1 times or 68%). Using MPOM in G1 group helped to strengthen the natural resistance: number of red blood cells, hemoglobin, α-, γ-globulins increased, with an insignificant decrease in the number of leukocytes, β-globulins and albumins. The results of the study showed that the live weight of calves obtained from the cows of the G1 group at birth exceeded the control group by 5.72%, and by the 30th day by 9.34%. Intermittent feeding of MPOM at these doses was beneficial as to the reproductive ability and the strengthening of the natural resistance of the female organism, and it also had a positive effect on the weight of the newborn calves.

P 321 | Prospecting for new markers of physiological and pathological ovarian follicles development in the pig

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The mechanism and selection time of the follicles that become the preovulatory follicles (PF) or follicular cysts (FC) is not known in the pig. The challenge with exogenous gonadotropins (eCG and hCG) of altrenogest primed prepubertal pigs induces pathological development of some follicles. The purpose of the present study was to investigate potential developmental markers of PF and non-induced and hormonally induced FC. PF were collected from gilts on day 20 of the estrous cycle (n = 4), non-induced FC from gilts (n = 4) in the follicular phase at the slaughterhouse and induced FC from prepubertal gilts (n = 4) exposed to altrenogest and eCG/hCG and ovariectomized 5 days after ovulation. Concentrations of estradiol (E2), testosterone (T4), androstenedione (A4) in cystic fluid of experimentally induced FC were lower compared to healthy PF and non-induced FC ($p < 0.01$). The highest concentration of progesterone (P4) was found in postovulatory FC ($p < 0.05$). Relation between particular steroids in follicular cystic fluid showed very dynamic changes in T4/E2, P4/T4 and P4/E2 ratios. Walls of experimentally induced FC showed a very high expression of STAR and HSD3B1 but low of HSD17B1 which explains the high concentration of P4 in cystic fluid. Healthy PF showed the highest abundance of HSD17B1 mRNA, the enzyme converting A4 to T4 which simultaneously with the highest expression of CYP19A1, guarantees the high E2 synthesis. Observations of selected miRNA abundance in the follicular fluid of FC and healthy PF indicate that 4/8 miRNAs were differentially expressed; miR21, miR266, and miR34a showed higher expression in both types of cysts versus PF in contrast to miR224. These preliminary observations are very promising in terms of finding miRNAs serving as markers for ovarian cysts.

P 322 | Induction of sexual activity in anestrus goats using a single or differed dose of eCG

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The aim of this study was to evaluate the possible effect of a single or differed dose of equine chorionic gonadotropin (eCG) upon estrus induction efficiency in anovulatory goats during the end of the anestrus season. Mixed-breed dairy goats (n = 29) from the Mexican sub-tropical region (26°N) were divided into three groups (T100 n = 10, T50 + 50 n = 10 & TP4 n = 9) with homogeneous body weight (36.9 ± 1.4 kg) and body condition score (2.2 ± 0.07). In June, each goat from the three experimental groups received 25 mg progesterone and, 24 h later eCG either 100 IU (T100), 50 + 50 IU 12 h later (T50 + 50), while the TP4 group received 0.5 ml of saline solution. All treatments were administered intramuscularly. After eCG administration, estrus behavior was quantified using sexually active male goats (n = 3), which were exposed to females twice daily (0800 & 1800 h; 15 min × 7 days). Goats showing estrus signs were mated during the first 12 h after the beginning of estrus. Ovulation and pregnancy detection was performed by transrectal ultrasonography on days 10 and 45 after matting, respectively. The number of estrus females, females ovulating, pregnancy and kidding percentages, were analyzed through Chi square. The estrus, ovulating and pregnant females' percentages were similar ($p > 0.05$) between T100 (100; 100; 70%) and T50 + 50 (100; 100; 80%), but differed ($p < 0.05$)

regarding the TP4 group (56; 56; 22%). No differences (p > 0.05) occurred regarding kidding rate (20; 60; 22%). Results show that both hormonal treatments, either 100 and 50 + 50 IU of eCG, could be efficiently used to induce estrus, ovulation, and pregnancy in anovulatory goats towards the end of the anestrus season. Nonetheless, it is worth to note that the T50 + 50 treatment tended (p = 0.11) to depict an increased kidding rate.

P 323 | Is addition of seminal plasma increasing quality of frozen-thawed semen from poor freezing stallions?

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The aim of this study was to evaluate the effect of the addition of seminal plasma (SP) to frozen-thawed (F-T) spermatozoa of poor freezing stallions. Ejaculates from 10 stallions were frozen with L-EDTA extender and SP was collected from a good freezing stallion (motility after thawing 45%). Criterion for poor freezing stallions was <15% of motile spermatozoa in the F-T sample. The F-T samples (n = 40) were halved and supplemented right after thawing either with SP-TALP (control group, C) or seminal plasma (SP) both in concentration 30% w/v. The samples were evaluated after thawing (T0) and 30 minutes of incubation (T30) at 37°C. The k-means cluster analysis was used to classify sperm into subpopulations (SUB) slow, medium fast, fast and Euclidean distances were computed from four variables – BCF, VAP, VCL, VSL obtained by CASA. The viability (eosin/nigrosine) and plasma membrane integrity (PMI; HOS test) were also evaluated. There was no positive effect of SP addition on distribution of SUB, viability and PMI (P>0.05) right after thawing. However after 30 minutes of incubation the percentage of sperm belonging to medium fast and fast SUB was about 11% lower and 11% higher in SP vs C samples, respectively (P<0.05). In both SP and C samples the slow SUB was represented by 56% at T30 (P>0.05). The incubation period did not affect viability (P<0.05) but in SP samples the PMI decreased by about 5% compared to C samples (P<0.05). In conclusion we demonstrated a positive effect of SP addition after thawing on distribution of sperm subpopulations, however addition may have a negative effect on plasma membrane integrity in frozen-thawed ejaculates of poor freezing stallions.

P 324 | Acute Phase Protein Response in Dogs Undergoing Ovariohysterectomy, Ovariectomy and Laparoscopic Ovariectomy

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The aim was to compare acute phase response in dogs following ovariohysterectomy (OHE), ovariectomy (OVE) and two-portal laparoscopic ovariectomy (LOVE). Twenty-one healthy dogs were randomly allocated into study groups. Dogs were premedicated with medetomidine and surgeries were performed under propofol-isoflurane anesthesia by an experienced surgeon. No intra or postoperative complications were observed. No rescue analgesia was used. Total surgery times were recorded. Serum samples were collected pre and postoperatively (1, 6, 24, 48, 96, 144h) for measurement of acute phase proteins: C-Reactive Protein (CRP), haptoglobulin (Hp), ceruloplasmin (Cp) and paraoxanase (PON)-1. Repeated measures ANOVA was used to evaluate the difference between groups. Bonferroni was used as Post hoc test. Total surgery time was not different between groups (OHE:19.2 ± 5.27; OVE:15.52 ± 3.99; LOVE:18.16 ± 1.91 min, p > 0.05). Serum CRP levels within 24h were significantly different between groups (Median OHE:0.642; OVE:0.261; LOVE:0.083 µg/L; p < 0.05). Serum Hp levels were lower in LOVE group at all time points (Median OHE:245.74; OVE:291.30; LOVE:211.99 ng/mL; p < 0.05). Significant differences were observed in Cp levels between groups (Median OHE:0.935; OVE:0.775; LOVE:0.520 U/mL; p<0.05). Serum PON-1 activity was lower in LOVE group at all time points (Median OHE:992.35; OVE:913.55; LOVE:663.04 U/mL; p<0.001). Serum PON-1 activity significantly increased from baseline in OHE group at 48h then decreased (p < 0.05). In conclusion, OVE induced less acute phase response compared to OHE. In addition, LOVE, in which similar surgical times are obtained by an experienced operator compared to conventional OHE and OVE, induces less acute phase response and it may be more appropriate for an outpatient setting.

P 325 | Effect of different mucolytic drugs on the viscosity of the cervical mucus and the route of F/T spermatozoa through the cervix in ewes

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Cervical artificial insemination (AI) with F/T semen has a very limited succes rate in sheep. The aim of this study was to address the hypothesis that systemic mucolysis can affect the viscosity of the cervical mucus and consequently the transport of the spermatozoa through the cervix. The estrous cycles of crossbred primiparous ewes (n = 23) were synchronized with intravaginal sponges (Chronogest CR (Fluorogestone acetate)). The animals in group A received Acetylcysteine (10 mg/kg BW; n = 8) and in group B Bromhexine

(0.5 mg/kg BW; n = 8) four times between sponge removal and AI. Animals in group C (n = 7) did not receive any additional treatment and served as a control. Insemination was performed 56h after sponge removal. Mucus was collected just prior to insemination and analyzed for viscosity. Animals were euthanized 5 hours after AI, reproductive tracts were removed and three segments were flushed with a Tris-based extender: mid-region of uterine cervix, left uterine horn and left oviduct. Flushes were analyzed for the presence of spermatozoa under a phase contrast microscope using a Makler chamber. Bromhexine significantly reduced (student *t* test) the viscosity of mucus compared to that of the control group (P = 0.03). More spermatozoa were recovered from the genital tract in animals of group B than in those of group A and practically none in group C animals. Pearson's correlation coefficient demonstrated a weak to medium correlation between cervical mucus viscosity and sperm passage to uterus.

The results suggest the possible use of a systemic mucolytic to reduce the viscosity of the cervical mucus and to increase the penetration of F/T ram spermatozoa through the cervical canal of the ewe in estrus.

P 326 | Complex viability, acrosome and morphology evaluation of frozen stallion semen batches used for AI with low pregnancy outcome

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The quality and fertility of frozen sperm can be different among freezing dates resulting in low pregnancy outcome after using different batches. In stud farms with limited laboratory equipment or in field practice motility and progressive motility are the most commonly used parameters in the evaluation of stallion semen. In the cases presented the motility of the frozen/thawed sperm at the place of insemination did not seem to be decreased, the pre-calculated doses seemed to be correct; however the artificial insemination using the selected batches were unsuccessful. Chicago sky blue - neutral red - Giemsa staining method was used for simultaneous evaluation of acrosome integrity, membrane integrity of equine spermatozoa subdomains and overall morphology. Using the complex evaluation sperm with intact head, tail and acrosome membrane were found in very low proportion (the lowest was 10%), besides sperm with intact head, tail membrane but damaged or lost acrosome was in high rate (more than 10 -15% in some stallions) and in some cases a fairly high percentage of the spermatozoa had damaged head but intact tail membrane. The highest ratio was 21%. The proportion of sperm having damaged head membrane and/or damaged/lost acrosome altogether was more than 40% of the sperm having intact tail membrane which might be motile but lost its functional integrity. The results have pointed out to the importance of recalculating the insemination dose of frozen/thawed semen based on ratio of membrane-intact and morphologically normal spermatozoa.

P 327 | Treatment with GnRH on day 5 after AI may increase the conception rate in lactating dairy cows during the hot season

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The present study aimed to evaluate conception rate (CR) in dairy cows during the hot summer season treated with 100-µg gonadotropin-releasing hormone (GnRH) at d 5 after AI. Lactating Holstein cows from one commercial herd during Aug-Sep 2017 were scored for body condition (BCS) and submitted for first AI to Presynch-Ovsynch protocol (2 injections of $PGF_{2\alpha}$, 14 days apart, and 10 days later an Ovsynch; GnRH 7 days before and 56 h after $PGF_{2\alpha}$, and TAI 16 hours after the second GnRH) starting at d 35±3 postpartum. After confirmed ovulation, at d 5 post-AI, cows were randomly allocated into 2 groups: control group (CON, n=45), without any additional treatment, and treatment group (GnRH, n=50), treated with 100 µg GnRH. Cows were examined on d 21 and d 30 after AI by ultrasound examination. On d 21 after AI, ovaries were scanned to diagnose non-pregnant cows with a high probability (no CL). Average daily temperature and relative humidity values were collected from a meteorological station, located near the herd, and temperature-humidity index (THI) was calculated according to the following formula: THI = (1.8 x T + 32) – ((0.55 – 0.0055 x RH) x (1.8 x T – 26)), where, T = Temperature and RH=Relative Humidity. Categorical values were analyzed with Non-parametric categorical test (Binomial). The average THI during the experiment was 79.5 ± 0.6. At d 21, more GnRH cows had more than one CL compared to CON (89.1% vs 6.8%). In addition, the GnRH group had a significantly higher CR than the CON group (64.0% vs. 42.2%, $p < 0.05$). BCS did not affect CR ($p > 0.72$). According to the results of the present study, cows had an increased conception rate if treated with GnRH at d 5 after AI, even when exposed to summer hot season.

P 328 | Otocephaly in an Istrian Pramenka lamb

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Otocephaly is a rare congenital abnormality in which the upper and lower jaw, tongue, and frontonasal bones are absent or hypoplastic, and the pinnae are often fused in the midbasal part of the head. In

veterinary medicine, until now only two cases of otocephaly were described in a puppy and in a lamb. In both cases, otocephaly was accompanied with abnormalities of the brain. This report describes the occurrence of otocephaly in an Istrian Pramenka *lamb*, *an* autochthonous sheep breed of Slovenia. The breed is used for milk and meat production and is important for the prevention of overgrowing of the agriculture land.

In February 2018, an 8-year-old ewe delivered a male lamb by c-section, weighing 6 kg at term. The ewe was from a flock of 500 sheep, which is under constant veterinary control.

The lamb died in less than a minute after birth and had severe craniofacial deformations: very small oral aperture (*microstomia*), the tongue and a lower jaw were absent (*agnathia* and *aglossia*) and it had a cleft palate. The ear pinnae were located at the medioventral part of the head and neck (*synothia*) without a connection to ear canals. The oesophagus had a segmental stenosis in the length of 1 cm, and the segment proximal to the stenosis was cystically dilated. The segment distal to the stenosis was uniformly dilated. Furthermore, two cysts filled with yellow, translucent fluid were found in the liver. The brain was normally developed and no other anomaly was observed. The morphology and gross lesions of the lamb were consistent with the otocephaly syndrome, which develops as a consequence of migration failure of neural crest cells from the hind brain.

P 329 | Postoperative Pain and Stress in Dogs Undergoing Ovariohysterectomy, Ovariectomy and Laparoscopic Ovariectomy

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The aim was to compare postoperative pain and surgical stress following ovariohysterectomy (OHE), ovariectomy (OVE) and laparoscopic OVE (LOVE) in dogs. Twenty-one healthy dogs were randomly allocated to study groups. Dogs were premedicated with medetomidine and surgeries were performed under propofol-isoflurane anesthesia by an experienced surgeon. No intra- or postoperative complications were observed. No rescue analgesia was used. Serum cortisol, glucose, tumor necrosis factor (TNF)-α, interleukin (IL)-6 concentrations and WBC were measured preoperatively and 1, 6 and 24h postoperatively. Repeated measures ANOVA was used to evaluate the difference between groups. Bonferroni was used as Post hoc test. University of Melbourne pain scores (UMPS) were monitored at 1, 6, 24 and 48 h. Kruskal-Wallis test was used to determine differences between groups over time. Cortisol concentrations significantly increased at 1, 6 and 24 h in OHE and OVE groups ($p < 0.001$). Glucose concentrations increased from baseline in all groups at 1, 6 and 24h ($p < 0.05$). WBC increased at 6 and 24 h in all groups ($p < 0.001$) exceeding reference values in OHE and OVE groups. TNF-α increased from baseline at 1, 6 and 24 h in OHE group ($p<0.001$). IL-6 values were similar at all time points in OHE and OVE groups and decreased at 1, 6 and 24h in LOVE group ($p < 0.05$). UMPS were significantly lower for LOVE group at 1, 6 and 24 h (0.43; 0.14; 0.0) compared to OHE (2.29; 5.0; 3.14) and OVE (2.29; 2.58; 0.57) groups ($p < 0.05$). UMPS were lower at 6 and 24h in OVE group compared to OHE group ($p < 0.05$). In conclusion, OVE is superior to OHE in terms of pain and surgical stress. In addition, LOVE caused less pain and surgical stress than both conventional OHE and OVE and it may be more appropriate for an outpatient setting.

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