Expression of vaspin and GRP78 in corpus luteum are dependent on estrous phase in Large White pigs

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Reproduction in Domestic Animals WILEY

P(St)1 | Melatonin added to freezing medium improves cryosurvival of English Bulldog spermatozoa

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In dogs, pregnancy rates obtained by artificial insemination using frozen-thawed spermatozoa are still unsatisfactory. Cryopreservation compromises sperm fertilising capacity due to a series of alterations in sperm structure and physiology; use of antioxidants such as melatonin (MLT), added to freezing media, may help to reduce sperm cryoinjury. To test the effect of MLT on dog sperm cryosurvival, semen (n = 24 ejaculates) from 8 English Bulldog males (2 to 6 years of age) was collected, assessed, centrifuged, resuspended in a standard freezing medium (EYT), cooled to 5°C in two hours, and added EYT to obtain a final concentration of 200 × 106 cells/ml and 5% glycerol. Diluted sperm were added MLT (0, 0.5, 1.0 and 3.5 mM), and packaged in 0.25 ml straws which were further cooled to −5°C (0.15°C/min) employing saline ice at −12°C, then were exposed to nitrogen vapour for 15 min, and plunged in liquid nitrogen. Thawing was carried out at 70°C for 5 sec in a water bath, then sperm (at 37°C) were assessed for: progressive motility (visual), viability (eosin/nigrosine), plasma membrane integrity (SYBR14/PI), acrosome integrity (PSA/FITC), capacitation status (CTC assay), and plasma membrane fluidity (MC540). Data were arcsine transformed to normalize it before ANOVA (MLT doses). There were significant differences ($p < 0.05$) in the percentage of sperm having hyper-fluid membranes, intact acrosome, capacitated acrosome-intact, and acrosome reacted, being the better values for "high" (2.0 & 3.5 mM) than for low (0 & 0.5 mM) MLT doses. In conclusion, 3.5 mM MLT improved cryosurvival of sperm from English Bulldog males. Supported by UNAM (PAPIIT IA204917, IA220419, PIAPI1615/1649/1810).

P(St)2 | Aquaglyceroporins rather than orthodox aquaporins are involved in boar sperm cryotolerance

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Aquaporins (AQPs) are transmembrane proteins that work as water channels and are classified into orthodox AQPs, aquaglyceroporins (GLPs) and superaquaporins (superAQPs). Mounting evidence indicates AQPs are involved in mammalian sperm cryotolerance, probably through volume and osmolality regulation. This study aimed to unravel the functional relevance of AQPs in boar sperm cryopreservation through different inhibitors. A total of eight ejaculates (one per boar) were cryopreserved in the presence of either acetazolamide (AC, 250 μM and 500 μM), an orthodox AQPs inhibitor; phloretin (PHL, 250 μM and 500 μM), an inhibitor of GLPs; or 1,3-propanediol (PDO, 1 mM and 10 mM), which inhibits orthodox AQPs and GLPs. Sperm quality was evaluated in fresh samples and after 30 min and 240 min of incubation at 37°C after thawing, in terms of total (TMOT) and progressive motility (PMOT) through a CASA system, and of viability and membrane lipid disorder through flow cytometry. The treatment with 10 mM PDO showed higher ($p < 0.05$) TMOT, PMOT and viability (%SYBR14 + /PI- sperm), and lower membrane lipid disorder (%M540-/YO-PRO-1- sperm) than the control at both 30 and 240 min post-thaw ($p < 0.05$), whereas the lowest concentration only showed higher TMOT and PMOT at 30 min post-thaw ($p < 0.05$). Both PHL concentrations caused a decrease ($p < 0.05$) in all post-thaw quality parameters, whereas AC had no effect. These results suggest GLPs, but not orthodox AQPs, are relevant in boar sperm cryotolerance. The positive effect of PDO suggests it exerts a cryoprotective effect itself. Further studies are needed to evaluate other quality parameters in presence of AQP inhibitors to unveil their role in sperm cryotolerance. This work was supported by MCIU, Spain (Grants: RYC-2014-15581 and AGL2017-88329-R).

P(St)3 | Changes in nitric oxide synthase isoforms localization in the presence of melatonin seem not to be related with changes in nitric oxide levels during ram spermatozoa capacitation

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We have recently demonstrated that the three isoforms of nitric oxide synthase (nNOS, iNOS, eNOS) are present in ram spermatozoa. This work aims to determine whether melatonin regulates NOS expression and localization during in vitro capacitation, as this hormone plays a fundamental role in ram sperm physiology and nitric oxide (NO·) seems to be important during this process. Swim-up selected spermatozoa were incubated in capacitating conditions in TALP medium (control) and TALP with cAMP-elevating agents (cocktail) plus melatonin (100 pM and 1 μM). Data were statistically compared by Chi-square test. Indirect immunofluorescence assays revealed two immunotypes for each NOS isoform, whose percentages changed during capacitation (evaluated by chlortetracycline staining). The addition of the cAMP-elevating agents led to a decrease in the percentage of sperm showing nNOS and iNOS labelling on the spermatozoa apical edge, and the presence of melatonin (100 pM and 1 μM) modified those values ($p < 0.05$). Western blot analysis revealed bands associated with nNOS (~120 kDa) and eNOS (~100 kDa), but iNOS could not be detected. No differences between treatments were observed. Finally, NO· levels (evaluated by DAF-2-DA/PI staining and flow cytometry) increased ($p < 0.05$) in control and cocktail samples, but no significant effects were observed in the presence of melatonin. Thus, it seems that melatonin provokes changes in NOS isoforms localization, but not in their levels or NO· production, so that could be an indirect effect associated with any other change that melatonin induces during capacitation. Grants: AGL-2017-83799-R, DGA 2016-A26, BES-2015-072034.

P(St)4 | Semen quality and genetic differences in boars of high and low field fertility

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The aim of the study was to determine semen quality and genetic parameters of boars with high and low field fertility (farrowing rate > 85% or < 80%, respectively). Ejaculates from 6 adult boars, 3 of high (group A) and 3 of low fertility (group B), were collected twice a week over 4 consecutive months. Ejaculates with ≥ 1 × 10*8 sperm/ml and ≥ 70% progressive motility were extended by a commercial medium to 30 × 10*6 sperm/ml. Aliquots of diluted semen were assessed for sperm motility by computer-assisted sperm analysis, sperm chromatin integrity (acridine orange), viability, morphology (eosin-nigrosin stain) and biochemical activity of membranes (HOS-Test). Possible polymorphisms were investigated in 5 genes P450scc, P45017a, P450arom, 3b-HSD, StAR, after blood sampling. In order to isolate DNA from the blood of each boar, PCR polymerase chain reaction was performed and the existence of polymorphisms in the 5 genes was investigated. Data were analyzed with a mixed model that included group as a fixed effect and boar as a random effect. The results showed higher values in progressive motility ($p = 0.019$), rapid moving ($p = 0.023$) and HOST+ spermatozoa ($p = 0.001$), VCL ($p = 0.003$), VSL ($p = 0.023$), BCF ($p = 0.005$) and WOB ($p = 0.025$) in group A compared to B. Moreover, significantly higher values of tail morphological abnormalities ($p = 0.002$) and slow moving spermatozoa ($p = 0.007$) were noticed in group B compared to A. Finally, polymorphisms and mutations in the selected genes were not detected and this fact might require further research. In conclusion, higher farrowing rates can be related to higher progressive motility, sperm membranes' biochemical function, VCL, VSL, BCF and WOB. This supports the based on sperm quality data selection of high fertile boars for artificial insemination.

P(St)5 | Restoration of IGF-1 after weaning in hyper-prolific primiparous sows: effect of protein loss on IGF-1 concentration

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Severe lactational sow bodyweight loss is associated with lower IGF-1 concentration and compromised follicle development at weaning. However, it is difficult to assess whether the protein losses or the lipid losses have more impact on IGF-1 concentration. For this reason, we investigated the effect of backfat thickness (BF) and muscle loin depth (LM) loss on IGF-1 concentration during the weaning to estrus interval. We retrospectively assigned 56 primiparous sows (DanAvl) into high- (HBF, > 22 %, n = 31) or low- (LBF, ≤ 22 %, n = 25) BF loss, and into high- (HLM, > 8%, n = 28) or low- (LLM, ≤ 8 %, n = 28) LM loss during lactation. After farrowing and at weaning, BF and LM were measured at 6 cm from the midline over the last rib. Blood samples for IGF-1 were taken at the day of weaning, three days after weaning and at estrus. MIXED procedure (SAS 9.4) was used for analysis. IGF-1 concentrations were low at weaning but thereafter increased (192.3 ± 16.9a vs. 273.7 ± 16.9b vs. 279.0 ± 17.2b ng/ml respectively, for D0, D3 and estrus, $p < 0.01$). IGF-1 concentrations at D0 were negatively correlated to lactational BF loss and LM loss ($\beta = -2.2$ (ng/ml)/%

and β = −5.8 (ng/ml)/%, respectively, p < 0.01) but no relationships were found with IGF-1 levels at D3 or at estrus. IGF-1 concentrations never differed between HBF and LBF sows. However, HLM sows had lower plasma IGF-1 concentrations than LLM sows at D0 (171.0 ± 15.4 vs. 221.6 ± 15.3 ng/ml, p < 0.01). To conclude, lactational protein loss during lactation appears to be connected to lower IGF-1 at weaning in hyper-prolific primiparous sows. However, IGF-1 levels are rapidly restored after weaning, regardless of body condition losses. Thus, sow management based on LM may be recommended for preventing lower IGF-1 at weaning.

P(St)6 | The use of curcumin or resveratrol in boar semen storage medium improves sperm quality parameters

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The use of pure bioactive substances in andrology has become a more popular solution in the prevention of oxidative damage to spermatozoa. Besides that curcumin (CUR) and resveratrol (RES) abound antioxidant activity, they also improve cell viability and provide organelle-specific beneficial effects. Therefore, the aim of our study was to investigate the in vitro effect of various concentrations (0, 100, 50, 25, 10 and 5 μmol/L) of RES and CUR on boar semen in different time periods (0, 24, 48 and 72 h). The sperm motility was measured using the computer-assisted semen analysis (CASA system). Mitochondrial activity was evaluated using the mitochondrial toxicity test (MTT). The formation of superoxide radicals, as a marker of oxidative stress, was analysed by nitroblue-tetrazolium (NBT) test. The results showed significantly increased (p < 0.001) motility after 24, 48 and 72 h, when using 5–25 μmol/L of RES, respectively a significantly increased (p < 0.01) motility, when using 25–10 μmol/L of CUR. On the other hand, when using 25–10 μmol/L of CUR, the scavenging of superoxide radicals was significantly improved (p < 0.001) after 24, 48 and 72 h. In case of RES at 25 μmol/L, a significantly decreased (p < 0.001) superoxide quantity was observed after 48 h (respectively concentrations of 25–10 μmol/L after 72 h). A significant amelioration (p < 0.001) of the mitochondrial activity came with using 25–5 μmol/L even after 24, 48 and 72 h. Similarly, 25–10 μmol/L of CUR significantly improved (p < 0.01) the mitochondrial activity. Both substances come with a beneficial effect on the quality parameters of spermatozoa, thus they may prolong the storage period and improve the fertilizing properties of preserved boar spermatozoa.

P(St)7 | Monthly changes in testicular ultrasonography and their association with ram sperm quality

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Testes ultrasonography is a non-invasive, complementary technique used for reproductive assessment. Doppler analysis provides information on blood flux from the testicular artery. Whereas seasonal changes in sperm quality and testicular size have been detected in ram, information about seasonal variations in testes echotexture and blood flow is scarce. This study aimed to evaluate seasonal differences in testis ultrasonography parameters provided by the ECOTEXT software (Humeco, Spain), resistive (RI) and pulsatility indexes (PI) by Doppler, and their relationship with current and future sperm quality. Mature rams were subjected to monthly (from October to January) ultrasonography using a portable ultrasound scanner (EXAGO, France) connected to a 7.5-MHz transducer. Semen was collected the same day, four and eight weeks after testicular analysis. Motility (M), viability (V), capacitation status, phosphatidylserine translocation, DNA damage, reactive oxygen species level (ROS), and morphology (Mo) were evaluated. Statistical analyses revealed monthly differences (p < 0.05) in Ecotex2 (white pixels), tubular density, PI and RI. In addition, Ecotext 2 correlated (p < 0.05) with several sperm parameters of the semen samples obtained in the same day (ROS, r = 0.338) and four weeks after testicular evaluation (M, r = −0.456; V, r = 0.352), and with morphology parameters (Mo, r = −0.463) eight weeks after evaluation. Blood flow measurements correlated with Mo (r = 0.37) at the moment of analysis and V after 4 (r = −0.405) and 8 (−0.459) weeks. In conclusion, ultrasonography could be a useful method for predicting testicular functions and current or future sperm quality. Grants: AGL-2017-83799-R, DGA-A07_17R.

P(St)8 | Evaluation of membrane integrity in bovine sperm by ethidium bromide

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The aim of the study was to evaluate the quality of bulls' semen by a fluorescent method. The method is based on the ability of ethidium bromide to enter cells with compromised membrane integrity and

causes the core luminescence. Ethidium bromide does not penetrate into spermatozoa with an intact membrane. Sperm was collected from 30 bulls. 1 ml of diluted in citrate Na semen was cultured with 10 μl of ethidium bromide (at a concentration 1.25 mg/ml) and the fluorescence intensity was measured. Fluorescence was measured on a LUMAM fluorescence microscope. The excitation wavelength is 366 nm, the registration wavelength is 560 nm. The total number of spermatozoa was determined by fluorescence after adding digitonin to the sperm sample, which destroys the membrane at a concentration of 50 μg/ml. Sperm samples were divided into 2 groups: motility 50–79% and concentration 600–800 mln/ml (G1) and motility more than 79%, concentration more than 800 mln/ml (G2). Commercial ejaculates are selected for freezing if motility is more than 80% and a concentration more than 800 mln/ml in accordance with the instructions in Russia. The number of cells with a damaged membrane in G1 and G2 did not differ significantly (14.6 ± 2.65% and 17.5 ± 2.22%, respectively; mean ± SD). The number of cells with an injured membrane ranged from 5.2% to 22.4% in G1 and from 4.8 to 22.5% in G2. The Spearman's rank correlation between the fertilizing ability of sperm and the number of cells with damaged membranes was high ($r = -0.5$, $p < 0.05$). The relationship between the number of cells with damaged membranes and motility was low ($r = -0.03$, $p < 0.05$). Thus, fluorescence assessment with ethidium bromide is an additional criterion for the quality of bull semen. Project № AAAA-A18-118021990006-9.

P(St)9 | Serological response to Coxiella burnetii and pregnancy duration, age and outcome in cases of abortion and stillbirth in dairy cows

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The purpose of this study was to detect the relation of pregnancy duration, age of the animal during abortion/stillbirth (A/S) and outcome of animal with serological response to Coxiella burnetii (C. burnetii). C. burnetii is an etiological agent of Q fever, a zoonosis that can cause abortion, stillbirth, premature delivery and weak offspring in dairy cows. In this study serum samples from 30 cows and heifers with reported abortion/stillbirth from July 2017 until November 2018 were tested by ELISA. Data of length of pregnancy, animal's age and outcome were collected from Agricultural Data Centre of Latvia. Results showed suspicious (n = 3), positive (n = 17) and negative (n = 10) serological response to C. burnetii. No differences in length of pregnancy during A/S in seropositive and negative animals were detected (245.8 ± 55.6 and 240.8 ± 37.2 days of pregnancy, respectively). Differences were detected in the age of seropositive and negative animals (2.6 ± 1.2 and 4.5 ± 1.5 years, respectively). No differences were detected in rate of culled/dead outcome between seropositive and negative animals (47% and 50%, respectively). Conclusions are that A/S occur in late pregnancy in both seropositive and negative animals. Abortions/stillbirth related with serological response to C. burnetii occur in younger animals more often. Rate of culling due to too long lactation (abortion before dry period) or due to a nonstarted lactation in heifers, was not different between seropositive animals and seronegative ones. Nevertheless, presence of C. burnetii in dairy herds is economically disadvantageous, because seropositive animals are younger, when suffering from A/S and are, therefore, excluded from herd earlier than the seronegative ones.

P(St)10 | Lineage differentiation of rabbit preimplantation embryos in sequential, single step and single step with renewal in vitro culture systems

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Rabbit embryos are able to develop from zygote to blastocyst in wide range of in vitro culture media, including those used in human IVF clinics. This capacity to cope with different in vitro culture conditions requires more than morphology examination of proper embryo development. Here, we show how different media and in vitro culture systems impact one of the key events in preimplantation mammalian embryo development – differentiation of first cell lineages: pluripotent epiblast (EPI), primitive endoderm (PrE) and trophectoderm (TE). Rabbit zygotes (19 hpc) were cultured in vitro in: two types of sequential culture media (a. G-1:tm: PLUS/G-2:tm: PLUS (Vitrolife); b. G-1:tm:/G-2:tm:), two single-step with renewal media (a. RDH + 0.3% BSA; b. RDH), and two types of single-step media (Global (Life Global:registered:) supplemented a. with 0.3% BSA b. w/o supplement. After 120 hpc, embryos were fixed and immunostained. Cell number and particular lineage differentiation were based on localization of lineage-specific transcription factors: CDX2 for TE, SOX17 for PrE and SOX2 for EPI. Interestingly, rabbit zygotes cultured in sequential and single-step with renewal media not only more often reached the blastocyst stage than embryos cultured in single-step media (82% vs 89% vs 47%) but also, on average, had more cells (105 ± 4 vs 113 ± 5 vs 72 ± 4, errors are SEMs) and more Sox2-positive cells (8 ± 2 vs 8 ± 3 vs 0.3 ± 0.2). The average number of Cdx2-positive cells was the highest in embryos cultured in single-step with renewal media (3 ± 1 vs 18 ± 2 vs 0.1 ± 0.1). Number of Sox17-positive cells seems to be independent of the applied medium; however, the numbers were higher in media without addition of a protein component (20 ± 2 vs 5 ± 1).

P(St)11 | Management and resolution of a large vaginal leiomyoma in a bitch causing severe dysuria without urinary or vaginal surgery: a case report

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In the bitch, tumors affecting the vagina or vulva are uncommon, with the majority being leiomyomas or fibromas (Brodey RS et al. J Am Vet Med Assoc 1967;151, 1294–3071). Ovarian hormones may be involved in the pathogenesis and medical treatment with aglepristone (AGLE) or ovariectomy are used with good results (Rollón et al. J Small Anim Pract 2008; 49:41–4). Clinical case: An 11-year old, intact Labrador Retriever bitch was referred to the clinic with a large vaginal mass prolapsed that appeared 3 days after proestrus onset with concomitant muco-purulent discharge. Serum progesterone (P4) concentration was 4.94 ng/ml. To prevent the mass from increasing during estrus, 10 mg/kg AGLE was administered on the day of referral, the following day and every other 7 days (Rollón et al. J Small Anim Pract 2008; 49:41–4). Eight days later the bitch showed difficulty in urinating and defecation. On ultrasound follicles of 0.7 cm were observed on both ovaries, urinary bladder and both ureters were dilated with evidence of hydronephrosis. On day 11 a CT-scan was performed showing a vaginal mass of 15 × 6 × 8 cm. Following a transcutaneal biopsy, histological diagnosis of leiomyoma was made. On day 18, because of the apparent lack of efficacy of AGLE a prostaglandin treatment was started (alfaprostol 0.02 μg/kg BID). On day 22, hydronephrosis was still present. Due to the increased values of P4 and unachieved luteolysis, ovariohysterectomy was performed on day 35 post-referral. The left ovary showed 11 corpora lutea (CL) and other 12 CL in the right ovary. A luteoma tumor was diagnosed in the left ovary. 10 days post-surgery P4 levels were undetectable, vaginal mass was not palpable and the bitch was able to urinate without a problem. We suspect a correlation between the ovarian tumor and the unachieved luteolysis.

P1 | Abstract withdrawn

P2 | DNA tests for early detection of bull-carriers of genetic defects in Aberdeen Angus cattle

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One of the indicators of beef cattle breeding profitability is the output of calves, the rate of which is 90–100 calves per 100 cows. One of the reasons that this indicator is decreasing is the manifestation of genetic anomalies causing appearance of stillborn or unviable calves. The use of a limited quantity of bull sires during artificial insemination leads to spread in populations of mutant alleles associated with inherited diseases. In this regard, early detection of bull sire-carriers of genetic anomalies is particularly relevant. The aim was to develop DNA tests for the detection of animal-carriers of genetic defects of Arthrogryposis multiplex (AM), Osteopetrosis (OS) and Developmental duplication (DD) and the analysis of the spread of the AM-, OS- and DD-carriers among Russian populations of Aberdeen Angus bull sires. DNA samples of Aberdeen Angus bull sires (n = 446) of three populations belonging to different farms of the Russian Central district were used. For DNA Analysis, AS-PCR (AM, OS) and PCR-RFLP (DD) methods were chosen. For the RFLP analysis, we used the restriction enzyme BstMWI. DNA tests for the analysis of genetic defects (AM, OS and DD) were developed. The population analysis revealed 0.22% of the bull sires were carriers of AM, 0.89% carriers of OS and 7.17% carriers of DD. Although mating of these bulls with cows free from the mutations ends in "healthy" offspring, such bulls should not be used any more to prevent spreading of the disease. The data display the need to control use of bull sires carrying AM, OS and DD genetic defects in Russia by using the tests developed. The study was funded by RFBR according to the research project №19-016-00007.

P3 | Dietary effect of different level of Omega-3 Fatty acids from linseed oil on fresh and post-thaw sperm quality in Holstein bull

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Previously, we have shown that DHA (a member of n-3 fatty acids family) from fish oil are important for membrane integrity, quality and cold sensitivity of Bovine sperm. The goal of this study was to investigate the dietary effect of different level of omega-3 fatty acids from linseed oil on fresh and post-thaw sperm quality in Holstein bull. 24 bulls were randomly assigned to 4 groups and received encapsulated fat for 12 weeks as follows: group P fed 300 g palm oil, group Pl fed 200 g palm + 100 g linseed oil, group pL fed 100 g palm + 200 g linseed oil, and group L fed 300 g linseed oil. The fresh and post-thaw semen quality including sperm motion characteristics, membrane integrity, hypo-osmotic swelling test, and malondialdehyde (MDA) content was evaluated. Data were analyzed by proc MIXED of SAS program. There were no significant differences in volume, sperm concentration, and sperm quality parameters of fresh semen. After freezing-thawing process, the total and progressive motility in group Pl (66.06 ± 1.95;47.53 ± 2.48), and pL (65.67 ± 1.95;47.48 ± 2.48) and L (65.36 ± 1.95;47.62 ± 2.48) were significantly greater than the group P (59.61 ± 1.95; 40.19 ± 2.48), but there were no significant differences between

the groups PI, PL2 and L. Membrane integrity and activity significantly increased in groups PI (62.73 ± 2.15; 48.93 ± 2.17), pL (64.06 ± 2.15;50.01 ± 2.17) and L (64.47 ± 2.15;49.68 ± 2.17) compared to P (55.79 ± 2.15;42.19 ± 2.17), but there were no significant differences between PI, pL and L. Sperm abnormality in the groups PI (21.55 ± 1.62), pL (21.69 ± 1.62) and L (20.90 ± 1.62) were significantly less than group P (25.99 ± 1.62). Therefore, feeding of 100 g linseed oil could be an effective strategy to improve the sperm cryotolerance in Holstein bulls.

P4 | Sexually inexperienced photo-stimulated mature males display high sexual behavior exposed to anestrous female goats

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Lack of sexual experience is diminishing sexual behavior. The aim of this study was to determine if sexually inexperienced photo-stimulated mature males display lower sexual behavior than experienced ones exposed to anestrous females. Five sexually inexperienced males were isolated from females since weaning at 40 days of age and 5 acquired sexual experience at 15 months of age. At 23 months of age, the males were submitted to a photoperiodic treatment (16 h light/day/2.5 months). At 27 months of age, the males were exposed to females (50 each) for 15 days. Males′ sexual behavior was registered on days 0–3 from 0800–0900 h and 1800–1900 h after introduction to females. Males′ sexual behavior was compared by X2-test for goodness of fit. On day 0 nudging did not differ between males ($p > 0.05$), on days 1–2 it was more often seen in the inexperienced males, and on day 3 more often in the experienced ones ($p < 0.0001$ each). On days 0–1 ano-genital sniffing was more often seen in the experienced males, whereas on days 2–3 in the inexperienced males ($p < 0.0001$ each). On days 0 and 3 mounting attempts did not differ between males ($p > 0.05$), but on days 1–2 more mountings were observed in the inexperienced ones ($p < 0.0001$ each). On days 1–3 mounts with intromission did not differ between the two groups of males ($p > 0.05$). In conclusion, sexually inexperienced photo-stimulated mature males show comparable sexual behavior as the experienced ones when in contact with anestrous females.

P5 | Effect of Berkan preparation on cow's uterine involution post partum

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The aim of the study was to test the way Berkan preparation accelerates uterus involution of Black-and-white cows in conditions of the breeding farm “Alga” located in the Krasnokamsk district of the Bashkortostan republic. Rectal studies established the location, size and consistency of the cervix and horns of the uterus of cows after parturition. During six days n = 12 cows of the experimental group were injected with 10 ml of Berkan medicine intramuscularly in the pararectal fossa once a day. N = 15 cows of the control group were not injected with this preparation. Berkan medicine is a water extract of birch wood, bark and bast. It was found that the cows treated with Berkan medicine had the shorter period of uterine involution (27.1 ± 3.21 days). The duration of uterus involution of the untreated cows was 39.1 ± 5.16 days. Fidelity assessment was made according to Student's test: $p < 0.05$. Application of Berkan plant medicine improved uterine involution. Pregnancy in the experimental group was 42% after the first insemination and 92% for the whole experiment. Pregnancy of cows untreated with Berkan preparation was 33% after the first insemination and 80% for the whole experiment (3 months; $p < 0.05$). Thus, using Berkan plant medicine, uterine involution period might be shortened.

P6 | Precision supplementation of protein enriched Opuntia cladodes in anestrous goats exposed to the male effect: Luteogenesis and embryo implantation

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The possible supplementation effect of protein enriched Opuntia megacantha Salm-Dyck cladodes on embryo implantation rate (EIR, %), corpus luteum diameter (CLD, mm) and serum progesterone concentration (SPC, ng ml^{-1}) in adult anestrous goats exposed to the male effect, was evaluated. In early May, Alpine-Saanen-Nubian × Criollo adult goats (n = 45, 15 per group; 26°N) were randomly assigned to: 1) Protein-enriched Opuntia (PEO; 44.5 ± 1.7 kg live weight (LW), 2.5 ± 0.14 units body condition score (BCS); 29.8% CP, 2.27 Mcal ME kg-1), 2) Non-enriched Opuntia (NEO; 41.9 ± 1.5 kg LW, 2.5 ± 0.1 units BCS; 6.4 % CP, 1.8 Mcal ME kg-1), and 3). Control (CC; 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 Scaromices cerevisiae, + 1% urea + 0.1% of ammonium sulfate). After exposure

to a 10d experimental breeding with testosterone-treated bucks, all goats continued the supplementation schedule during 30-d post-breeding. Neither LW ($p > 0.05$) nor BCS ($p > 0.05$) or either the P4-average (3.73 ± 1.6 ng ml^{-1}) or the P4-values across time differed ($p > 0.05$) among treatments. Yet, increases ($p < 0.05$) in EIR % and CLD mm occurred in the PEO and NEO vs. CONT (67 ± 20%, 57 ± 19%, 28 ± 15% and 1.7 ± 0.3, 0.9 ± 0.3, 0.6 ± 0.2 mm, respectively). Peri-implantation targeted Opuntia cladodes supplementation in anestrous goats exposed to testosterone-treated males (male effect) generated both augmented EIR and CLD, with no differences regarding SPC around the embryo peri-implantation stage. The last being potentially important to increase not only the reproductive outcome, but also the economic return and sustainability of extensive goat production systems under marginal conditions.

P7 | The influence of selection for fur blackening on reproductive ability in sables (Martez Zibellina L.)

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The first farm-bred sable population in Russia was formed in 1928–1936 on the basis of natural populations from Siberia. The selection process was primarily directed at fur blackening; the first black sables were documented in 1968. The effects of directional selection on the reproductive capacity of females were studied from 1992–2001. Female fertility was defined for four possible types of animal crossings, in which males and females differed in the intensity of fur colour (in total, 4918 females). In A group (crossing of males and females with a light fur), an average litter size (LS) was 1.9 cubs per female, and the proportion of mated females without litters (Pr) was 0.31. In B and C groups (reciprocal crossing), LS was 1.2–1.7, and Pr was 0.37–0.53. The lowest reproduction rate and the highest proportion of females that did not give offspring were found when selecting pairs of animals with the darkest fur colour (D group): LS = 0.4 and Pr = 0.8. In addition, the dynamics of these traits were analysed over a ten-year period. In the D group, LS increased from 0.1 to 0.9, while in others, it grew from 1.2 to 2.6. An analysis of the dynamics of the trait 'the proportion of females without a litter' revealed a general downward trend in all the groups. In the A group, Pr decreased from 0.5 to 0.09; in B group: from 0.5 to 0.1, and in the C and D groups, Pr decreased by half. We showed that the reproductive capacity was influenced by two factors: the type of crossing and the generation of directional selection. However, the effect of the interaction of two of the factors was significant only for litter size ($p = 0.0093$). In general, the results showed that breeding for darker fur is promising; further selection will allow normalisation of the reproduction of dark sables.

P8 | Impact of season and old age on Chios ram semen quality characteristics

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The aim of the present study was to provide information about the impact that season and old age may have on Chios ram semen quality. The research was performed biweekly during a year, including both breeding and non-breeding periods, on 7 Chios breed rams, separated in two age groups: 3 rams of 2–6 years old (mature) and 4 rams of 9–13 years old (old). After collection, semen volume and sperm concentration were measured. Motility and kinetics were assessed by Computer Assisted Sperm Analyzer. Viability was evaluated by Eosin-Nigrosin stain, morphology by SpermBlue®, DNA integrity by Acridine Orange Test and sperm membrane biochemical functionality by the Hypo-osmotic swelling test (HOST). Two separate statistical analyses, one between ram age and one between period groups, were performed by two-way repeated measures ANOVA. Motility and viability did not significantly differ neither between seasons ($p > 0.05$), nor between age groups ($p > 0.05$). Non-breeding period negatively affected semen quality showing higher rate of hyperactivated spermatozoa ($p = 0.011$), head abnormalities ($p = 0.001$), and lower percentage of HOST positive spermatozoa ($p < 0.001$). Tail abnormalities were increased during breeding period ($p = 0.022$). The following results refer to the comparison of semen traits between ram age groups, independently of the season. Both semen volume and concentration had significantly higher values in old, compared to mature ram group ($p = 0.001$ and $p < 0.001$, respectively). Ram age had no effect on sperm morphology, DNA integrity and HOST positive spermatozoa percentage ($p > 0.05$). In conclusion, old rams up to the age of 13 years preserve high semen quality, while non-breeding season seems to negatively affect Chios ram sperm membrane biochemical functionality and head morphology.

P9 | The reproductive and economic impact of postpartum uterine diseases in dairy cattle herds

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The aim of this study was to quantify the impact of postpartum uterine diseases on reproductive parameters, and to estimate their economic losses on large dairy farms. The data of 3,660

calving events that occurred in 2016 and 2017 on five Hungarian Holstein-Friesian farms were analysed. Information about retained placenta (RP; recorded on the day after calving), uterine inflammation (metritis and endometritis; first assessed on day 5 after calving, and weekly thereafter), and their treatments were collected. Intrauterine antibiotics, intrauterine solution containing iodine, and PGF2α were administered, based on the mucus quality. Reproductive parameters were calculated based on cow-level data (n = 3,660), and analysed by linear and logistic regression, and Dunnett test. Cost of open days, excess semen usage, and treatments were considered in the economic calculations (1 EUR = 320 HUF). Uterine treatment was required in 42.7% of the cows; 13.3% because of RP, and 29.4% due to uterine inflammation. Uterine treatments and RP were more likely in multiparous cows (odds ratio: 1.22 and 2.05, p = 0.0098 and p < 0.0001, respectively). Calving to conception interval increased by 2.7 and 28.3 days, services per conception by 0.9 and 2.2, and first service conception risk was reduced by 4.9 and 4.0% points, in cows with RP and uterine inflammations, respectively. Each case of RP caused 38.8 EUR loss, of which treatment cost had the largest share (46.4%). Uterine inflammations caused 122.8 EUR loss per case, with days open having the largest share (57.6%). Postpartum uterine diseases are serious economic concerns, therefore, control strategies should be implemented in large dairy herds.

P10 | Serological variability of leptospira cultures aborted cows

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Leptospirosis, a disease of many species of animals and humans, can lead to loss of productivity and abortion in cows, the birth of non-viable offspring, mastitis, an increase in the number of somatic cells. In cattle, the possibility of infection with leptospirosis during sexual contact is described. Leptospirosis is a disease caused by various antigenic variants (serovars) of the pathogen. The aim of the work is to establish the etiological structure of leptospirosis in reproductive pathology of cattle in the farm of the North-Western region. The selection of blood for research was carried from aborting cows. Microagglutination test (MAT) with sera of aborting cows was performed according to State Standard 25386-91 with diagnostic strains of seven serogroup Leptospira (Pomona, Hebdomadis, Grippotyphosa, Icterohaemorrhagiae, Canicola, Tarassovi, Sejroe). Leptospira agglutination was detected in the dark field of the microscope (magnification 20 × 10 or 40 × 7). As a result of monitoring of leptospirosis, we found positive samples in 2005–2006 in cattle on average in 11.95%, for small ruminants 7.95%, for horses 45.82%. For comparison, in the period from 1973 to 1985, positive samples were found in cattle on average in 21.15%. In aborting cows from 2005 – 2006, in microagglutination test (MAT), serum showed agglutinating antibody titers of 1:100–1:200. A positive reaction is interpreted when 1:400–1:800 in paired sera over the first 2 or 3 weeks are found. The maximum serum titer depends on the serovar, but is achieved by about 3–4 weeks of illness. Our study indicates the potential role of leptospira serogroups in the reproductive pathology of cows.

P11 | The occurrence of subclinical endometritis (SEM), and effect on pregnancy rate in Norwegian Red cows

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Internationally, there is great concern about increased embryo loss in the dairy cow and the occurrence of SEM as a major cause. The Norwegian Red cows have good fertility, but the prevalence of SEM is unknown. The objective of the study was to assess the prevalence of SEM at 1. AI PP, and the effect on pregnancy rate (PR). Totally, 991 cows located in 120 herds were included in the study. Vaginal mucus obtained by Metricheck was scored, and BCS recorded. Endometrial cytology samples were collected by the cytotape method. Totally, 300 cells were counted at 400× magnification, and the proportion of polymorphonuclear neutrophils (PMN) calculated. Pregnancy was diagnosed by rectal palpation or PAG analysis. The average interval to 1. AI was 71.3 d (SE ± 0.95), and at least 1 PMN was counted in 58.8% (583/991) of the samples. Defining SEM ≥ 1% PMN (Pascottini et al., JDS 2016;100:588–97), the proportion of SEM positive cows was 43.7% (433/991). The overall PR was 65.9% (653/991), with 67.2% (375/558) and 64.2% (278/433) for cows with < 1% PMN and ≥ 1% PMN, respectively, and hence no significant difference in PR by the Chi-square test. The PR was not associated with the occurrence of PMN at the cut-off level of 0.1% or 5%, or PMN as a continuous variable. Univariate logistic regression analyses clustered on herd level, revealed that only barn type and AI-technician were associated with PR to 1. AI. However, barn type, season, calving to 1. AI interval, AI-technician, parity, average milk yield per cow-year, season, mucus score, and the occurrence of red blood cells in the uterine smears were associated with the occurrence of PMN (0/1). In conclusion, the prevalence SEM (≥ 1% PMN) at 1. AI was high, and association to PR is unclear in Norwegian Red.

P12 | Birth and afterbirth period in mares of the Yakut breed

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Horse-breeding for meat production is a traditional branch of the agriculture of the Republic Sakha (Yakutia). The Yakut horse is the local breed which is on pasture all round the year in severe natural climatic conditions of the North-East of Russia. The live-stock compromises around 180'000 horses. The aim of the investigations was to study the peculiarities of the delivery process and afterbirth period of the horses of the Yakut breed and the adaptation mechanism to the environmental conditions. We have investigated n = 190 mares who gave birth in the period 2017–2018. Before parturition, the mares are transferred to pens, where they can be monitored. The preparatory stage of the delivery took place from 14:00 p.m. till 24:00 p.m., the largest quantity of foal delivery (66.7%) took place from one in the morning to six in the morning, no foal birth was observed from 15:00 p.m. till 24:00 p.m. The second stage of the delivery in the mares of the Yakut breed lasted 15 ± 2.85 minutes. During the foal birth, the afterbirth separation of the foetus capsule took place as a result of umbilical cord stretching when the mare stood up. When the foal was born without the capsule, in 3 or 5 minutes, sparse afterbirth labours and attempts occur, and in 5 or 10 minutes the separation of the foetus capsule was observed. The foal birth season lasted 60–75 days, however, most of the parturitions took place in a short period from April 25th till May 20th, depending on the level of pasture feed, fatness of the mares, and meteorological conditions of the year. Conclusion: early terms of foal birth in March and at the beginning of April are not desirable, and also late foal birth at the end of May and in June are not desirable because of the natural climatic conditions.

P13 | A vaginal fibroleiomyoma in a ten-year-old spayed pug dog – a case report

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A ten-year-old pug, spayed two years before, was presented for a bleeding vaginal mass. The owner reported tenesmus, dysuria progressing to anuria and a mass protruding intermittently from the vulva. There was no report of signs of heat after ovariectomy or exogenous estrogen administration. Antimüllerian hormone and progesterone were 0.01 and 0.1 ng/ml respectively. Vaginal examination revealed a single, painful mass with areas of calcification and necrosis, measuring 5 × 5 cm, involving the dorsal wall of the caudal vagina. Vaginal cytology showed no sign of estrogen impregnation. No ovarian tissue was identified at abdominal ultrasound. The mass was resected through an episiotomy and a partial thickness incision was made around the pedicle. Because of diffuse bleeding, hemostasis was controlled with vaginal mesh left in situ during 48 h. Histology revealed a fibroleiomyoma with some atypical areas with anisokaryosis. Estrogen receptors alpha ER 1D5 were found by immunohistochemistry in the tumor, including atypical areas, healthy vaginal epithelium and smooth muscles. Half of the benign vaginal tumors have estrogen receptors. In this case, there is no current evidence of residual ovarian syndrome. The first hypothesis is that the vaginal mass was already present at the time of ovariectomy. Therefore vaginal examination before late neutering should be recommended. The second hypothesis is that the mass appeared and grew after sterilisation without estrogen stimulation, even if estrogen receptors were present. In fact, these were found in healthy vaginal tissue. It is suspected that they persist without estrogen stimulation. Contrary to mammary tumors, malignancy of vaginal tumors cannot be predicted in spayed bitches.

P14 | Application of Genome Resource Banking (GRB) with lipid-rich embryos in cats

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Preimplantation embryos of some mammalian species including domestic cat are lipid-rich and this is an obstacle for their cryobanking. We compared different options for Felidae preimplantation embryos cryopreservation using domestic cat as a research model. In-vitro-derived 4–8-cell stage embryos were obtained after in vitro fertilization (IVF) of in vitro matured (IVM) cat oocytes with the domestic cat epididymal spermatozoa. A total of 37 embryos were frozen with a standard controlled-rate slow freezing method using a programmable freezer (CL 8800; CryoLogic, Australia) and 0.25 ml straws (Cryo Bio System, France). Ham's F-10 medium supplemented with 10% propylene glycol (PG) and 0.125 M sucrose was used as a cryoprotectant solution. A total of 22 embryos were vitrified with the PG (20%), dimethyl sulfoxide (DMSO) (20%), and sucrose (1 M) cryoprotectant mixture. A total of 22 embryos were used as a non-frozen control group. It was found that both cryopreservation options lowered the probability of the post-warming development of cat embryos to the morula stage (64.9% and 68.2%, respectively) compared with the non-frozen group (95.5%). Nuclear fragmentation rate in

the slow freezing group (24.78 ± 4.85) did not differ from controls (8.90 ± 2.45), but vitrification caused the higher nuclear fragmentation rate (40.53 ± 10.74) as compared to the control group. Our results indicate that the slow freezing with PG provides less DNA damage in cat embryos in comparison to vitrification. A degree of intracellular lipid unsaturation examined in this study with the use of Raman spectroscopy was relatively high (0.108 ± 0.013) for cat embryos. We consider this as one of the reasons for the successful applicability of slow freezing for the lipid-rich domestic cat embryos.

P15 | The influence of the structure of teat / udder of cows on development of the teat end hyperkeratosis

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The purpose of our work was to study the branching of major milk ducts, the structural features of the gland cistern, teat cistern and the concomitant finding of teat end hyperkeratosis. Ultrasound scan was done in 167 quarters of the udder of lactating cows with the transducer placed on the base of the teats. Results: Large volume of single major ducts in the lower part of the gland cistern were mainly recorded in the anterior quarters of the mammary gland (59.1%), average volume gland cistern with large diameter major ducts were identified with comparable frequency in the front and rear quarters. Gland cisterns with no cavity with different diameter major ducts were identified mainly in the back quarters of the udder. Established absence of a very rough teat ring was noticed in the quarters with large volume gland cistern and single major ducts. More than 50% of the quarters with rough and very rough teat ring had multiple small milk ducts and an absent gland cistern cavity. The wall of the teat cistern can have a different number of longitudinal folds, which affects the condition of the teat end. The more pronounced the teat cistern wall folds, the more often the very rough teat ring is recorded. For example, a large number of longitudinal folds of the teat cistern wall was recorded in 26.7% of cases with a very rough teat ring and only 1.8% with no ring teat. Conclusion. High risk of development of teat end hyperkeratosis occurs in cows with a gland cistern with a lack of a cavity and multiple small milk ducts and with an increased folding of the wall of the teat cistern.

P16 | Expression of vaspin and GRP78 in corpus luteum are dependent on estrous phase in Large White pigs

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Vaspin is an adipokine involved in the development of obesity, insulin resistance or pathogenesis of inflammatory reactions in the body. Vaspin receptor is still unknown, however, in endothelial cells vaspin can prevent apoptosis by binding 78-kDa glucose-regulated protein receptor (GRP78). In our previous study, we observed vaspin expression and its regulation by several factors like gonadotropins, steroid hormones and insulin in the porcine ovarian follicles. The aims of the present study were to investigate mRNA and protein expression as well immunolocalisation of vaspin and GRP78 in corpus luteum (CL). CLs were collected from mature Large White sows during the early (CL1), middle (CL2) and late (CL3) luteal phase. Vaspin/GRP78 mRNA, protein levels and immunolocalisation were determined by real time PCR, Western Blot and immunohistochemistry, respectively. Statistical analyses were performed using Graph Pad Prism 5 software and the data were analyzed using a one-way ANOVA, by Tukey's honest significant difference test. We observed that mRNA expression of vaspin and GRP78 were higher in CL2/3 than CL1, however on protein level vaspin expression was smallest in CL3, while GRP78 was higher in CL2/3 ($n = 4$, $p < 0.05$). There was vaspin staining in the cytoplasm of both small and large luteal cells ($n = 3$). Taken together, expression of vaspin/GRP78 in CLs are depending on the estrus cycle suggest that this proteins can be regulated by progesterone levels. Overall, the expression of both components (ligand and receptor) of the vaspin signaling system provides the opportunity for an analysis of the direct role of vaspin in porcine corpus luteum function and tissue development. Supported by DS/MND/WB/IZ/11/2018.

P17 | The first application of the four-channel EMG telemetry system for uterine activity measurements in the large animal model

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Recently reported three-channel EMG (electromyography) telemetry systems are not suitable for studying uterine and cervical activity simultaneously because of the limited number of channels.

Cervical dysfunction causes or contributes to infertility, premature birth or loss of the pregnancy. Therefore the measurement of the electrical activity in the cervix in communication with the uterus is possible with the application of the new four-channel measuring system. The experiment was conducted on mature Polish Landrace sows (n = 6). Four silver bipolar electrodes connected to the new four-channel telemetry transmitter (L04) were surgically positioned in caudal and cranial part of the cervix in the muscle layer with 6 cm distance between electrodes and in the muscle layer of right and left uterine horn 10 cm from the uterine body. The EMG signals were recorded simultaneously during in vivo experiment using the new digital software platform (Ponemah) and the semiautomatic analytical software (NeuroScore 3.3.1). The first results obtained in diestrus were characterized by high repeatability between animals in parts of the reproductive tract. No differences ($p > 0.05$) in (mean ± SD) amplitude (A) (0.15 ± 0.06 mV), RMS (0.02 ± 0.006 mV), and duration of activity (D) (5.32 ± 2.51 s) between animals were noted in cranial and caudal part of the cervix (A: 0.18 ± 0.09 mV; RMS: 0.02 ± 0.01 mV; D: 6.25 ± 3.39 s) and both horns (right: A: 0.22 ± 0.14 mV; RMS: 0.02 ± 0.01 mV; D: 4.42 ± 4.05 s; left: A: 0.21 ± 0.03 mV; RMS: 0.02 ± 0.009 mV; D: 4.35 ± 3.44 s). The four-channel EMG telemetry system is accurate and useful in the determination of the cervical activity in the large animal model.

P18 | Holographic presentation of swine ovaries in "mixed reality" reconstructions derived from 3T MR images -feasibility study

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Magnetic resonance (MR) is an advantageous tool allowing a complex pelvic exam with high tissue contrast. 2D and 3D datasets can be derived and often used for 3-dimensional reconstructions. The aim of the study was to present a novel method for MR data post-processing and presentation of the ovaries in the pig using a mixed reality holographic approach. MR examinations were performed in six Polish Landrace gilts under general anesthesia in a standard sternal recumbency on 3T MR scanner (Discovery MR750w 3.0T, GE Healthcare, Milwaukee, USA). T2-weighted images in sagittal, coronal and axial planes were acquired in PROPELLER k-space acquisition mode with the following parameters: TR/TE eff > 2500/100 ms; acquisition matrix: 384 × 224; slice thickness 3 mm. The images were imported into CarnaLife Holo Software (MedApp S.A., Kraków, Poland). 3D reconstructions of pelvic organs were created and displayed in mixed reality by means of holographic head mounted display (HoloLens, Microsoft, Richmond, USA). The resulting holograms from all studied animals allowed for a detailed 3-dimensional localization of right and left ovaries in all studied subjects. The main landmarks used for ovaries respective orientation were bladder and lumbosacral spine. Obtained holograms could be manipulated by means of operator's head movements, voice control and hand gestures allowing advanced image manipulations. In this study, we have proven the usefulness of holograms as new method for 3-dimensional MRI image post-processing and presentation. The information could be potentially useful during operations and percutaneous procedures. Further tests will include superimposing of holograms on animal body intraoperatively. Funded by KNOW (05-1/KNOW2/2015).

P19 | Catch-up growth in pigs with intrauterine growth retardation syndrome (IUGR) is related to impaired glucose metabolism and preferential use of fat as a source of energy

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IUGR syndrome is a consequence of reduced glucose and amino acid supply via placenta which leads to compensation of growth postnatally. The aim of the study was to better understand the catch up growth (CG) phenomenon in IUGR pigs. IUGR pigs (n = 20) were compared to normal birth weight pigs (n = 20; NBW) for 6 months after birth. Two weeks after weaning half of the pigs started to receive high energy diet (HED, 150% ME requirements). Each month body mass control and oral glucose tolerance test (OGTT) was performed. Uniformity of the SD and Kolmogorov-Smirnov A test was performed to evaluate the normal distribution. Student's t-test was used to compare data from the two groups (GraphPad Prism software v. 5). Body mass ratio (BR) IUGR/NBW after standard at day 0 was 0.63. After weaning, BR decreased dramatically to 0.33. CG in IUGR pigs was observed between third and fourth month of life, when BR increased to 0.5 and 0.85, respectively. Increased glucose level in OGTT after 1 and 2 h was observed in IUGR in the second month compared to NBW (247 ± 38 vs. 142 ± 9 and 208 ± 35 vs. 99 ± 17, $p < 0.05$). HED increased body weight of IUGR pigs in second and 3rd month. HED had a negative effect for growth in NBW group at all times measured. In conclusion, consequences of IUGR in pigs are observed during all life as strong retardation of growth after weaning and limitations to glucose uptake. However, HED in IUGR pigs during this time may improve increase of weight. These data are convergent to our previous results, that, in IUGR fatty acids may be preferential as source of energy. Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

P20 | First overall report of seroprevalence of Leptospira in horses in Poland

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The prevalence of leptospirosis is unknown, but serologic evidence indicates a higher incidence than is apparent clinically. Almost all epidemiological studies have relied solely on serology, with the reported differences in serovar prevalence according to geographical region. In the present study, we have aimed to estimate seroprevalence of Leptospira which is the early stage of a larger project to determine the occurence of Leptospira species in the population of horses in Poland. A total of 5405 horse serum samples were collected from 2015 to 2018 in the monitoring program from all 16 provinces of Poland. All the serum samples were tested by microscopic agglutination test (MAT) with the panel of 8 serovars representative of 7 serogroups, most often found in horse population in Poland: Icterohaemorrhagiae, Grippotyphosa, Sejroe, Pomona, Canicola, Bratislava, Hardjo and Poi. The minimum sera dilution was 1:100. During 4 years, the seroprevalence of Leptospira in horses was as follows: 2015 (20.3%), 2016 (14.73%), 2017 (12.95%), 2018 (11.22%). The most common serovars between 2015–2018 were: Sejroe (~ 4%), Grippotyphosa (~ 3.9%) and Pomona (~ 1.9%). The remaining positive horse serum samples in the MAT showed positive reactions with serovar Poi (~ 1.4%), Bratislava (~ 0.7%), Canicola (~ 0.6%) and Hardjo (~ 0.4%). Numerous cross-reactions between two or three serovars have been observed. The results of our study show the high presence of seropositive samples tested for serovars of Leptospira in horses in Poland. A map of Poland showing seroprevalence of Leptospira in horses has been created. The positive correlation of the occurrence of antibodies related to the age of animals (> 5 years) and type of keeping animals (stud farms versus individual) was confirmed.

P21 | Anti-Müllerian hormone concentration and ovarian function in dairy cows

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Anti-Müllerian hormone (AMH) concentration can be used as a marker for the total number of morphologically healthy follicles in ovaries. Produced by granulosa cells of growing follicles, expression of AMH starts as soon as follicles are initially recruited. The aim of the study was to analyze correlation of AMH with pregnancy status, number of follicles and retrieved oocytes in Holstein cows. A total of nine pregnant (P, 44-85 d) and 15 non-pregnant (NP, 46–68 d pp) cows were used as donors. To obtain oocytes, ovum pick-up (OPU) procedure was used. Prior to OPU, cows were restrained and epidural anesthesia was carried out using Xylazine (0.05 mg/kg body weight) diluted in 5 ml of saline. Ovaries were scanned with a 7.5 MHz micro-convex transducer and follicles ≥ 3 mm were punctured using an 18G needle coupled to the aspiration device and vacuum system. Follicular fluid was transported to the laboratory within one hour. On the day of OPU, blood samples were collected. After centrifugation at 1000 *g* for 10 min, plasma was collected and stored at-20°C until further analysis. AMH was determined with ELISA (Laboklin, Bad Kissingen, Germany). In P cows 58 follicles were punctured, 30 (51.7%) cumulus-oocyte complexes (COCs) aspirated and in non-pregnant cows 98 follicles and 31 (31.6%) COCs aspirated. Per OPU session, in P and NP cows a mean of 6.4 ± 2.6 and 6.5 ± 4.3 follicles were punctured (p = 0.741 Wilcoxon test) and 3.3 ± 2.2 and 2.1 ± 1.8 COCs aspirated (p = 0.171), respectively. In P and NP cows, concentrations of AMH were 0.28 ± 0.18 and 0.23 ± 0.12 ng/ml (p = 0.255), respectively. In conclusion, between P and NP cows there was no difference in the concentration of AMH, number of follicles and retrieved oocytes per cow/session. Supported by ARIB project F160079VLBS.

P22 | Influence of semen storage on the responsiveness to a sensitizer of receptor-gated intracellular calcium channels in boar spermatozoa

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Calcium (Ca2 +) has been identified as a key component in sperm function. Ca2 + -homeostasis is tightly regulated by intracellular calcium stores. The aim was to test whether hypothermic semen storage affects the response of boar spermatozoa to thimerosal (Thim), a sensitizer of inositol 1,4,5-trisphosphate and ryanodine receptor-gated intracellular Ca2 + channels. Semen (n = 6 boars) was diluted in Beltsville Thawing Solution and stored at 5°C or 17°C. Fluo-4/AM-loaded aliquots of stored samples were incubated in a bicarbonate– and Ca2 + -free Tyrode's solution at 38°C. After 3 min, 100 μM Thim was added and subsequent changes in the free intracellular Ca2 + concentration ([Ca2 +]i; Fluo-4) were monitored in viable (propidium iodide negative) spermatozoa using continuous flow cytometry measurements for 6 minutes. Data were analyzed with ANOVA for

repeated measurements and Tukey's test (SAS Enterprise Guide v7.1). The initial [Ca2 +]i was higher in all samples stored at 17°C compared to 5°C ($p < 0.001$). At 24 h and 72 h, the rise of [Ca2 +]i indicated by an increase of relative Fluo-4 fluorescence intensity (F1/F0) after 3 min was higher ($p < 0.05$) in samples stored at 5°C compared to 17°C (x-fold: 24 h, 5°C: 2.0 ± 0.2 vs. 17°C: 1.7 ± 0.2; 72 h: 5°C, 2.2 ± 0.4 vs. 17°C: 1.8 ± 0.1); at 120 h, there was no difference in F1/F0 between the temperature groups. At 72 h and 120 h, the [Ca2 +]i remained higher in samples stored at 17°C until 5 min after the addition Thim ($p < 0.05$). Storage duration did not influence the response to Thim within the temperature groups. In conclusion, viable boar sperm stored at 5°C maintain their sensitivity to Thim, a stimulator of intracellular Ca2 + channels, but [Ca2 +]i remains at lower level compared to spermatozoa stored at 17°C.

P23 | Meaning of adhesion of Staphylococcus aureus in bovine mastitis in the North-East of Poland

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Adherence of S. aureus to the mammary gland epithelium is an important factor of pathogenicity of staphylococcus which leads to occurrence of mastitis. It allows tissues colonization along with development and spread of infection. It is particularly important in mammary gland infection because S. aureus attached to epithelial cells makes it impossible to flush bacteria with flowing milk. The occurrence of injuries in the entrance of the teat canal causes increased adhesion and more severe symptoms of mastitis. In the investigation, the adherence ability of S. aureus isolated in milk from cows with clinical and subclinical mastitis in the Nord-East Region of Poland (the Podlaski Region) was tested. Epithelial cells were sampled right after slaughter with cytobrush straight from milk sinus and teat sinus from cows in lactation. Measurement of adherence ability was conducted with Frost method. 45 S. aureus strains were tested. In the statistical analysis, the average number of individual S. aureus strains adhering to a single epithelial cell was diversified and varied from 5.61 to 14.36 and mean value was 9.05 ± 0.4. Genetic tests with ADSRRS–fingerprinting method showed nine patterns of bands. The most frequently isolated in Podlaski Region was genotype D (65%) and strains belonging to this predominant genotype had higher ability to adhere compared to strains that belong to other genotypes, which could be the reason for increase in S. aureus isolation in this region. The examination confirmed that S. aureus were showing higher adhesion towards epithelial cells which were acquired from teats with abrasions. Funded by KNOW (Leading NRC) Scient. Consort. “Healthy Animal – Safe Food”. decision of Min. of Science and Higher Education No. 05-1/KNOW2/201.

P24 | Comparison of two estrus synchronisation protocols in Polish Limousin beef cattle herds: preliminary results

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Demand for genetic improvements and more efficient work procedures causes growing interest in estrus synchronization and AI protocols in beef cattle. The purpose of the study was to evaluate the applicability and to compare the efficiency of the Ovsynch (OVS) and PRID with eCG in Polish Limousin beef cattle. The study included 62 clinically healthy cows from three herds in the north-eastern Poland. Cows from Group A (n = 19) were regularly examined with ultrasound paying particular attention to the ovarian function, starting from day 60 after parturition. After finding follicles of a diameter of 8 mm they were injected with buserelin acetate. Cloprostenol was given 7 d later and buserelin 2 d after. 18 hrs after the 2nd buserelin injection AI was done. In group B (n = 22) a progesterone releasing intravaginal device (PRID) was inserted, and 6 d later PGF2α was injected. On day 7 PRID was removed and eCG was administered at the same time. Insemination was done 12 hrs after the first signs of estrus. The animals of the control group C (n = 21) were inseminated during spontaneous estrus by the 90th day after calving. All cows were checked for pregnancy using ultrasound on day 30 and day 50 after AI. In addition, tolerance reflex was checked with a help of a vasectomized bull. Statistical analysis was performed by ANOVA summary with Tukey's multiple comparisons test. Results in groups A, B, C showed pregnancies in 7, 10 and 14 cows ($p > 0.05$), respectively and tolerance reflex was present in 8, 14, 19 cows ($p < 0.05$ group A from B and A from C), respectively. Protocols of estrus synchronization can be used in Limousin beef cattle. OVS or PRID with eCG give comparable results, however the second method seems to be more suitable for natural mating due to a better tolerance reflex.

P25 | Intra-ovarian platelet rich plasma before treatment enhances superovulation response in Holstein cows: preliminary results

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Superovulation is still impaired by a high variability of individual response. Aim of the present study was to investigate the role of intra-ovarian administration of platelet rich plasma (PRP) for the improvement of ovarian function. Platelets contain many factors known to inhibit apoptosis and fibrosis, enhance angiogenesis and stimulate mitosis. Eight Holstein-Friesian cows, 3 to 4 years old,

with a history of normal fertility, were enrolled in this study. Two days before a luteolytic PGF2alfa dose, in each animal the right ovary was considered as control while the left one was injected by ultrasound guidance with 5 ml of autologous PRP. Nine days after induced estrus, all cows were superovulated with 50 mg Folltropin in ten decreasing doses and inseminated twice by the same cryopreserved semen. Seven days after AI, right and left uterine horns were separately flushed. All data were evaluated by Student's T-test. By ecography, before PRP treatment, the average number of follicles, on the right ovaries was 9.18 ± 1.35 and on left ones 7.32 ± 1.67 ($p > 0.05$). Two days post-treatment with PRP, the average number of follicles on control ovaries was 7.67 ± 2.52 and in treated ovaries 8.00 ± 2.00 ($p > 0.05$). At the last Folltropin injection, i.e. onset of proestrus, in the control ovaries the average of follicles was 11.33 ± 2.89 and in treated ovaries 20.00 ± 9.17 ($p < 0.05$). By flushing, 6.67 ± 2.31 grade 1–2 blastocysts were collected from the uterine horn omolateral to control ovaries and 14.67 ± 9.29 from the treated ones ($p < 0.05$). Our data suggest that PRP could stimulate latent follicles and in vivo embryo production.

P26 | The molecular and tissue regulation of progesterone receptors in endometrium in progesterone-treated dairy cows in anestrus type II

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Hormonal treatment of anestrus type II includes a progesterone (P4) supplementation resulting in the regulation of cell development, differentiation, and endometrial function. P4 autoregulates production of endometrial receptors (PR, isoforms A and B) at the level of transcription and the hormone binding in target tissues. We determined the response of PR in bovine endometrium on the exogenous P4. The study was conducted on Holstein-Friesian non-pregnant lactating cows, both cyclic (n = 10) and in anestrus type II (n = 23). Uterine biopsy samples were collected before application of intravaginal P4 insert (1.55 g of P4; 8 days) (d1) and one day after removing the insert (d9). In corresponding samples, PR mRNA was isolated and multiplied using specifically paired starters (PRAB-GAPDH; PRB-GAPDH), PR expression was quantified using scanning cytometry after immunofluorescent staining (primary Ab anti-PRAB), and the Spearman's rank-order correlations were calculated. In cyclic cows at d1 positive correlations were observed for mRNA expression between PRAB/PRB (Sp = 0.64; p = 0.04) and PRAB/PRA (Sp = 0.74; p = 0.012), and at d9 between PRAB/PRB (Sp = 0.64; p = 0.04) and PRA/PRB (Sp = 0.61; p = 0.003). In anestrus type II at d1 positive correlations were found for mRNA expression between PRAB/PRB (Sp = 0.75; p = 0.0003) and PRA/PRB (Sp = 0.54; p = 0.007), and at d9 between PRAB/PRB (Sp = 0.57; p = 0.004) and PRA/PRB (Sp = 0.62; p = 0.001). No other correlations, especially between PR expression in the tissue and mRNA levels were observed. In progesterone-treated cows in anestrus type II, the differences in the mutual relations between the A and B isoforms of PR indicated different P4-dependent autoregulation pathway than in cyclic cows. Funded by KNOW (No. 05-1/KNOW2/2015).

P27 | The quantification of the histological features in equine endometrosis based on the Masson's Trichrome staining

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Equine endometrosis represents degenerative lesions of endometrium considered as an important cause of infertility in mares mainly older than 12-years old. Histological features of endometrium allow assigning mares into I, IIA, IIB or III category, associated with decreasing fertility. The aim was to propose a quantification method for histological features crucial in the diagnosis of equine endometrosis. Endometrial biopsy samples (n = 63) were stained with hematoxylin-eosin (HE) and Masson's Trichrome (MT) protocols. HE-stained slides were examined under a light microscope and representative group of samples in category I (n = 10) and III (n = 10) were selected. MT-stained slides were then scanned using semiautomatic analysis of slides on brightfield system (TissueFaxs Plus). In each sample unaffected/affected regions were distinguished based on the morphology of glandular epithelium (normal/ fibrotic foci), color of cytoplasm (dark-pink (DPC)/light-pink (LPC)), and the shape and location of nuclei (small, round, near the basement membrane/large, oval, central in the cell). The significant difference in a palette of colors between DPC and LPC allowed the quantification of areas of each color using advanced HistoQuest PLUS image analysis software. The area of LPC was higher ($p < 0.001$) in category III (22.97% ± 4.56) than in category I (3.62% ± 1.09), with no differences ($p > 0.05$) in the area of DPC (12.81% ± 4.52/11.53% ± 3.76). The degree of fibrosis (blue stained fibers) was higher ($p = 0.0122$) around LCP (49.97% ± 12.18) than DPC (24.61% ± 12.68). We suggested the use of TissueFaxs Plus, on MT stained biopsy sections, as a diagnostic tool possible to consider in further investigations, that allow quantifying the histological features of the endometrium. Funded by KNOW (05-1/KNOW2/2015).

P28 | Clinical picture and ultrasound examination of cystic endometrial hyperplasia (CEH) in lion (Panthera Leo) resulting from long lasting medroxyprogesterone acetate (MPA) – a case report

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Cystic endometrial hyperplasia (CEH) is a common lesion in the uterus in bitches which predisposes to bacterial infection, however, it occurs more rarely in female felids. Some studies reported CEH occurrence in captive lionesses; however, every new case description broadens the knowledge about this specific condition. A 7 years old lioness treated with Medroxyprogesterone Acetate (MPA) for 5 years presented periodic muco-bloody discharge from vulva for one year without any other clinical symptoms. Ultrasound showed oedema in corpus (CU) and horns (HU) of uterus without any fluid. In CU (diameter 3.12 cm) hypoechogenic, round foci were found, which indicated CEH. Ovaries were also examined: left (5.68 × 2.56 cm) was homogenous, while right (4.58 × 2.65 cm) contained follicle/cyst with thin wall and size 1.88 × 1.54 cm. Traditional ovariohysterectomy (OVH) was conducted, after which lioness has fully recovered. Uterus was pale-pink, with longitudinal folds and macroscopic changes similar to those detected in queens with CEH after MPA treatment. Ovaries were evaluated after extraction, two corpora lutea and large follicular cyst on the right ovary were found. We stated hypoplasia and hyperaemia of endometrium and presence of mucus with blood in the lumen of uterus. Above observations suggest similar pathway of receptor response on MPA treatment in lions, but with lower intensity. MPA can lead to CEH, so in case of lionesses OVH can be considered at younger age. Evaluation of endometrium and ovaries via ultrasound seems to be equally useful as in domesticated felids. Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

P29 | Postpartum uterine involution in free farrowing sows examined by ultrasound

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Puerperal disease, e.g. metritis, is one of the most important disorders leading to culling of sows. The inflammation of the uterus is suspected to be associated with delayed uterine involution influencing the further reproductive performance of the sow. The aim of the present study was to describe the continuous regression of the uterine diameter in healthy sows after farrowing by means of ultrasonography. Therefore, 46 sows were examined daily from day two until day 14 after parturition and on the day before weaning. In 90% of the sows, the uterus diameter could be detected on all study days, while on the day before weaning a significant reduction to 71% detected uteri was observed. The median of the uterine diameter declined from 32.4 mm (min: 18.6 mm, max: 52.3 mm) on day 2 to 9.0 mm (min: 7.6 mm, max: 12.7 mm) one day before weaning. A significant reduction of the uterine diameter from day two until day ten was identified with an exception from day five to day 6 and day 8 to day 9. In addition, a significant reduction of the uterine diameter from day 14 (median: 9.9 mm; min: 8.0 mm, max: 13.4 mm) in comparison to day 28 (median: 9.0 mm; min: 7.6 mm, max: 12.7 mm) was detected. The ultra-sonographic examination is a practical and adequate tool to describe the uterus involution in sows. Contrary to findings in the literature, the diameter of the uterus seems to decrease until weaning and not only until day fourteen. Further studies are needed to evaluate the influence of the uterine involution on the further reproductive performance of the sow.

P30 | The evidence of bio-distribution of multimodal oxide nanoparticles to gonads using the rat model – a preliminary study

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The MRI method is appropriate to detect pathological lesions such as ovarian edema, ovarian vein thrombosis or small testicular tumors. Some of these potential applications make use of dynamic contrast-enhanced procedures. Using contrast agents improve the quality of imaging. However, according to recent research, just a single application of the contrast agent has a negative impact on the quality of the sperm. Our team runs the researches on the innovation intragastric non-Gadolinium contrast agents. Those tested nanoparticles (NPs) were developed in the Institute of Physics, PAS. The NPs core was based on ZrO2, doped with Eu, as well as ZrO2 with Gd as a positive control. For the preliminary study, we used adult rats as model, which were oncological patients (n = 2 male and n = 3 female). Examined rats underwent the preliminary MR screening followed by oral application of NPs (10 mg/ml, 1 ml/rat). MRI was conducted 24 and 48 h before tumors were surgically removed. To all imaging sequences: T1 FSPGR 3D, T2 Cor were used. The study used the GE MR Discovery mr750w 3.0T GEM Flex coil (GE Healthcare, Milwaukee, USA). Obtained MRI

images were subjected to quantitative analysis of mean relaxation time in testicles, ovaries and tumor. Lung tissue was used as a control. The results are average brightness of VOI (volume of interest) expressed in organs in comparison to background in DICOM format images. Image analysis demonstrates an essential statistically significant change of the gonads luminosity after 24 h from the application (ovaries 144.7% ±12.3% compared to the measurement before administration) and a non significant decrease of the luminosity after 48 h from the application (ovaries 273.4%±25.3%, testicles 249.7%±18.4%). Funded by KNOW (No. 05-1/KNOW2/2015).

P31 | Rare complications after laparotomic ovariohysterectomy in a bitch – a case report

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Minimally invasive techniques of canine spaying such as laparoscopic ovariohysterectomy (LOHE) are gaining popularity among pet owners (Hsueh et al., 2018;47:015–025). LOHE seems to be beneficial for the animal's health due to reduction of pain, surgical stress and recovery time. Case report: An 8-years old chow-chow bitch was referred to the vet clinic due to vaginal discharge. The ultrasound (US) showed cystic-endometrial hyperplasia. LOHE was recommended. Before surgery, the bitch had antibiotic therapy (AT) (Enrofloxacin 5 mg/kg QD, 10 days) based on vaginal smear culture (multiple E. coli) and antibiogram. Blood analysis was performed revealing Leukocytosis (24.9 × 10*9/l). The surgery was successful with minor problems with electrocauterization (EC). On the 3rd day post-surgery the bitch started to vomit and had abdominal pain. US showed no abnormalities, blood analysis revealed severe anemia (Ht = 19.27%) and leukocytosis (59.03 × 10*9/l). Endoscopy and bone marrow biopsy were done to exclude comorbidities. Treatment with tramadol (2 mg/kg BID), ceftriaxonum (25 mg/kg BID), metamizol (50 mg/kg BID), Ringer lactate (50 ml/kg/ 24 h) was induced. However, blood test results worsened (Ht = 15.15%) and blood transfusion was performed. The dog started to recover gradually. We think that the major reason of this post-surgical complication were micro-hemorrhages due to micro-damage of vessels related to EC problems during surgery, although no bleeding was visible on US. Conclusions: Although LOHE is considered a very novel and minimally invasive technique of surgical spaying in dogs, it carries certain risk of complications. Anemia without internal hemorrhage due to problems with EC during surgery was not previously described. Publication was funded by KNOW, 05-1/KNOW2/2015.

P32 | Vascular changes evaluation in equine endometrosis using novel measurement methods in the Masson's Trichrome (MT) staining

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Endometrosis comprises chronic degenerative changes in the endometrium of older mares. Fibrosis affects glands, but also blood and lymphatic vessels. The aim of the study was to assess degenerative and fibrotic changes in and around blood vessels of endometrium in different stages of endometrosis, depending on the Kenney and Doig classification. Full-thickness sections of corpus uteri were taken in the luteal phase (n = 24), fixed, cut and stained with hematoxylin-eosin (HE) and Masson's Trichrome (MT). Samples were classified into categories I, IIA, IIB and III on HE. MT-stained slides were scanned using semiautomatic analysis of slides on brightfield system (TissueFaxs Plus) and analyzed with HistoQuest software. Areas (μm^2) of all blood vessels with a diameter > 40 μm in samples' endometrium were marked, including lumen, wall and perivascular fibrosis. Results (mean ± SEM) were compared between groups with Kruskal-Wallis and Dunn's multiple comparisons tests. The lumen of vessels was significantly ($p < 0.01$) larger in category III (2623 ± 580 μm^2) than in IIA (956 ± 127 μm^2) and IIB (869 ± 122 μm^2). Wall area was also significantly ($p < 0.01$) larger in category III (2301 ± 592 μm^2) than in IIA (715 ± 152 μm^2) and IIB (739 ± 124 μm^2) as well as fibrosis area in III (3981 ± 838 μm^2) than in IIA (984 ± 221 μm^2) and IIB (1059 ± 199 μm^2). However, the occurrence of vessel type (determined by lumen/wall ratio) did not differ significantly between groups ($p > 0.05$). We concluded that MT is useful in the evaluation of vascular degeneration and perivascular fibrosis in the course of endometrosis due to good connective tissue differentiation. TissueFaxsPlus allowed to determine the character of vessels remodeling along with the increase of endometrosis severity. Funded by KNOW (05-1/KNOW/2015).

P33 | Abstract withdrawn

P34 | The dynamics of body weight of sheep of the Volgograd breed with a different kinship ratio

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The aim of this study was to investigate how body weight changes of the youngstock sheep depends on the kinship Ratio in purebred and crossbreed lambs. N = 480 ewes of Volgograd sheep were

artificially inseminated using semen of rams: group 1 (n = 240) ewes with the semen of purebred rams (Volgograd breed), and group 2 (n = 240): ewes with the semen of crossbreed rams (1/4 North Caucasian breed). Semen quality was determined manually by morphological assessment of at least 500 sperms per sample. During the whole period of the study, all ewes and lambs were in one flock. The body weight of lambs was determined by individual weighing at birth, at 2.5 months and at 4.5 months (weaning). According to the study, the ejaculate volume of purebred rams was 1.4 ml, and the ejaculate volume of crossbreed rams was 1.3 ml. However, semen quality of crossbreed rams was higher than semen quality of purebred rams. Group 1 gave birth to 233 lambs, and group 2 gave birth to 235 lambs. There were 115 and 118 male lambs, and 118 and 117 female lambs, respectively. In the study, male lambs' body weights at birth were not different (3.71 kg and 3.69 kg) whereas female crossbred lambs were heavier than purebred ones (3.45 kg vs 3.3 kg). At the age of 2.5 months, male crossbred lambs outweighed purebreds by 6.94% (22.17 kg and 20.73 kg) ($p \leq 0.05$); female lambs' body weights were not different (19.98 kg and 19.88 kg). During weaning, male crossbred lambs outweighed purebred lambs by 20.85% (32.33 kg and 26.75 kg) ($p \leq 0.01$); female crossbred lambs outweighed purebred by 7.38% (27.20 kg and 25.33 kg) (students t-test). It can be concluded that the use of crossbreed rams with ewes of the Volgograd breed increases the weight of lambs at weaning.

P35 | Therapy of retained fetal membranes in cattle: comparison of two treatment protocols

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This prospective study was carried out to compare two treatment protocols of retained fetal membranes (RFM) in cattle. The routine therapy in Switzerland consisting of intrauterine tetracycline boluses (group Te) was compared with the administration of ozone foam (group Oz), both intrauterine therapies without attempts to manually remove the placenta. Primiparous and multiparous dairy cows of the breeds Holstein Friesian, Red Holstein, Brown Swiss, Simmental and their crossbreds with RFM were included. Pregnancy duration had to be over 265 d. Group assignment was done according to ear tag number (n = 70 Te, n = 65 Oz). Therapy consisted in 3 g Tetracycline or 8–10 ml ozone foam on days 1,4,7 post partum. Baseline parameters as age, breed, yearly milk yield, parity, pregnancy duration, calving season and number of routine intrauterine treatments were not significantly different between the groups. Number of days with rectal temperature over 39.7°C within the first 10 DIM were higher in the ozone group (Oz: 0.36d versus Te: 0.04d; p = 0.002). Number of escape therapies (based on rectal temperature over 39.7°C and reduced food intake) consisting of the parenteral administration of tetracyclines and NSAIDS in the first 10 DIM were not different between the groups, though (Oz: n = 4, Te: n = 5; p = 0.94). Neither number of inseminations/pregnancy until 200 DIM nor culling for fertility reasons were significantly different between the groups. Multiple logistic regression analysis revealed no significant risk factors for fever days except for treatment group. Ozone foam might be an alternative to Tetracycline boluses as therapy for RFM.

P36 | Noscapine inhibits collagen deposition induced by neutrophil extracellular traps components in equine endometrium

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Endometrial fibrosis (endometrosis) may cause mare subfertility/infertility. We have shown that neutrophil extracellular traps (NETs), besides killing pathogens, may also contribute for endometrosis. Elastase (ELA), cathepsin G (CAT) and myeloperoxidase (MPO) are NETs components stimulating in vitro endometrial collagen deposition. The aim was to evaluate noscapine (N, an antimitotic drug) ability to inhibit pro-fibrotic effects of NETs components in equine endometrium. Follicular-FP (n = 8) and mid luteal–MLP (n = 7) phase explants were cultured for 24 or 48 h with ELA(0.5–1 μg/ml), ELA(0.5–1 μg/ml) + N(45 μg/ml), CAT(0.1–1 μg/ml); CAT(0.1–1 μg/ml) + N(45 μg/ml); MPO(0.1–0.5 μg/ml) or MPO(0.1–0.5 μg/ml) + N(45 μg/ml). Collagen type I (COL1) transcripts were evaluated by qPCR and protein expression by western blot. Data were analyzed by ANOVA. N inhibited stimulatory effects of ELA0.5, ELA1 and CAT1 on COL1 mRNA and protein expression at different phases and incubation times ($p < 0.05$). At FP, ELA0.5 increased protein expression ($p < 0.01$), but N decreased it ($p < 0.05$). N inhibition on CAT0.1 COL1 transcripts was also visible (FP 24–48 h; MLP 48 h; $p < 0.05$). Protein expression of COL1 raised with CAT0.1 (MLP 24 h; FP 48 h; $p < 0.01$) and CAT1 (FP 48 h; $p < 0.01$), while N decreased this effect ($p < 0.05$). MPO0.1 upregulated COL1 transcripts (MLP 48 h) but N downregulated this stimulatory effect ($p < 0.01$). N incubation also lowered MPO0.5 effect on COL1 transcripts (FP 24 h) and protein expression (FP 48 h) ($p < 0.01$). Inhibition of NETs components by N may reduce the establishment of mare endometrosis, acting on myofibroblasts differentiation. Funding: PTDC-CVT-REP-4202-2014; UID/CVT/00276/2019.

P37 | A novel tail-mounted calving sensor – pilot study

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Accurate prediction of the onset of calving would potentially prevent dystocia and stillbirth and facilitate prompt colostrum feeding and calf removal, particularly in herds with paratuberculosis. The objective of this study was pilot-test a new biosensor to predict the onset of calving in dairy cows. A monitor was developed containing an accelerometer and other gravitational measurement devices. It was attached to the upper side of the cow's tail approximately 6 cm below the anus. Three behavioural changes were monitored: (1) tail raise frequency and duration (2) angle of tail raise and (3) bouts of standing/lying down. Measurement of these behaviours was taken every 2 seconds and the data transmitted to a receiver base. The device was tested on 20 dairy cows. The time of calving was established by 24 h staff supervision and CCTV. Of the 20 cows, 12 calvings were monitored, (6 primiparae, 6 pluriparae); 5 unassisted, 6 easily assisted and 1 difficult. Prolonged elevation of the tail (>30–45 degrees, > 20 seconds and 4 repetitions within 60 minutes), either alone or in combination with an abnormal standing pattern (within a 30 min. period) were observed within 4 h of calving (unassisted calvings 2–3.3 h; assisted calvings 45 mins – 3 h). Prolonged tail elevation combined with increased restlessness was predictive of imminent calving. The monitor was able to detect and record the pattern of calving behaviours and the algorithm was able to detect distinct onset of calving-specific behavioural change up to 4 h before birth. This would allow farm staff to observe stage two of calving and intervene if necessary to prevent calving problems and provide prompt neonatal care to the newborn calf/ves.

P38 | Effect of extender on reindeer (Rangifer tarandus) sperm quality during freezing

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The aim of the study was to compare 3 extenders for freezing reindeer (Rangifer tarandus) sperm. A total of 15 ejaculates from 6 adult males were collected by electroejaculation. The collected ejaculate was divided into three parts, diluted with different extenders and frozen. We used glucose and sodium citrate based extender (E1), a milk-based extender (E2) and a TRIS and fructose based extender (E3). Total and progressive motility was measured by CASA. Morphology was measured by phase-contrast microscopy. The data were processed by software STATISTICA 10. Results are depicted as means±SDs and ranges. The best results were obtained by freezing in E3 compared with E1 and E2 ($p < 0.01$). The total motility in E3 was 46.1 ± 17.6% (mean ± SD) (variations from 21 to 64%) compared to E1 (13.3 ± 10.31%, variations from 0 to 45%) and E2 (18.0 ± 15.20%, variations from 2 to 40%). The progressive motility in E3 was 24.2 ± 13.09 (from 8 to 45%), and in E1 and E2 9.7 ± 8.72 (from 0 to 32) and 13.3 ± 3.80 (from 2 to 27%), respectively. There was a total loss of motility after freezing in E1 and E2 in some cases. And no change in progressive mobility after cryopreservation in E3 was observed in some cases. Significant differences in the effect of the extender on the sperm morphology were not found. Thus, the best results were obtained by freezing reindeer sperm in a TRIS and fructose based extender. This extender may be recommended for cryopreservation of reindeer sperm. Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01023.

P39 | Plasminogen activator activity in follicular fluid and maturation medium collected from different sized bovine follicles

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Plasminogen activators/plasmin system is involved in several biochemical and physiological processes, such as fertilization and tissue remodeling. According to in vivo studies (Papanikolaou et al. Reprod Fertil Dev 2004, 20:320–7), plasminogen activator activity (PAA) was increased in bovine pre-ovulatory follicular fluid (FF). Our aim was to study PAA in the FF and maturation medium (MM) of different sized bovine follicles and its relationship with oocyte quality. Circa 2900 COCs were aspirated from abattoir ovaries, divided into 3 groups according (visual check) to follicular size [Small (SF) 2–5 mm; Large (LF) 6–8 mm; Control i.e. as routinely performed (CF) 2–8 mm] and into 3 groups according to their quality (grade: A, B-C and D). COCs underwent 24 h IVM and samples of MM were collected from all groups, including MM with no ova. In FF and MM, PAA (IU/ml) was measured spectrophotometrically. Data were analyzed by ANOVA and regression, in SPSS. More grade-A COCs were collected from SF and more grade-BC from LF group ($p < 0.05$). In FF, PAA was lower ($p < 0.05$) in SF group (89.30 ± 6.69) than in LF (145.55 ± 10.75) or CF (126.91 ± 14.66), probably due to increased release of plasminogen activator inhibitors from COCs. PAA was positively associated ($p < 0.05$) with the ratio of grade-A COCs in SF group. In MM, PAA was lower ($p < 0.05$) in LF (119.22 ± 28.94) or CF (121.72 ± 20.86) than in no ova (182.68 ± 18.85) group. In conclusion, PAA in FF relates to follicle size and, in SF group, PAA in FF relates to oocyte quality.

P40 | The effect of Mepron®, lipoic acid and L-carnitine on thyroid function in cows during the transit period

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During the transition period, the liver of the cows is under a great load. After calving, mobilization of fat from the depot is activated. Many cows are prone to fatty dystrophy. However, most animals overcome this difficult period in a natural way. The goal of our research was to study the effect of protected methionine, L-carnitine and lipoic acid on the function of the thyroid gland during the transition period. Two groups of 10 dry cows were formed on the principle of analogs. Cows of the first group received daily Mepron (5 g/d), L-carnitine (2 g/d) and lipoic acid (0.5 g/d) during 4 weeks before calving and 4 weeks after calving. Blood was collected for analysis three times: 4 weeks before calving, 20–25 and 60–70 days after calving. The concentration of total T3 and total and free T4 was investigated. Statistical processing of the research results was carried out using Student's t-test. At the beginning of the experiment, there were no differences in the concentration of hormones in both groups. After 20–25 days after calving, an increase in the concentration of thyroid hormones in the first group was detected, a significant difference of 22% was determined for T3 ($p < 0.05$). Two months after calving, the concentration of T3, T4 total and T4 free in the first group was 3.47 ± 0.14 nmol/l, 100.95 ± 3.06 nmol/l and 14.54 ± 0.41 pmol/l, respectively. In the control group, the values of these parameters were 2.75 ± 0.21 nmol/l, 90.98 ± 2.8 nmol/l and 12.66 ± 0.72 pmol/l, respectively, which differs from the experimental group by 9.9–20.8% ($p < 0.05$). Studies have confirmed the positive effect of Mepron®, lipoic acid and L-carnitine on the functional activity of the thyroid gland.

P41 | Expression of the osteopontin (SPP1) and calbindin (CALB1) mRNAs in the egg shell gland of layers supplemented by Cynara Scolimus L

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The improvement of the eggshell quality is an important task for the poultry industry. The successful mineralization during the eggshell formation requires an adequate quantity of inorganic minerals in the blood as well as a properly expression of genes, responsible for the mineralization. The present study aimed to evaluate how the layer diet supplemented with dry artichoke affects the egg shell quality and mRNA expression of the calcium-binding proteins osteopontin (SPP1) and calbindin (CALB1) in the egg shell gland, and the concentration of total calcium (Ca) and total inorganic phosphorus (P) in blood plasma. Forty ISA Brown layer hens at 38 weeks of age and 40 Lohmann Brown Classic hens at 42 weeks of age were randomly divided into 3 groups fed a control diet and two experimental diets with 0.22% and 0.30% of dry artichoke during 5 weeks. The mRNA expression of SPP1 and CALB1 was analyzed by RT-PCR. The eggshell weight and the thickness did not differ between groups at the end of the experiment. SPP1 gene was up-regulated whereas the CALB1 gene was down regulated in the egg shell gland of the experimental hens. Enhanced concentration of total Ca in the blood plasma of the experimental groups ($p < 0.05$) corresponded to increased level of Ca in the egg shell ($p = 0.06$). In conclusion, the inclusion of dry artichoke in the layer diet influenced the eggshell mechanical property by increasing the calcium in blood and egg shell, also concentration and mRNA expression of the calcium-binding proteins SPP1 and CALB1 was changed. Acknowledgement: this research was supported by Grant DM16/4–20.12.17 of NSF-MES Bulgaria.

P42 | Morphometric traits of boar spermatozoa depending on breed type

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Studies show that cryoresistance, nuclear DNA fragmentation, and activity of sperm cells depend on their morphometric parameters. The aim of this study was to investigate the morphometric characteristics of boar semen depending on the breed. Sperm samples of three breeds were studied: Large White (n = 52), Landrace (n = 37) and Duroc (n = 24). Activity, morphology and DNA fragmentation of spermatozoa were evaluated using CASA system. A Diff-Quik kit was used to dye sperm. The slides were visualized with a microscope Nikon Eclipse Ni-U and camera Nikon DS-Qi2. Morphometric parameters were measured in NIS-Elements BR 4.30 (Basic Research) using statistical analysis (SPSS v.15.0). The morphometry of spermatozoa was assessed on the basis of the following indicators: overall sperm length, length of sperm head and tail, sperm head perimeter and area. One-way analysis of variance (ANOVA) was performed. The factor to consider was the breed of boar. The results of the analysis of variance show that the influence of breed factor on the overall sperm length is statistically significant. The average length of spermatozoa amounted to 52.55 ± 1.55 μm, for the Large White breed 52.83 ± 1.21 μm, Landrace and Duroc 51.83 ± 2.27 μm ($p < 0.05$). The length of the spermatozoon flagellum of Duroc was 42.22 ± 2.92 μm, which is 1.25 μm less than that of the Large White breed and 1.19 μm less than that of the Landrace breed. The width of the sperm head of the Large White breed was the largest and was 5.04 ± 0.26 μm, which is 0.41 μm more than that of Duroc and 0.46 μm more than that of Landrace. Thus, our study proves that one of the biotic factors affecting the morphometric characteristics of boar sperm is their breed.

P43 | Preventing respiratory viral infections in calves during prenatal and early neonatal periods

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The state of post-vaccination immunity to infectious pathogens in the dam during gestation is the decisive factor in the formation of antiviral protection of newborns in the early postnatal period. Objective: to study the dynamics of colostral immunity against respiratory viral infections (ARVI) in calves born from vaccinated cows. The immunity in dams vaccinated during pregnancy (n = 132) with an inactivated Russia-produced multivalent vaccine against ARVI pathogens was determined by titers of specific antibodies in blood serums. The titer of antibodies to Bovine herpesvirus 1 (BoHV-1) was 5.82 ± 0.23 log; to bovine parainfluenza virus type 3 (BPIV3)–9.02 ± 0.34 log, to bovine viral diarrhea virus (BVDV)–6.76 ± 0.28 log. The serum titres in calves (n = 132) given colostrum from these dams, were determined between day 1 and 3 of life: titres to BoHV-1 was 4.58 ± 0.16log; BPIV3– 7.64 ± 0.28log; to BVDV – 5.28 ± 0.16 log. Up to 10 days there were no changes in titres. By day 20, a decrease was registered in the antibody titers to BoHV-1 t (2.54 ± 0.22log; BPIV3 – up to 4.84 ± 0.31log; BVDV – up to 2.84 ± 0.24log). These titres are regarded as minimum protective levels. By day 30, the antibody titres in 87.5% of calves to ARVI pathogens were further decreased (BoHV-1 – 1.8 ± 0.14log; BPIV3 – 3.2 ± 0.17log; to BVDV – 2.19 ± 0.19log.) None of the calves showed clinical symptoms during the study period. These data suggest that regular vaccination in the third semester of the pregnancy of cows provides humoral immunity against AVRI in the calves in the neonatal period. If this immunity is protective against AVRI must be tested in a proper challenge study.

P44 | Effect of EGF on viability of cryopreserved beef bull semen

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Different concentration of Epidermal Growth Factor (EGF): 0 (control) 50, 100, 200 and 400 ng/ml, before cryopreservation was used to improve the vitality of diluted bull semen. Semen was collected weekly for 8 weeks from 4 Piedmontese beef bulls, pooled and extended with Bullxcell extender. After dilution, semen was cooled, equilibrated and finally frozen in the liquid nitrogen. After thawing at 37°C for 40 s, semen was assessed for sperm motility and velocity parameters with CASA after 0, 1, 2, 3 and 4 h of incubation at 37°C; in addition to sperm vitality, acrosome, plasma membrane and DNA integrities, apoptosis, mitochondrial membrane potential, mucus penetration distance and superoxide dismutase activity, were performed. The data were analyzed as mean ± SEM with ANOVA. Duncan test was used for multiple comparisons and Pearson correlations for different parameters correlations. P value was set at < 0.05 to define statistical significance. EGF significantly ($p < 0.05$, $p < 0.01$) improved the total sperm motility after 0, 1,3 and 4 h incubation mainly with the concentrations 100 and 200 ng/ml. The progressive motility after 1 and 2 h and the rapid motility after 1, 2 and 3 h of incubation ($p < 0.01$) mainly with the concentration 200 ng/ml were improved. The EGF significantly ($p < 0.05$, $p < 0.05$) improves the different velocity parameters after the different incubation periods mainly with the concentrations 100, 200 and 400 ng/ml. EGF significantly improved the sperm vitality ($p < 0.01$) and decreased sperm apoptosis ($p < 0.05$) with the concentrations 100, 200 and 400 ng/ml without affecting acrosome, plasma membrane and DNA integrities. In conclusions, incorporation of EGF especially at concentrations 100 and 200 ng/ml could improve the vitality parameters of cryopreserved bull semen.

P45 | Results of vaginal samples in cows in the post partum period

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One of the main reasons for the reduction of fertility in farms are infectious diseases of cattle, leading to disturbances in the reproductive system of cows, resulting in pregnancy problems, fetal death, abortion at any time during pregnancy and stillbirth. The material for this study was 110 vaginal swabs and scrapings of cows in the post partum period. We used microscopic methods as well as cultivation on nutrient media. Identification was performed using test systems API 20E, BioMerieux, France, as well as using MALDI Biotyper, Helena Biosciences Europe, United Kingdom. Bacteriological analysis from vaginal samples revealed a wide range of microorganisms: E. coli in 12.9% of cases, K. pneumoniae (8.3%), Proteus sp. (8%), C. albicans (6%), P. aeruginosa (6%), Staphylococcus sp. (4%), Streptococcus sp. (3%), Bacillus sp. (3.2%), Serratia sp. (1.4%), Corynebacterium sp. (4.2%). Polymerase chain reaction in microchip format for the diagnosis and monitoring of pathogens of urogenital infections in cattle was also performed from clinical samples. For amplification, RT-PCR cycler AriaDNA with a microchip with lyophilized reagents developed by IC Lumex was used. In the samples, the microorganisms Mycoplasma and Ureaplasma were simultaneously identified in 55% of cases; and Mycoplasma, Ureaplasma and Chlamydophila in 5%. Microbes in an ascending direction might cause the development of inflammation in the vagina, cervix, and then also in the uterus if immune protection is decreased. The period of risk of infection with microorganisms is during the passage through the birth canal at parturition. Laboratory diagnostics allow timely treatment and prevention of diseases by management optimising.

P46 | Comparison of three methods to synchronize ewes in estrus during the breeding season

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Three treatments to synchronize ewes in estrus during the breeding season at 41°N (Zaragoza, Spain) were compared. Ewes were divided into three groups: Group 2PG (n = 28), receiving two injections of 10 mg PGF2α (PROSYL®, CEVA, Barcelona, Spain) 10 d apart + 480 IU eCG (SYNCRO-PART® PMSG, CEVA) at the second injection; Group 7-PG-eCG (n = 28), a 30 mg fluorogestone intravaginal sponge (SYNCRO-PART® esponjas, CEVA) for 7 d + 10 mg of PGF2α+480 IU eCG at sponge withdrawal; and Group 14-eCG (n = 26), receiving the same sponge + 480 IU eCG at sponge withdrawal. Five rams were introduced in each group, 24 h after the second injection for the 2PG group, or after pessary withdrawal for the other groups. Rams were rotated among groups every 12 h, and kept for 72 h. They were equipped with aprons and crayons for estrous detection; marks were verified each 6 h. Pregnancy was diagnosed by transrectal scanning at d30. Anova and chi-square tests were used. No differences were observed for the proportion of ewes presenting estrus, although ewes of the 14-eCG group presented an earlier estrus (2PG: 45; 7-PG-eCG: 44; 14-eCG: 32 h; $p < 0.001$). The proportion of pregnant ewes at d30 was lower in the 2PG group (57; 93; 72%, resp; $p < 0.01$). The highest percentage of lambing ewes was observed in the 7-PG-eCG group (46; 89; 54% resp; $p < 0.01$), which had the highest mean (± S.E.M.) litter size (2PG: 1.7 ± 0.2; 7-PG-eCG: 2.4 ± 0.2; 14-eCG: 2.3 ± 0.2 lambs/lambing $p < 0.05$) and fecundity rate (2PG: 0.8 ± 0.2; 7-PG-eCG: 2.2 ± 0.3; 14-eCG: 1.2 ± 0.3 lambs/ewe; $p < 0.01$) of the three treatments. In conclusion, the treatment with intravaginal sponges for 7 days accompanied by PGF2α and eCG has demonstrated a high efficiency, with the best results in terms of lamb production of the three treatment under study.

P47 | Miranda breed donkey fetal age determination by real-time ultrasound measurement of the fetal eye – a preliminary study

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In equids, when breeding date is not known, ultrasonographic measures of the fetal eye have been used to estimate gestational age. The study of the relation between the fetal eye size and gestational age has not been performed in the Miranda donkey breed and, in horses, breed variations on this parameter have been described. In some donkey populations of this and similar breeds, reproductive management is done by free range breeding; thus, a method for gestational age determination will be useful for management and welfare reasons. A total of 27 transrectal ultrasounds were performed from days 125 to 343 of gestation in seven jennies, with ages ranging from 5 to 17 years old (9.28 ± 3.53 years). The weight of the jennies ranged from 284.5 to 425.5 kg (363.2 ± 54.08) and the height from 125.6 to 144.2 cm (134.3 ± 7.45 cm). Each gestation was on average checked 3.8 ± 0.3 times (2 to 5 times). The fetal eye was successfully measured in 22 of 27 ultrasounds (81.5% success rate). The measure obtained was the longitudinal length of the eye vitreous body, in cm. There was a significant correlation between gestational age and the eyes measures ($p < 0.001$, $R^2 = 0.959$). The relation between gestational age and diameter of the vitreous body was linear and the formula that better estimates ($R^2 = 0.9193$) the gestational age in this sample was $y=(x-0.3864)/0.009$, being y the gestational age in days and × the longitudinal length of the eye vitreous body, in cm. In this sample, this formula allowed the estimation of the gestational age with an average error of 17.5 ± 11.83 days. The variations on the eye diameter were independent of mother's weight ($p = 0.155$), height ($p = 0.115$) and age ($p = 0.985$). Future studies are necessary to confirm these findings, with a higher number of measurements.

P48 | Melatonin does not improve cryosurvival of sperm from Belgian & German Shepherd dogs

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Working dogs represent a valuable resource for human society; hence, artificial insemination with frozen-thawed spermatozoa may help to increase the number of offspring of superior males. However, cryopreservation reduces the fertilizing capacity of sperm. To assess the effect of melatonin (MLT) as antioxidant, and pre-freeze cooling to −5°C on cryosurvival of sperm cells from Belgian & German Shepherd dogs, semen was collected throughout autumn, winter, and spring. Semen was assessed, centrifuged and resuspended in a freezing medium (EYT); sperm were cooled to 5°C in 2 h, and more EYT was added (final concentration 200 × 106 cells/ml, 5% glycerol). Then MLT (0, 1, or 2 mM) was added, and diluted sperm were packaged in 0.5 ml straws. Half of the straws at 5°C was exposed 15 min to nitrogen vapor, and then were immersed in liquid nitrogen. The other half was further cooled to −5°C (0.15°C/min) employing saline ice at −12°C, and frozen as mentioned. Straws were thawed in a water bath at 37°C for 30 sec; then, progressive motility (PM), plasma membrane integrity, viability, capacitation status, and acrosome integrity were assessed. Data were arcsine transformed for normalization before an ANOVA was done to assess the effect of MLT levels, while the "t" test was used to compare cooling temperatures. There were no significant differences on sperm quality by either cooling temperatures or MLT doses, nor by the interaction cooling × MLT. There were however significant differences in sperm progressive motility between seasons

(pre-freeze values): smaller in autumn than in winter and spring. In conclusion, pre-freeze cooling and MLT did not improve Belgian & German Shepherd sperm cryosurvival. Supported by UNAM (PAPIIT IA204917, IA220419, PIAPI1615/1649/1810).

P49 | The effect of pre-freeze cooling and thawing temperatures on dog sperm cryosurvival

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To test the combined effect of 3 different pre-freeze cooling and 2 thawing temperatures on dog sperm cryosurvival, semen from 6 Belgian Shepherd males was centrifuged, resuspended in a standard freezing medium (EYT), and slowly cooled from 23 to 5°C. Then, a second fraction of EYT was added (final concentration of 200 × 106 sperm/ml and 5% glycerol). Diluted sperm were packaged in 0.5 ml plastic straws and kept at 5°C during 16 h. Then, one third of the straws at 5°C was frozen over nitrogen vapours, and stored in liquid nitrogen; the rest was further cooled to either −3°C or to −5°C, frozen and stored as mentioned. Straws were thawed in a water bath at either 37°C for 30 sec or 70°C for 5 sec. Progressive motility, viability (eosin/nigrosine), capacitation status (CTC assay), and acrosome integrity (PSA-FITC) were assessed. Data were arcsine transformed to normalize it before ANOVA. There were no differences in motile, viable, acrosome-intact, and non-capacitated acrosome-intact spermatozoa between any of the treatments. However, there were significant differences in the percentages of capacitated acrosome-intact spermatozoa: +5°C × 70°C produced the highest value (55.3 ± 3.44%) which was different from the others except that of −5°C × 70°C (46.7 ± 3.82%). Also, there were significant differences in the percentages of acrosome-reacted spermatozoa; treatments involving thawing at 70°C (cooled to + 5 or −5°C) produced the smallest values which were different from that of −3°C × 37°C, the biggest. In conclusion, thawing temperature was more important than pre-freeze cooling temperature on dog sperm cryosurvival. Supported by UNAM (PAPIIT IA204917, IA220419, PIAPI1615/1649/1810).

P50 | Abstract withdrawn

P51 | Slow and ultra-rapid freezing protocols for cryopreserving roe deer (Capreolus capreolus) epididymal sperm collected at different times of year

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Viable epididymal spermatozoa can be cryopreserved from dead males of several wild species. The present study reports the effect of two cooling rates on the quality of frozen-thawed roe deer epididymal sperm. Testes were collected, 24–48 h after death, from January to September (n = 106 males). A Tris-citric acid-glucose + 6% egg yolk-based medium was used for the conventional slow freezing in straws; glycerol was added to a final concentration of 5%. The same diluents were used for ultra-rapid freezing in pellets, but replacing the glycerol with 100 mM of sucrose. Sperm variables (motility: CASA; acrosome and membrane integrity: PNA-FITC/PI; morphometry: ASMA; morphological abnormalities) were analysed before and after cryopreservation. In addition, histological sections of testes allowed evaluating the spermatogenic activity. Several testes from January (100%) to May (10%), and in September (20%) were azoospermic and without spermatogenic activity. The values after slow freezing-thawing were not significantly different from ultra-rapid freezing-thawing values for the percentage of viable sperm (26.3% vs 16.1%) and the percentage of sperm with morphological abnormalities (45.1% vs 32.2%). Acrosome integrity (43.3% vs 25.0%) and all kinetic sperm variables were more desirable after slow compared to ultra-rapid freezing protocols (e.g. VSL 19 μm/s vs 4 μm/s, $p < 0.05$). Post-thaw sperm heads were smaller ($p < 0.001$) compared with pre-freeze for both freezing methods. In conclusion, June-July would appear to be the best season for collecting and freezing the sperm of roe deer, because all animals show spermatogenic activity. Ultra-rapid freezing resulted in lower motility and acrosome values than conventional sperm freezing (Supported by MINECO grant AGL2017-85753-R).

P52 | The influence membranes damage and activity of roosters' sperm on the fertilization of eggs when using cured cryopreserved sperm

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The most likely causes of a decrease in frozen/thawed sperm fertility are membrane integrity problems and a significant decrease in overall motility. The aim of the study was to establish the reliability of the effect on fertilization of eggs of each of the 2 factors separately. The study on the quality of frozen/thawed sperm on the level of activity and damage to sperm, as well as on fertility of eggs, as a sign that determines the level of fertility of sperm was conducted in 2 replications. We used Rhode Island cocks (♂n = 13 (exp.1) and ♂n = 8 (exp.2), at the age of 54–56 weeks, 1♂:20♀, by the method of freezing individual ejaculates in pellets, insemination sperm dose was 0.02 ml. Sperm membrane damage was assessed by Sperm VitalStain colorant and Axio Imager imaging system at 1:100, counting 200 cells/sample. Individual cell damage differences in exp.1 ranged from 15%–55%, in exp.2 from 17%–59%, activity indicators of frozen/thawed sperm ranged from 43%–85% and from 40%–70%, respectively, fertility in exp.1 was 30.8 – 88.9% and in exp.2 it was 21.1–86%. The influence of each factor was determined using regression analysis. The relationship between damage and fertility: y = −0.24x+62.12 (exp.1) and y = −0.34x+88.87 (exp.2), where *x* is the indicator of membrane damage. An analysis of the formulated equations shows that even with *x* = 50, the % fertilization (y) will be at a high level of ~75%. The regression equation, where *x* is an indicator of sperm activity: y = 4.7x+43.2 (exp.1) and y = 17.3x-42.2 (exp.2). Fertility prognosis with an average of *x* = 60%, will be > 65%. Of the 2 factors assessed, the level of activity of spermatozoa influences the fertility rate more. Research conducted on the topic AAAA-A18-118021590132-9 biomaterial from Genetic collection of the RRIFAGB.

P53 | Correlation between the age of heifers' first successful insemination and LV-poliformism of somatotropin gene

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The purpose of the study was to determine the effect of allelic variations in the somatotropin gene on number of days of successful insemination in heifers. The somatotropin or the growth hormone (GH) is a significant regulator of the animal somatic growth, which produces a lactogenic and a lipid-mobilizing effect. The gene polymorphism in L and V alleles, identified with Alu I restriction endonuclease, has three genotypes: LL, LV and VV. The research studied the correlation between the age when Holstein black-and- white heifers were first successfully inseminated and the somatotropin gene polymorphism. The somatotropin gene polymorphism was studied using a PCR-RFLP technique (polymerase chain reaction – restriction fragment length polymorphism) (Alu I restriction endonuclease) and further electrophoresis in 3% agarose gel. Statistical indicators were calculated using IBM SPSS Statistics 23 software. The somatotropin gene in the sampled animals (120 heads per group) was represented by two gene variations: LL-84% and LV-16%. It was revealed that animals with LL variation were inseminated successfully on the 419th day and reached the weight of 398 kg, and animals with LV variation were inseminated successfully by the 436th day and reached the weight of 396 kg. The difference was 17 days ($p \leq 0.1$). Heifers with LL variation of the somatotropin gene grew and developed better, and it the fact that they reached body weight necessary for the insemination earlier can be explained by that. Therefore, the use of animals with LL variation of the somatotropin gene in herds will enable to reach the age of the first successful insemination earlier than the use of animals with LV variation.

P54 | Duration of the period from calving to fruitful insemination of cows depending on their genetic similarity to servicing bulls

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The purpose of the research was to study the interval between calving to successful insemination of cows with different genetic characteristics. The study was conducted on black-and-white holsteinized breeding cattle (Bos taurus). A range of microsatellite loci recommended by the International Society for Animal Genetics (ISAG) were identified in the cows and bulls (BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126, TGLA227). Microsatellite analysis was conducted using AB 3130 genetic analyzer and the following software: Data Collection v.3.1 and GeneMapper v.4.0. The genetic similarity index in pairs "a bull – a cow" was calculated. The sampled cows were (n = 200) divided into three groups (I-n-65, II-n-70, III-n-65) according to the index of genetic similarity to the bulls: I. (≤ 0.20), II. (0.21–0.40), III. (≥ 0.41). The outcome of the experiment showed that the cows with the genetic similarity index below 0.2 were inseminated earlier than those with the genetic similarity index above 0.41. The interval between calving to successful insemination of the cows in group I was 143 days and in group II 163 days, in group III 198 days. The correlation ratio between the genetic similarity of the cattle pairs and the interval

between calving to successful insemination constituted $r = 0.68$ with $p \leq 0.001$. The insemination of cows by servicing bulls which do not have genetic similarity to the cows enables to reduce the duration of the period from calving to successful insemination.

P55 | Effect of rearing autumn-born ram-lambs in contact with sexual-active adult rams or exposed to artificial long days. 1. Effect on live weight, scrotal circumference and plasma testosterone levels

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In mid Jan, 40 ram-lambs born in Oct were divided into 4 groups (n = 10): Group FA, exposed to 16 h of light for 2 months (15 Jan-15 Mar); Group SAR, housed with 3 adult sexually-activated rams from 15 Mar to the end of the experiment; Group NAR, housed with 3 adult non-activated rams; and Group C, control, isolated from adult rams during the experiment. Adult rams were rendered sexually active by exposing them to long days (16 h light) from 15 Jan to 15 Mar. Blood samples were collected weekly to determine plasma testosterone (T) concentrations, and live weight and scrotal circumference were recorded fortnightly, until June. No live weight differences among groups were observed. Rearing method significantly affected scrotal circumference and plasma T levels ($p < 0.001$). Thus, NAR ram-lambs consistently had the lowest scrotal circumference throughout the study, although the 4 groups gradually increased them (± SEM) to reach similar values in June (FA: 28.6 ± 0.6; SAR: 28.6 ± 0.5; NAR: 27.6 ± 1.2; C: 29.2 ± 0.6 cm). Overall mean plasma T levels (ng/ml) throughout the study were higher in the C (4.4 ± 0.2) and SAR (4.3 ± 0.2) groups compared with FA (3.4 ± 0.2) and NSA (3.4 ± 0.2). The four groups presented a gradual increase in their T levels, although at the end of the experimental period, in June, all the groups but C experimented a drop (FA: 3.1 ± 0.2; SAR: 4.4 ± 0.2; NAR: 4.7 ± 0.2; C: 7.3 ± 0.2; $p < 0.001$). In conclusion, rearing system did not affect live weight or scrotal circumference, although ram-lambs reared isolated from adult rams, or those in permanent contact with sexual-active rams, presented higher levels of T during the prepuberal period in spring. However, only isolated ram-lambs did not seem to enter into sexual rest as the other groups did at the end of the experiment.

P56 | Effect of rearing autumn-born ram-lambs in contact with sexual-active adult rams or exposed to artificial long days. 2: Effect on sexual activity and fertility at 8-months of age

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In mid Jan, 40 ram-lambs born in Oct were divided into 4 groups (n = 10): Group FA, exposed to 16 h of light for 2 months (15 Jan–15 Mar); Group SAR, housed with 3 adult sexually-activated rams from 15 Mar to the end of the experiment; Group NAR, housed with 3 adult non-activated rams from 15 Mar; and Group C, control, isolated from adult rams during the experiment. Adult rams were rendered sexually active by a exposing them to long days (16 h light) from 15 Jan to 15 Mar. Individual and group ram serving capacity tests were performed on 20 June, when ram-lambs were 8-month-old, using 40 ewes synchronized in oestrus. For 20 min, each ram was exposed to 5 ewes, and the number of attempts of mounting and mountings were recorded. After that, rams were regrouped and the same observations of events, with the same 10 ewes used for the individuals test, were recorded. After that, ram-lambs could mate the ewes for 48 h. GLM, anova and chi square tests were applied. During the individual tests, half of the attempts of mountings (6, 17, 22, and 55%, $p < 0.01$) and mountings (4, 15, 31, and 50%, for the FA, SAR, NAR and C groups, resp, $p < 0.01$) performed per ram were recorded in the control group. A similar distribution was observed in the group test (attempts of mounting: 9, 11, 24, 57%, mountings: 0, 18, 27, 55%, for the FA, SAR, NAR and C groups, resp, $p < 0.001$). However, fertility rate obtained by ewes mated by SAR (90%) and C (100%) ram-lambs was higher ($p < 0.01$) than the FA (50%) and NAR (40%) groups. In conclusion, rearing autumn-born ram-lambs in isolation of adult congeners of the same species results in a high degree of sexual activity at 8-months of age, although their fertility is similar to ram-lambs reared in the presence of sexually-active adult rams.

P57 | Cases of bacterial infection of the urogenital organs from gilts and boar

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The aim of the study was to find out the causes of urogenital disease of landrace pigs (n = 4, age 6–6.5 mo) and one boar (age 9 mo) from a farm of Russian North-West region. The methods used were clinical examination and post-mortem viscera inspection and bacteriological examination. In the last 3–4 weeks, the following

clinical signs were found in the females: anxiety, sagging of the back, polyuria, and vulvae discharge of serous mucous exudates (catarrhal vestibulitis). Pigs were treated with antimicrobial drugs for 10 days. By the 10th day frequent urination, depression, loss of condition and lack of estrus were still observed. The libido of the boar was 1 point on a five-point scale (1–the boar has no interest, 5–the boar Mounts on an artificial sow and allows the collection of sperm). In the boar's clinical examination, no abnormalities in the development of the external genital organs were identified. For 3 weeks, the animal had a depression, anxiety, and a hunched position when urinating. All animals were culled and subjected to post-mortem viscera inspection. Both gilts and boars were diagnosed with inflammation of the urinary bladder, and the boar also had an inflammation of the seminal vesicular gland. The vesicular gland was hyperemic and increased in volume. A cloudy, light-red exudate with flakes flowed from the incision surface. E. coli was isolated from the urinary bladder wall of all animals and the vesicle gland in the boar by bacteriological examination. As a result, the urogenic infection reduced libido of the boar and the gilts, and finally caused their disposal. We believe that the cause of the disease was a decrease in immunity due to the low content of protein in the diet and unsatisfactory hygienic conditions.

P58 | Immunobiology of the mammary gland in mice in the phases of lactation and physiological rest

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The functioning of the breast is closely related to the activity of the immune system. Leukocytes are present in colostrum and milk. The goal of our study was to evaluate the quantitative and qualitative composition of leukocytes in the mammary gland of lactating and non-lactating mice. Two groups with 5 animals in each were formed: First group of non-lactating mice, and a second group of lactating mice (lactation duration: 7 days). The mammary gland was isolated, cut and an imprint was made on the slide. The samples were dried, stained and examined using immersion optics. The study of smears-prints showed that fat and epithelial cells were predominant in the state of physiological rest of the mammary gland, whereas the adipose tissue was replaced by glandular tissue in the period of lactation. Leukocytes were not found in non-lactating mice. The presence of leukocytes was seen during lactation. Most of the leukocytes were lymphocytes (15 ± 4%). The presence of segmented neutrophils (7 ± 3%) and band neutrophils (2 ± 1%) was established. The appearance of leukocytes in the mammary gland during the lactation period is linked with passive transfer of macrophages in colostrum and milk, which is necessary to protect the newborn. The presence of cells in colostrum enhances the positive dynamics of the development of the immune system of animals. Immunity is transmitted not only passively, but a foundation is laid for the normal functioning of the immune system in the adult. In conclusion, quantitative and qualitative changes of immunocompetent cells were found in the mammary gland of mice.

P59 | Cells of immune memory in mice in the colostrums

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The issue of immune protection in newborns is extremely relevant. During the period of preparation for lactation, the cavity of the alveoli of the mammary gland is filled with leukocytes. We hypothesize the existence of a mechanism of cellular immunity. After drinking colostrum, leukogram of newborn mice changes its profile from neutrophilic to lymphocytic. Male cubs receive through mother's lymphocytes the immune information, leading to the appearance of immune memory cells in the bone marrow. The goal of our research was to trace the transition of immunocompetent cells from the mammary gland of the mother to the immune organs of the cubs. Studies have been conducted on newborn linear mice-males (n = 10) in the colostrums period. Bone marrow from the proximal femur was used. These samples were investigated using immersion optics. The phenotype of cells of adaptive immunity was determined using a set of fluorochrome-conjugated antibodies: CCD45-BV421, CD4-PerCP-Cy5.5, CD8-PE/Cy7, CD62L APC/Cy7, CD44-BV510, in the presence of True stain reagent, containing antibodies against CD16/CD32 for blocking non-specific binding of antibodies. Data collection was performed on a CytoFlex flow cytometer (Beckman Coulter, USA). The results were analyzed in the program Kaluza Analysis 2.1. In three-day mice, lymphocytes are dominant (up to 48%) over segmental neutrophils (up to 45%) and 11% of them were cells of mother origin containing Bar bodies. T-lymphocytes expressing the CD45, CD4, CD8 antigens were detected. Immunocompetent cells from the breast with colostrum and milk penetrate through the intestinal epithelium not only into the blood, but also into the marrow of the newborn. Colostrums lymphocytes play an important role in the formation of cellular immunity in the newborn.

P60 | Analysis of the antibiotic resistance genes of microorganisms in the milk of cows and goats

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In 2018, 158 cow milk samples and 21 goat milk samples from animals with subclinical mastitis from various farms in five regions of Russia were examined. The objective of the research was to identify DNA of pathogenic bacteria in the milk and to determine the presence of antibiotic resistance genes. We have conducted PCR studies of the biological material on the device "RotorGene-3000" (Australia), using a set of reagents "IDS" (Russia). In 94.3% of cow milk samples, the DNA of the following microorganisms was detected: Staphylococcus spp. (73.4%), S. aureus (23.4%), E. coli (34.1%), S. agalactiae (12.0%). The blaDHA gene, which determines resistance to inhibitor-protected penicillins and cephalosporins of the 3rd and 4th generations, was found in 83.0% of E. coli. Antimicrobial resistance of S. aureus against 2nd generation cephalosporins (MecA gene) was found in 29.2% of the samples. The gene of resistance to macrolides of the 1st generation (ErmB) was found in 20.0% of Staphylococcus spp. and 2.7% of S. agalactiae. Resistance gene for 1st generation cephalosporins (CTX-M) was found in 3.3% of E. coli. In 71.4% of goat milk samples, the following specific microbial DNA was detected: Staphylococcus spp. (23.8%), S. aureus (38.0%), E. coli (42.8%), S. agalactiae (9.5%). The blaDHA gene was found in 90.0% of E. coli. These data indicate a widespread antibiotic resistance of pathogenic agents in the milk of cows and goats. The high prevalence of blaDHA gene positive samples could be associated with the frequent use of penicillins and cephalosporins of the 3rd and 4th generations on the farms. Laboratory control of bacterial resistance to antibiotics is required. Improvement of udder health management including vaccines or alternative antibiotics should be considered.

P61 | The use of marker vaccines against IBR to control reproduction of dairy herds

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Eradication of bovine herpesvirus (BHV-1, responsible for infectious bovine rhinotracheitis = IBR) in dairy herds is optimally controlled using DIVA-strategy (differentiate infected from vaccinated animals). Differentiation of field and vaccine strains was performed using modified BHV-1-based monovaccines with a deletion of the gE gene. The recovery control was performed with gE and gB diagnostic ELISA test systems, where the gE gene encodes glycoprotein E, which is a virulence factor for all known Alphaherpesvirinae viruses, gB–vaccine marker, which is absent in the field strain. The effects of labeled vaccines on the reproduction in dairy herd with seroprevalence to a field strain were evaluated. It was found that with the use of labeled vaccines against IBR, seroprevalence of the wild strain in the herd decreased from 95% to 53% during the first year, and it has decreased to 14% during the second year after the start of vaccination. In 5 years the seroprevalence decreased to 5%. Regarding the reproductive performance, it was found that, with the introduction of vaccination against IBR, the period from calving to conception decreased significantly by 11.5% (from 130 to 115 days), the number of placental retentions decreased significantly from 21.9% to 15%. The number of abortions and calves born alive remained unchanged. The use of monovaccine for the prevention of IBR also makes it possible to reduce the appearance of infectious vulvovaginitis: before vaccination against IBR, 52.5% of the cows in the herd had clinical signs of vulvovaginitis (swelling of the vulva, pustules, redness). After the use of monovaccines against IBR, the prevalence of cows with vulvovaginitis was 7%.

P62 | Testing of natural antioxidant oregonin on ram semen quality

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Oregonin is a natural polyphenol, isolated from the Alnus incana bark, which possesses antioxidative and anti-inflammatory activities. The objective of this study was to test the effect of different doses of oregonin on the ram semen quality. The ejaculates were collected by artificial vagina from 3 rams. A sperm concentration was adjusted to 200 × 106 cells/ml by dilution with a commercial extender (Triladyl) without oregonin (control) or with 100 μM, 200 μM, 500 μM and 5 mM of oregonin. After that, equal aliquots from all semen samples were stored in a water bath at 37° C for six hours. Motility (CASA), plasma membrane integrity and morphological defects (eosin/nigrosine staining) were estimated at 0, 2 and 6 h after the final dilution. Non parametric Wilcoxon pairs test was used for the statistical analysis. In oregonin treated samples, the progressive motility increased for 4% (100 μM) and 6% (500 μM and 5 mM) between 0 h and 6 h compared to the control. The VCL (curvilinear velocity) and percent of linear moving sperm were enhanced for 9.1% (100 μM) and 6.7% (500 μM and 5 mM) and for 4.3% (100 μM) and 2.7% (500 μM and 5 mM) respectively at 2 h of the storage. There were no significant differences in the morphological defects (abnormal tail and cytoplasmic drops) between control and treated groups in average (6.35 ± 0.8% vs. 6.80 ± 0.73%; 3.20 ± 0.62 vs. 2.6 ± 0.81%). The plasma membrane integrity was 17% higher in average in the experimental samples compared to the control

samples at 6 h ($p < 0.05$). In conclusion, natural antioxidant oregonin in micromole doses improved the kinematic properties of ram spermatozoa without negatively affecting their morphology. Research was supported by NSP-REPROBIOTEH, MINISTRY OF EDUCATION AND SCIENCE, BULGARIA.

P63 | Longevity of cows depending on age at first service

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The aim of the research was to define the effect of age of cows at first service on their reproductive performance and longevity. The study was based on the data of the SELEX software product recorded for Bestuzhev cows for the last three years (2015–2017). The research was conducted in the breeding herd of the LLC "ALEX" located in the Nurimanovo district of the Bashkortostan Republic. The cows were divided into five groups depending on the time of their first service: group I (n = 22, up to 17.0 months), group II (n = 58, 17.1–18.0 months), group III (n = 46, 18.1–19.0 months), group IV (n = 34, 19.1–20.0 months) and group V (n = 20, ≥ 20.1 months). Milk yield of cows in group II over the entire period of life was 18'312 kg, compared to group I (4794 kg; $p < 0.001$), and group III (1244 kg), and group IV (2485 kg; $p < 0.01$) and group V (3516 kg; $p < 0.01$). Statistical Analysis was done by ANOVA, the reliability of the results was determined by the Student's table. In addition, they had higher rates of milk yield per day compared to cows inseminated later. Animals serviced at an earlier age (up to 17 months), having a lower live weight (372 kg), were culled earlier and could not realize their genetic potential. Cows of group II had a longer period of productive performance (5.6 lactations) than cows in the other groups and they were characterized by a long period of economic use.

P64 | DNA fragmentation in reindeer semen before and after freezing – a pilot study

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The aim of the study was to evaluate DNA fragmentation in reindeer semen before and after freezing. Many authors indicate that DNA fragmentation in sperm is one of the causes of infertility. Semen was collected from caudae epididymidae of six slaughtered and two castrated males. Semen was extended to a final concentration of 100 million spermatozoa/ml in Steridyl semen extender and frozen in 0.25 ml straws. The sperm Chromatin Dispersion (SCD) test was used to assess DNA fragmentation in semen. Sperm with fragmented DNA failed to produce a halo of dispersed DNA loops following acid denaturation and removal of nuclear proteins. The GoldCyto DNA kit was used for the SCD test. One hundred spermatozoa per male were measured by CASA (ArgusSoft – module DNA fragmentation). As a result, in fresh and frozen-thawed semen, the proportion of sperm cells with fragmented DNA varied from 3.3 to 23.5% (mean ± SD; 14.6 ± 7.02%) and from 4.7 to 25.1% (15.3 ± 5.49%), respectively. On average, after freezing and thawing, the number of sperm cells with fragmented DNA increased from 0.2 to 2.2% (0.9 ± 1.1%). Our preliminary results show that there were few sperm cells with fragmented DNA in reindeer semen, however, a high individual variability was observed. DNA was slightly damaged during cryopreservation. However, further research is required. (Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01023 (DNA fragmentation assay) and from NordForsk, ReiGN in Russia (sampling of slaughtered males).

P65 | Reproductive and productive health of pigs when using Azoxivet

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The aim of the study was to determine the effectiveness of using an immune-modulator Azoxivet (an azoximer bromide) for prevention of postpartum diseases in sows. Sows were divided in 3 groups: in group 1 (G1, n = 30) Azoxivet was applied intramuscularly from day 100 of pregnancy 3 times with 7-day intervals at a dose of 18 mg; in group 2 (G2, n = 30) Azoxivet was used on day 100 of pregnancy and on the day of farrowing at the same dose; sows of group 3 (G3, n = 30) served as control (no immune-Modulators). Azoxivet did not affect the duration of pregnancy, it lasted 114–115 days in all groups. For data processing, mean and sd was calculated and Students t-test used. The morbidity of sows with acute purulent-catarrhal endometritis in G1 and G2 was registered in 10.0% of the animals in each group, in G3 it was registered in 26.67% of sows, and in addition in G3 in 3.33% of the sows metritis-mastitis-agalactia syndrome was found (including inflammation of the uterus and udder, and milk flow dysfunction). Safety of the piglets (percent of live piglets from initial quantity on day 28 of live in each group of sows) in experimental groups was higher than in the control group by 0.9–2.6%. The body weight of the weaned piglets in G1 was 6.71 ± 0.23 kg, in G2 6.75 ± 0.20 kg, in G3 6.34 ± 0.23 kg. Therefore, the administration of Azoxivet to sows reduced the risk of development of postpartum diseases, and allowed to increase the viability of these sow's piglets.

P66 | The method of superlong sequencing to determine the nucleotide sequences of the cattle TLR genes

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The purpose of the study was to develop an approach to screen for the polymorphism in TLR genes in a representative set of modern cattle breeds from Russia. Conducting studies to determine the variability of surface TLR receptors is imperious to the creation of new genetic tests to identify animals with an increased risk of miscarriages in cows due to infections in the early stages of fetal development. N = 575 animals were used for the analysis. The samples from genomic DNA were sequenced on the PacBio platform. The general amplicon included the overlapping amplification products from the coding regions of all 10 bovine TLR genes. The identified structural variants of TLRs were annotated according to their biological significance. Both new and already identified sites of variability, already annotated and documented in dbSNP, were found. The sequences of the exon regions of ten TLR genes of cattle of nine different breeds within Russia were obtained. The next generation sequencing method allowed identification of up to 64 putative mutations in the coding sequences of the genes studied and up to 31 using the primer extension method. This information makes it possible to determine of SNV in the population, for example, using direct methods of genotyping by chips. As a result of the research, gene variability of the component 64/31 SNV was revealed. This information will be used to determine the connection of the identified variants with the possibility of termination of pregnancy due to intrauterine infections and other infectious diseases of cattle.

P67 | Radiation-induced pathology of the mammary gland in cattle

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The purpose of this study was to research the effects of Cs137 and Sr90 radionuclides that are most common in the environment, after various industrial disasters, in the mammary gland of cattle. Studies were performed on 100 lactating cows of the Holstein breed aged 4-5 years. It was found that the main source of penetration of Cs137 and Sr90 radionuclides into the body of dairy cows are coarse and succulent feeds of natural grasslands. The greatest ability to accumulate Cs137 and Sr90 is distinguished by legumes (lupine, etc.) and perennial grasses of natural hayfields and pastures. It was established that in the tested hay the maximum radionuclide content was 3300 Bq/kg (radiation tolerance level (RTL)-1300 Bq/kg); in the haylage-1200 Bq/kg (RTL-500 Bq/kg); in the silo-1000 Bq/kg (RTL-240 Bq/kg); in herbal mass – 880 Bq/kg (RTL-185 Bq/kg); in grain yield-270 Bq/kg (RTL-180 Bq/kg). The determination of Cs137 and Sr90 in the feed was carried out using the express method of "wet ashing" with further radiometric measurements using low-background radiometers. For the study of malignant neoplasm, anatomical dissection methods, morphometry, and the manufacture of histological sections were used (the biopsy material was placed in an automatic processing machine for histological Leica TM 1020 (Germany), with further section microscopy. According to our observations, the alimentary intake of radionuclides causes an accumulation of toxic metabolites and, as a consequence, the appearance of malignant neoplasms. Thus, in the farms of Minsk and Gomel regions, the number of udder neoplasms increased compared to 1986 from 5–7% to 28% and 29% in 2016, and to 63% and 74% in 2018, respectively.

P68 | Genetic diversity of small-scaled populations in Locus related with reproduction traits of chickens

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The genetic diversity of chicken (Gallus gallus) breeds, formed as a result of artificial selection, provides a variety of breeds adapted to certain environmental conditions. Selection pressure and genetic drift cause variants and combinations of genes representing source of unique genetic material in breeds. The study was conducted on genomic data from 293 chickens of Pushkin, Amrock, Rhode Island, Silkie White, Yurlov Crower and Russian White breeds from the CCU "Genetic collection of rare and endangered breeds of chickens" (St. Petersburg, Pushkin). Genotyping was performed using the illumina Chicken 60K SNP iSelect BeadChip. Egg production (EP) of chickens is an important reproductive trait in small-scaled populations. Several SNPs potentially related with EP regions were selected on different chromosomes, to search for new genomic markers. Quality control, linkage disequilibrium and haplotype studies were carried out in Plink 1.9 software. On chromosome (GGA) 4 and 20 several homozygous regions were found, located in the region of known QTL associated with egg-laying rate. Homozygous haploblocks were found in Pushkin, Russian White and Sussex breeds. This area should be studied in more detail using sequence data. Areas on GGA8 and 17 were defined as potentially associated with egg yolk diameter. Haploblocks in these regions are present in Silkie White, Sussex, Amrock and Rhode Island breeds. Two regions

on GGA16, were possibly associated with the mass of yolk and showed the presence of haploblocks in Silkie White hens. All haploblocks found are important in the search of genes associated with reproduction traits of gene-pool chicken breeds. Studies were funded by RFFI project № 18-016-00114 A.

P69 | Ovarian response to P4 injection plus hCG in multiparous non-cycling local and Alpine goats in northern Mexico

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The aim of this study was to compare the ovarian response in multiparous goats subjected to a protocol based on a progesterone (P4) injection plus human chorionic gonadotropin (hCG). Multiparous non-cycling local Mexican (n = 18) and Alpine (n = 19) goats received 20 mg of P4 plus 100 IU of hCG IM injections 24 h apart. The estrus response was determined twice a day during a 15-minute period (performed from 0 to 5 days after hCG administration) by using two bucks with an apron to avoid complete mating. A female was considered to be in estrus when she stood to be mounted by the buck. Follicular diameter was determined thru transrectal ovarian ultrasonography (7.5 MHz; Aloka SSD 500) at the time of the hCG administration, and then every 12 h from the estrus onset until ovulation occurred. Statistical analyzes for ovulatory rate were performed with a Student-T test for independent samples and the proportion of goats in estrus by means of the Genmode procedure of SAS. The proportion of goats in estrus and those that ovulated tended to be reduced ($p = 0.06$) in the Local than the Alpine (15/18, 83.3% vs. 19/19, 100%; respectively. In addition, no differences ($p > 0.05$) in ovulatory rate (1.6 ± 0.6 vs. 1.3 ± 0.5) and pregnancy rate (55.5% vs. 78.9%) occurred between the Local and the Alpine groups. To conclude, the stage of seasonal anestrus or breed does not modify neither estrus nor ovarian response in non-cyclic goats synchronized with a P4 injection plus hCG-based protocol.

P70 | Thyroid hormones levels evaluation in pregnant Saanen goats

P Polistovskaya; A Bakhta; L Karpenko; A Balykina; A Kozitcyna; A Enukashvili

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The aim of this research was to study blood levels of thyroid hormones in Saanen goats depending on month of pregnancy. The research was conducted in North-Western region of Russian Federation and in biochemistry and physiology department laboratory of FSBEI of Higher Education «SPbSAVM». The experimental group included 30 pregnant Saanen goats, 1–4 years of age, selected using matched pairs method; control group included 30 non-pregnant Saanen goats, same age. The blood samples were taken 5 times during pregnancy – 1 in each month. The blood levels of protein-bound iodine (toluene extraction method), TSH, T3 and T4 (RIA) were assessed. The study outcome revealed that protein-bound iodine level was lower in experimental group ($p < 0.05$) when compared with control group during the whole period of pregnancy – by 28.8% at 2nd month of pregnancy, by 32.2% ($p < 0.05$) at 3rd month, by 36.44% ($p < 0.05$) at 4th month and by 40.68% ($p < 0.05$) at 5th month. Blood levels of T3 and T4 remained within normal limits during the whole period of pregnancy, but the quotient changed – T3 levels were increasing since 3rd month of pregnancy and reached its peak at 5th month (higher than T3 levels of control group by 45.6% ($p < 0.05$)). The T4 level was declining by 5th month of pregnancy and almost reached the lower limit of normal values (lower than T4 levels of control group by 35.6% ($p < 0.05$)). Therefore, there are signs of development of compensated hypothyroidism due to iodine insufficiency during pregnancy in goats, which allows taking into consideration preventive measures for improvement.

P71 | Hematological characteristics in pregnant Saanen goats

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The aim of this research was to study hematological characteristics in Saanen goats depending on month of pregnancy. The research was conducted in North-Western region of Russian Federation, in laboratory of biochemistry and physiology department, FSBEI of Higher Education «SPbSAVM». The experimental group included 30 pregnant Saanen goats, 1–4 years of age, selected using matched pairs method; control group included 30 non-pregnant Saanen goats of the same age. The blood samples were taken 5 times during pregnancy – 1 in each month. The blood levels of hemoglobin, red blood cell count (RBCT), cell-color ratio, iron, total iron binding capacity (TIBC), latent iron binding capacity (LIBC), transferrin

saturation with iron were assessed by using the hematological and biochemical (BA-88A Mindray) analyzers. The study outcome revealed differences in these markers during the whole period of pregnancy. By the 5th month of pregnancy, there were the most visible changes in experimental group when compared with the control group. Blood levels of hemoglobin, iron and RBCT were lower in experimental group when compared with controls during the last month of pregnancy – by 20.1%, 37.5%, 16.5% respectively ($p < 0.05$). The cell-color ratio, TIBC, LIBC and transferrin saturation with iron were elevated by 18%, 25.1%, 28.5%, 33.56% respectively ($p < 0.05$). After analyzing all the data, it is possible to suggest the developing of iron deficiency anemia in the second half of pregnancy in Saanen goats. The blood levels of hemoglobin, iron and RBCT decrease and cell-color ratio, TIBC, LIBC and transferrin saturation with iron elevation are pointing at this. These results suggest to take into consideration preventive measures to prevent this outcome during the second half of pregnancy in goats.

P72 | Human chorionic gonadotropin increase the pregnancy rate in Morada Nova ewes

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The study was performed to evaluate if the use of hCG 7 days after the synchronized estrus in Morada Nova ewes increase the pregnancy rate. Morada Nova ewes (n = 87, mean weight of 36.5 kg, body condition score 3.1 on 1 to 5 scale) were used. Estrus was synchronized with intravaginal device (60 mg, Progespon®, Zoetis, USA) for 6× days and eCG (200 IU, i.m. Novormon®, Zoetis, USA) plus PGF2α analog (37.5 μg, 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 = 43) or normal saline (control group, 1 ml, im., n = 44) was injected. B-mode ultrasound examinations of the ovaries were performed using the equipment MyLab Vet 30 Gold (Esoate, Holanda) on Day 7 and Day 13, to quantify the number of corpora lutea present in the ovaries, and on Day 30, where we confirmed the pregnancy. X^2 test was performed to assess the pregnancy rates. 74.41% (32/43) of the hCG group made at least one accessory corpora lutea (ACL). On Day 7, the number of corpora lutea was not different ($p > 0.05$) between the hCG and control groups (1.50 ± 0.1 vs. 1.55 ± 0.09, respectively), however, on Day 13 it was higher ($p < 0.01$) in the hCG group (2.60 ± 0.13) than in the control group (1.67 ± 0.13). The total pregnancy rate was 45.45% (20/44) in the control group and 67.44% (29/43) in the hCG group ($p = 0.06$). Considering those ewes that successfully formed ACL, the pregnancy rate was 81.25% (26/32), and was different ($p < 0.01$) in comparison to the control group pregnancy rate. The hCG 7 days after the synchronized estrus in Morada Nova ewes increase the pregnancy rate when the ewe have at least one ACL. Financial support: CNPq and EMBRAPA (process nº 02.13.06.026.00.02).

P73 | Sperm concentration and volume of the ejaculate in quarter horses in two season of the year (spring and summer)

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Sperm concentration and volume of the ejaculate were quantified in quarter mile horses in two seasons of the year in Northern Mexico (27°N, 105° W). Equine stallions (n = 6, 9-year-old) with average body weight of 500 kg body and body condition score of 3 units (scale 1–5), were used. The study was carried out in two periods of the year: March to May 2016 (Spring), and December 2016 to February 2017 (Winter). While the volume of the ejaculate (ml) was determined through a graduated conic tube on a 1 ml scale the sperm concentration (x106/ml) was quantified with a SpermaCue Photometer (Minitube, Qro, Mexico). Data were analyzed in a completely random design evaluating the effect of the time (Spring or Winter) through a T-student test (SYSTAT 12.0). Statistical differences ($p < 0.05$) were observed in both the volume of the ejaculate and the sperm concentration observing the higher values during the spring period (102.6 ± 17.2 ml vs. 64.4 ± 4.2 ml, and 311.8 ± 43.8 x106/ml vs 130.6 ± 17.0 x106/ml). At 26° North, the horses of the quartermile show significant differences with respect to sperm concentration and the volume of the ejaculate, observing the best values during the spring season. The last should be important in the design the reproductive programs to this equine breed.

P74 | Milk composition as an indicator of the reproductive performance of Holstein cows

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The aim of the present study was to evaluate potential associations between the milk composition (including metabolic parameters) and reproductive performance of Holstein cows in Russia. Cows were divided in groups based on daily milk production and days open: up

to 80 days (n = 20); 81–120 d (n = 18), 121–200 d and 200 and more days (n = 12). Cows were fed according to the stage of lactation. Rations consisted of corn silage and haylage supplemented with concentrates, vitamins and minerals. On average the energy concentration in 1 kg of dry matter was 11.6–12.6 MJ. Milk was analyzed for 18 different components using the infra-spectrometric analyzer CombiFoss F + . In comparison to cows that became pregnant later than 200 days in milk, cows that became pregnant during the first 80 days, produced milk with a lower protein −0.29% ($p < 0.001$), casein −0.20% ($p < 0.001$), myristic (C14:0) −0.17 g/100 g ($p < 0.001$), palmitic (C16:0) −0.27 g/100 g ($p < 0.01$), stearic (C18:0) −0.17 g/100 g ($p < 0.001$), oleic (C18:1) −0.31 g/100 g ($p < 0.001$), Long chain fatty acids (LCFA) −0.53 g/100 g ($p < 0.001$), Short chain fatty acids (SCFA) −0.15 ($p < 0.001$), MonoUnsaturatedFA −0.19 g/100 g ($p < 0.001$) content. The strongest correlations with days open, were found with mass fractions of oleic acid, LCFA and MonoUnsaturatedFA ($r = 0.626$, $p < 0.001$; 0.643, $p < 0.001$; 0.529, $p < 0.001$, respectively). Conclusively, mass fractions in milk of LCFA and Mono-Unsaturated FA, protein as well as the fatty acid composition are all associated with the days open in Russian Holstein cows. The latter suggests these milk metabolites might be used in the selection for fertility in Russian Holstein cows. AAAA-A18-118021590129-9.

P75 | Somatic cells in milk and reproductive parameters of Red-Motley breed cows

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According to studies from around the world, there is a positive correlation between the somatic cell count (SCC) in cow's milk and fertility parameters such as service period (SP) and number of inseminations (NI). The aim of our research was to determine the association between the SCC in milk and the SP in cows in Russia. Studies were performed in AC Berezovskoe in the Krasnoyarsk region on Red-Motley cows of second lactation and older (n = 147). The cows were divided into classes based on their SCC (x1000 cells/ml) ≤ 400 (n = 95) and > 400 (n = 52), and their SP (days) ≤ 90 (n = 55) and > 90 (n = 92). Analysis of variance and correlation analysis were done to analyze the data. A not significant positive correlation ($p > 0.05$) was established between SCC and SP ($r = 0.11$), SCC and NI ($r = 0.15$) in the class of ≤ 400'000 SCC/ml, as well as a weak negative correlation (-0.11 and −0.12 respectively) in the class > 400'000 SCC/ml. Statistically significant positive correlations ($p < 0.001$) in the classes formed by the service period (≤ 90 and > 90 days) were found between SP and NI ($r = 0.44$ and $r = 0.68$). As a result of the dispersion analysis, a rather weak influence of SCC on SP (4%; $F = 6.5$; $p < 0.05$) and NI (3%; $F = 4.5$; $p < 0.05$) was found, while a significant effect of SP on NI (29%; $p < 0.001$) was established. Conclusively, we detected no significant influence of the somatic cell count in milk on the reproductive ability of cows, while a significant association between the length of the service period and the number of inseminations was found.

P76 | Effect of oregonin from Alnus incana bark on the motility and enzyme activity of cooled ram semen

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The maintenance of high vitality and motility of ram's spermatozoa during storage at low temperatures has a crucial role for successful fertilization. This study was designed to evaluate the effect of the natural antioxidant oregonin on the motility and enzyme activity of ram semen, stored at 5°C for 48 h. The ejaculates of 3 rams with volume > 1 ml; mass motility > 3.5 and abnormal sperms < 20% were used. Semen samples were split in two equal parts and diluted by commercial extender (Triladyl) with no oregonin (control) and supplemented with 100 μM oregonin (experimental) until adjustment of the sperm concentration to 200 × 106 sperm/ml. Equal aliquots of the control (n = 6) and experimental (n = 6) samples were placed in a glass with water at 37° C and stored in a refrigerator for 48 h. Motility parameters by CASA and lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in semen plasma by chemistry analyzer were evaluated at 0, 3, 24 and 48 h. The data were processed by StatSoft, v.10. Significant ($p < 0.05$) reduction in percentage of progressive and linear motile sperm between 0 h and 48 h was recorded in the control samples, while in the oregonin treated samples these parameters were close to the initially determined. The differences in a total curvilinear velocity (VCL) and VCL of rapid sperm indicated faster movement of the oregonin treated sperm (63.6 ± 1.9 μm/s vs.53.4 ± 2.0 μm/s and 126.8 ± 2.3 μm/s vs. 120.3 ± 1.4 μm/s respectively, $p = 0.02$). The activity of LDH and ALP did not differ significantly. In conclusion, supplementation of 100 μM oregonin to the extender had a positive effect on motility parameters of ram semen stored at 5°C for 48 h. Research was supported by NSP-REPROBIOTEH, MES, BULGARIA.

P77 | Effects of single layer centrifugation through EquicollTM on motile sperm subpopulations of donkey fresh semen

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The aim of the study was to determine whether single layer centrifugation (SLC) of fresh donkey semen has any impact on the proportions of motile sperm subpopulations. For this purpose, a total of 5 ejaculates from 3 donkeys (2 donkeys were collected twice, whereas the other was collected once) were obtained using an artificial vagina Hannover model equipped with an in-line filter to get a gel-free semen sample. An aliquot of the extended ejaculate was centrifuged through a SLC of a silane-coated silica colloid with species-specific formulation (EquicollTM, Sweden) and the remaining volume was used as control. Aliquots were incubated at 37°C under aerobic conditions for 3 h. Individual sperm kinematic parameters were evaluated at 0 h, 1 h, 2 h and 3 h, through a computer assisted sperm analysis CASA (Integrated Semen Analysis System, ISAS). A two-step clustering procedure based on log-likelihood and Schwarz Bayesian criterion was run with the 24,399 motile spermatozoa evaluated and four sperm subpopulations (SP) were identified. SP1 and SP2 exhibited the highest average path velocity (VAP), SP3 was characterized by moderate VAP and SP4 showed the lowest VAP. The effects of SLC-filtration upon sperm motile populations were evaluated through a Scheirer-Ray-Hare ranked ANOVA followed by Mann–Whitney test. After 1 and 2 h of incubation, SLC-washed spermatozoa showed significantly ($p < 0.05$) higher proportions of motile spermatozoa belonging to SP2 (1 h: 55.2% ± 7.2%; 2 h: 62.5% ± 4.8%) than the control (1 h: 26.4% ± 8.3%; 2 h: 23.1% ± 6.7%). We can conclude that previous centrifugation with EquicollTM likely selects donkey spermatozoa with high sperm velocity parameters.

P78 | CD9 pattern during bovine sperm maturation

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CD9 tetraspanin is one of the molecules which importance in fertilization process was confirmed. We have already described the presence of CD9 on plasma membrane of bovine ejaculated sperm and its probable role in tetraspanin web stabilization. The aim of this study was to investigate if the testicular form of this protein exists and if the reaction pattern of CD9 is influenced by epididymal maturation since the transfer of CD9-positive microvesicles to bull epididymal sperm was recently documented. Spermatozoa isolated from the caput, corpus and cauda of bull epididymis and within the testicular and epididymal tissue sections were used in our analyses. Epididymides and testes from three different bulls were analysed. Polyclonal antibody MRP1 (anti-CD9) followed by goat anti-rabbit IgG-FITC conjugate were applied in indirect immunofluorescence assay. The intactness of sperm acrosomes was assessed by Peanut agglutinin – TRITC conjugate. We detected the presence of CD9 already in equatorial segment of testicular sperm. The position of CD9 molecule was changed in epididymis distally and expands from equatorial segment of sperm from caput to strong visible pattern spreading to whole acrosomal area of sperm from corpus. The pattern was less visible on caudal sperm. It can be assumed, that CD9 tetraspanin could be additionally obtained by sperm during epididymal transport via CD9-positive vesicles, where it could act also as organizer of this transfer via association with membrane-curving proteins or lipids. Thus these vesicles could serve as a vehicle for other proteins essential for sperm maturation, due to its ability to link covalently other molecules. This work was funded by grants VEGA-2/0037/16, APVV-15-0196, bilateral projects SAS-CAS (15-05) and SAS-CAS (18-17).

P79 | Detection of cluster of differentiation molecule 63 in bull sperm

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The most of cell types including the mammalian gametes express specific subset of tetraspanin family proteins. Experiments mainly performed in mice showed that tetraspanins are also involved in fertilization process. Since we have already identified tetraspanins cluster of differentiation (CD) 9 and CD81 in bull sperm, knowing that CD9 is via epididymosomes transported to the sperm membranes and CD63 is an exosome marker, the goal of the current study was to analyse the expression of CD63 on bull sperm and consider its role in the sperm-egg interaction. In our analysis indirect immunofluorescent assay using polyclonal CD63-antibody and IgG (H+L) Fluorescein conjugate as a secondary antibody on permeabilized smears of intact, capacitated and acrosome-reacted sperm was performed. Capacitation of sperm was induced in TL medium specified for bull sperm capacitation. Sperm acrosome reaction was stimulated in vitro by 10 μmol/L calcium ionophore. Acrosomal integrity was verified by Pisum sativum agglutinin staining. Sperm from five different bulls were analysed. Our results showed CD63 presence in equatorial region of frozen-thawed ejaculated bull sperm without change of localization during the capacitation, whereas in acrosome-reacted sperm a much weaker or no signal of CD63-antibody was detected. Since the equatorial segment of mammalian spermatozoa is considered to be an organizing centre for molecular complexes assembly essential for sperm-egg interaction and following fusion, achieved results could contribute to the understanding of the events that occur upon

fertilization process in mammals, although further analyses using fresh semen are needed. This work was funded by grants VEGA-2/0037/16), APVV-15-0196 and bilateral projects SAS-CAS 15-05 and 18-17.

P80 | Ovarian morphology of Romanov sheep

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The Romanov sheep is characterized by natural multiple pregnancy and polyoestrous sexual period. The morphology and vascularization of the ovaries in postnatal ontogenesis was studied using gross anatomy, histology and computed tomography. In new-born lambs (n = 4) the thickness (T) of the rudimentary epithelium was 2.7 ± 0.3 μm. T of the cortical layer was 99.8 ± 9.6 μm and of the medulla 91.5 ± 9.3 μm. Primordial follicles were arranged in groups of 130–150 pc with diameter (D) of 4.5 ± 0.4 μm. Primary follicles were of oval shape 10–13 pc, D = 78.0 ± 9.8 μm. D of secondary follicles was 151.1 ± 15.0 μm and of tertiary follicles 210.2 ± 25.3 μm. In lambs during puberty (n = 4), the ovary is covered with an albumin membrane (T = 6.3 ± 0.6 μm). In the ovary, there were 130–140 primordial follicles (D = 29.0 ± 3.0 μm) and 21–25 primary follicles. Between the cortical layer and medulla, there were 17–20 secondary follicles. The size of tertiary follicles was 299.5 ± 21.5 μm. In the adult sheep (n = 5), ovarian thickening of the tunica was found in comparison with young sheep. The number of atretic bodies was increased ($p \leq 0.05$) and the number of primordial follicles was directly proportional. In the cortex, there were yellow bodies of the sexual cycle. The parenchyma of the corpus luteum consisted of luteocytes that feed on a dense capillary network. The size of both luteocytes (8.3 ± 2.9 μm) and the capillaries associated with them (11.1 ± 5.0 μm) was very diverse. D of the formed yellow body was 111.9 ± 11.0 μm. In newborn lambs, T of the muscular layer of the ovarian artery was 49.6 ± 5.0 μm and adventitia 39.6 ± 4.0 μm. The before-mentioned measures in 5–7 month sheep were 150.0 ± 15.0 μm and 109.5 ± 11.0 μm, and in adults 230.9 ± 24.5 μm and 182.4 ± 18.2 μm, respectively.

P81 | Abstract withdrawn

P82 | Evaluation of the activity of enzymes of pregnant and non-pregnant cows

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The objective of the current research was to study the activity of enzymes of pregnant and non-pregnant cows. The research was performed on clinically healthy cows of Kostroma breed of 3rd-4th lactation (n = 15): during the dry period (one month before calving); during the prenatal period (10–13 days before calving); during the postnatal period (until 20 days after calving). The activity of AST, ALT, CPK, LDH enzymes in the serum samples was checked by standard methods. The results of the research showed that AST activity was below normal range and changed fluctuating: one month before calving period cows had 24.43 ± 0.96 U/l, then values decreasing during the prenatal period by 21% with subsequent return to the initial index. ALT activity was within the normal range and decreased over the entire period from 22.32 ± 1.36 to 13.24 ± 1.12 U/l ($p < 0.001$). The month before calving, LDH activity was within the physiological range (510.63 ± 44.01 U/l), then increased before calving and exceeded the limit (950.72 ± 7.23 U/l; $p < 0.001$); however, during the postnatal period, it decreased to the normal range, but remained above the initial values (856.2 ± 12.61 U/l; $p < 0.001$). CPK activity of cows was above the normal range, in the pregnant it ranged from 332.9 ± 18.8 to 360.0 ± 8.89 U/l, and among non-pregnant animals it was 2.0–2.2 times lower ($p < 0.001$). These enzymes suggest metabolic changes in the cow during pregnancy that should be further investigated.

P83 | Storage of porcine morulae and blastocysts at 20 °C prevents hatching for at least 72 h

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Although vitrification is the ideal method for the cryopreservation of porcine embryos, the strict airline rules for the transportation of liquid nitrogen (LN2) dewars and the obstacles experienced when vitrified embryos are transferred by non-surgical methods have forced the introduction of alternative storage methods. Here, we examined the effects of storage temperature and medium supplementation on the embryonic viability (EV) and development (ED) of in vivo-derived morulae (MO) and blastocysts (BL). Embryos (N = 401) were stored for 72 h at 17°C or 20°C in NCSU23-HEPES containing 50% FCS or 4% BSA. At the end of storage, EV and ED were assessed. Then, the stored embryos were conventionally cultured (NCSU23-0.4% BSA-10% FCS, 38.5°C, 5% CO2) for additional 48 h (MO) or 24 h (BL) to reassess EV and ED and to evaluate their hatching competence. Fresh MO and BL conventionally cultured were used as controls. Data were compared using the Fisher's exact test and the Kruskal–Wallis test. Neither FCS nor BSA was able to counteract the negative effect of low temperatures (17°C) on EV after storage. In contrast, 4% BSA in the medium at 20°C successfully arrested the ED of both MO

and BL at 72 h. Over 80% of the arrested embryos restarted ED by conventional culture and progressed to further stages, including hatching. However, EV was lower ($p < 0.03$) and ED was slower ($p < 0.001$) in these stored embryos compared to the controls. In conclusion, porcine MO and BL can survive and remain unhatched (a sanitary requirement) during at least 72 h when stored at 20°C in a liquid state. This strategy offers new options for the use of porcine embryos worldwide in the absence of LN2. Supported by MINECO-FEDER (AGL2015-69735-R), Seneca Foundation (19892/GERM/15) and FORMAS (2017-00946).

P84 | Prevalence of retained fetal membranes after elective cesarean section in Belgian Blue cattle

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In cattle, a variety of risk factors is associated with retained fetal membranes (RFM) including dystocia, induction of parturition, abortion and cesarean section (CS). As more than 95% of Belgian Blue cattle (BB) give birth by CS, the occurrence of RFM is expected to be higher in this breed. The aim of this study was to evaluate the prevalence of RFM after elective CS and whether parity is a risk factor for RFM in BB. Over a 1000 surgery reports from CS performed in the teaching hospital between November 2011 and January 2017 were used as input for this study. These reports contain information about the cow before, during and after the CS, including the moment of placental expulsion. Only cases where the calf was born alive and where complete data were available were included (N = 882). The results of the descriptive analysis show that 29.5% of the animals retained their fetal membranes within 12 h post-partum. After 24 h, 18.8% of the animals still didn't expel their placenta and were diagnosed with RFM. Using a logistic regression model (SAS 9.4®), it was shown that the more CS a cow had, the higher the risk to suffer from RFM ($p < 0.001$). This study shows that when elective CS are performed, 18.8% of the cows suffer from RFM, which is higher than after a normal parturition but less than previously described in non-elective CS (35–40.8%) (Bouchard et al., Can Vet J. 1994; 35(12):770–774; Newman & Anderson, Vet Clin Food Anim 21 (2005):73–100) and the number of CS the cow had already undergone prior to calving, increases the risk for RFM.

P85 | The activity of the cytochrome system p450-dependent monooxygenases in calves with liver pathology of antenatal origin

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The aim of the study was the activity of the cytochrome system of P450-dependent monooxygenases in clinically healthy calves (n = 10) and with liver pathology of antenatal origin caused by malnutrition in pregnant cows (n = 10). Twenty Holstein calves were examined at the age of 1 h, 1, 15, 30, 60, 90, 120, 150 and 180 days. Colostrum was given to calves 90 min after birth and for 3 more days; from 4–60 days their diet consisted of milk, hay and mixed cattle feed, followed by only forage. On the first day, the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), succinate dehydrogenase (SDH) and glutamate dehydrogenase (GLDH) was determined in blood. When assessing the activity of the cytochrome P450 system, blood volume was determined, and a loading test with antipyrin (0.15 mg/l of blood) was performed. The cytolysis syndrome was revealed in calves with perinatal pathology: ALT activity was increased (by 25%), AST (by 71%), GGT (by 39.5%), SDH (by 6.7 times) and GLDH (by 5 times). In sick calves at the age of 1 h the clearance of antipyrin was 3.70 ± 0.053 ml/min (2.11 ± 0.025 in healthy calves), 1 day 2.60 ± 0.017 (2.25 ± 0.030), 15 days 1.86 ± 0.0035 (4.50 ± 0.020), 30 days 9.04 ± 0.050 (13.50 ± 0.28), 60 days 17.0 ± 1.30 (25.9 ± 0.45) 90 days 39.0 ± 5.06 (45.0 ± 1.31), 120 days 43.9 ± 6.00 (56.7 ± 5.58), 150 days 53.7 ± 4.63 (58.1 ± 7.52) and 180 days 55.0 ± 3.97 (57.0 ± 4.79). It has been revealed that newborns with congenital liver pathology are characterized by premature activation of the cytochrome system of P450-dependent monooxygenases, but then its prolonged depression is observed, which probably increases the sensitivity of organism to toxins of endogenous and exogenous origin, including the increased risk of drug complications.

P86 | Interferon-tau in the blood of cows as an indicator of the emerging pregnancy

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Interferon-tau (INFT) produced by embryonic trophoblast cells, plays a special role in mechanisms of immunoendocrine control of embryo formation and development in cattle. The INFT ensures the physiologically necessary conditions for maternal recognition of pregnancy. The aim of the study was to evaluate the INFT status of

cows during embryos' normal development, delayed development and embryonic death. There were 25 cows included in the study. Blood samples were obtained on 8–9, 11–13 and 15–17 days after insemination. INFT content was determined in serum using Bovine Interferon-Tau Elisa Kit (Cloud-Clone Corp., Katy, TX, USA). The state of the emerging embryo was retrospectively assessed by transrectal ultrasonography of the genital tract at 30–32 and 60–65 days after insemination. It could be shown that in cows with normally developing pregnancy, the INFT concentration was 364.0 ± 22.1 pg/ml at 8–9 days, increased 2.43 times by day 11–13 to 886.0 ± 47.5 pg/ml, and at 15–17 days increased again to 1403.2 ± 71.8 pg/ml. At delayed embryo development, it was statistically lower ($p < 0.05$) by 23.5% (278.4 ± 19.4 pg/ml), 25.4% (1046.4 ± 62.7 pg/ml) and 22.0% (634.2 ± 45.8 pg/ml), respectively, and at embryonic death was lower by 21.6% (285.5 ± 20.8 pg/ml), 39.8% (845.2 ± 51.7 pg/ml) and 29.7% (571.9 ± 38.2 pg/ml). The confidence interval of INFT concentration variations on days 15–17 was 1330–1475 pg/ml, 980–1110 pg/ml and 790–900 pg/ml, respectively. Based on the data obtained, it can be concluded that the determination of INFT concentration in the blood of cows during the period for maternal recognition of pregnancy gives an indication of the nature of embryo's development and predict its possible death.

P87 | The influence of alpha and gamma interferon on cyto-morphometric profile of sow's vaginal mucosa in different physiological periods

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The studies for the presence of latent inflammatory processes in reproductive organs were carried out on 27 sows, which were tested on day 103–105 of pregnancy and divided into two groups. Sows of the first group (n = 13) eight to ten days before farrowing were administered parenterally alpha and gamma (α- and γ) recombinant pork interferons 10 ml per animal three times with the interval of 48 h. The second group (n = 14) served as a control. Swab samples of the vagina were collected and stained according Romanovsky-Giemsa. The data were statistically processed using IBM Statistics 20.0 (IBM Corp., USA) and Students t-test. The number of parabasal cells in animals of the experimental and the control groups was 30.0% and 27.0%, respectively at cyto-morphometric examination of vaginal mucosa 103–105 days before farrowing. The number of intermediate cells was 44.0% and 40.8%, respectively; surface cells were 26.0% and 32.2%, respectively. The number of parabasal cells in the lactating animals of the first group decreased by 20.0% (24.0%) on 3–4 days after farrowing. No significant changes in number of parabasal cells were observed in sows of the control group (26.2%). The number of intermediate cells increased to 70.6%. The number of intermediate cells in the control group remained practically unchanged (40.8%). The pool of surface layer cells in the animals of the first group decreased by 4.6 times. In the control group, the number of surface vaginal epithelial cells decreased by 1.3 times, and it was 24.8%. The decrease in the number of deep layer cells in the endometrial mucosa of the experimental group might indicate the prevention of a potential inflammation. An increased number of intermediate and superficial layer cells might indicate remodelling.

P88 | The effect of the probiotic preparation bioplus-2b on the microbiocenosis of the genital tract and sow productivity

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We have studied the influence of probiotic "Bioplus 2B" (is a complex of lactose and a lyophilized spore-forming bacteria Bacillus subtilis, and Bacillus licheniformis, in a ratio of 1:1) on the microbiome of the reproductive tract and on the productive health in 50 lactating sows. The experimental group (n = 27) received two weeks before weaning "Bioplus 2B" in a mixture with feed in a dose of 0.4 g/kg. Sows of the second group (n = 23) served as control. The vaginal mucus was taken 2 weeks before and just before weaning. Postpartum diseases were assessed throughout the entire lactation period. Statistical data processing was carried out using IBM Statistics 10.0 (IBM Corp., USA). The microbial landscape of the reproductive tract of experimental sows in relation to the control contained 1.9 times more lactobacillus, 2.6 times more bifidobacteria, 2.1 times more enterococcus, 30.9 times more bacillus (Bacillus spp.) and 4.2 times more Escherichia. At the same time, Staphylococcus aureus and Pseudomonas aeruginosa were additionally isolated only from the control animals in 70% and 20% of the cases, respectively. The analysis of this clinical study has shown that 7 sows of the experimental group and 9 sows of the control group suffered from postpartal diseases, including acute purulent-and-catarrhal endometritis in 6 sows (22.2%), in 6 sows (26.1%), and in 3 sows and in 1 sow metrite-mastitis-agalactium accordingly. By the end of the sucking period, the number of piglets per sow in the first and second groups was 9.5 and 9.1, and their survival was 85.6% and 78.9%, respectively. The use of the probiotic drug in sows contributes to the normalization of microbiocenosis of the genital tract and improves their performance.

P89 | The influence of geomagnetic storms on the sperm morphology

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In scientific literature, there is no consensus on the effect of geomagnetic storms on biological objects. An analysis of existing data suggests that the influence of geomagnetic activity on biological objects depends on many factors, including the features of these objects. The purpose of this study was to study the effect of geomagnetic storms on the pathology of spermatozoa of bulls. Samples of spermatozoa of bulls (n = 17) of seven breeds were investigated: Holstein, Simmental, Schwyz, Jersey, Kostroma, Yaroslavl, and Montbeliard. Samples were collected 6 times at magnetic storms and 12 times in its absence. Monitoring of geomagnetic activity (K-index) was carried out according to the Pushkov Institute of Terrestrial Magnetism, Ionosphere and Radio Wave Propagation Russian Academy of Sciences. CASA systems were used to assess sperm morphology. Statistical analysis was performed using SPSS v.15.0. A one-factor analysis of variance (ANOVA) was performed. In the period of our study from January to September 2018, several geomagnetic storms were observed, the maximum value K = 5. Sperm collection from bulls was performed on days with different levels of geomagnetic activity. On days with geomagnetic storms, the proportion of ejaculates with sperm pathology was 10.32 ± 1.28%; in the absence of disturbance, this proportion was 5.03 ± 0.66%, ($p < 0.05$). The most frequent pathology was seen in the flagella of spermatozoa, the difference between the average values of samples obtained on days with geomagnetic activity and the quiet period was 4.65% ($p < 0.05$). The survey is conducted under support grant №. 18-016-00128 A, provided by the RFB.

P90 | Cattle embryo development at elevated temperature before activation of embryonic genome

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Cattle embryos are sensitive to high temperature before activation of the embryonic genome. However, the embryos' response to elevated temperature can depend on embryo system cultures. The aim was to compare the response of embryos to high temperatures in coculture with bovine oviduct epithelial cells (BOECs) and in synthetic medium KSOMaa. The cattle zygotes were: 1) cocultured with BOECs and 2) cultured in synthetic medium KSOMaa at control temperature (38.5°C) for 168 h and transiently elevated temperature (40.5°C; 41°C) for 72 h, then at 38.5°C for a total of 168 h. The percentage of cleaved, 8 cell and blastocysts dependency on temperature (n = 6) was analysed by multiple pair-wise comparisons using Tukey's test Statgraphics 5.0 Centurion (USA). At control temperature of 38.5°C, there was no significant difference in percentage of cleaved (83.26 ± 2.96 vs 85.75 ± 4.43%), 8 cell (64.59 ± 3.11 vs 61.34 ± 2.11%) and blastocysts (30.21 ± 1.89 vs 29.21 ± 2.53%) between BOECs and KSOMaa. At 40.5°C, the embryos cocultured with BOECs developed significantly better compared with the KSOMaa system, as observed from the cleavage rate (70.45 ± 2.45 vs 65.13 ± 1.09%), minimum 8 cell rate (19.18 ± 1.37 vs 14.43 ± 1.07%) and blastocyst rate (5.08 ± 2.14 vs 0%), ($p < 0.001$). At 41°C, there was a significant difference in the cleavage rate (60.46 ± 0.98 vs 53.64%) and minimum 8 cell rate (10.32 ± 2.11 vs 7.25 ± 2.44%) in the BOECs coculture system compared with the KSOMaa system ($p < 0.001$) but no difference in the blastocyst rate. These data indicate that cattle embryos cultured at elevated temperatures until activation of the genome in the presence of BOECs develop better than embryos cultured in KSOMaa. KNOW 05-1/KNOW2/2015.

P91 | Effect of social rank upon the induction of the reproductive response of anestrous goats treated with eCG

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We evaluated if the social rank affects the reproductive response of anovulatory goats subjected to a hormonal treatment with IM progesterone (P4) plus equine chorionic gonadotropin (eCG) under an intensive production system. Goats (n = 70, 1–4 yr old) were subjected to a behavioral test to determine the social rank of each female by evaluating the aggressive interactions among goats at feeding (08:00–10:00, 13:00–15:00 and 18:00–20:00 h) during 7 consecutive days. Thereafter, a success index (SI) was calculated and three social ranks were defined: 0 to 0.33, low social rank (LSR); 0.34 to 0.66 medium social rank (MSR) and; 0.67 to 1, high social rank (HSR). According to their behavior, 18, 28 and 24 females were respectively grouped in each social rank. During the anestrous season (March, 26° North), 25 mg of P4 + 350 IU of eCG (24 h apart) were applied intramuscularly. Thereafter, estrus, ovulation and pregnancy were quantified and evaluated throughout a chi-square

test. The estrus percentage differed ($p < 0.01$) among social rank with the largest values occurring in the HSR goats (96%) regarding the MSR 71% and the LSR 56%. However, neither the ovulation percentage (LSR 78%, MSR 93% and HSR 96%) nor the pregnancy percentage (LSR 50%, MSR 50% and HSR 58%), differed among groups. Our results demonstrate that the social rank only affects estrus behaviour, but not ovulation and pregnancy rate; this should be important in delineating reproductive strategies within the reproductive herd.

P92 | Advantages of the bilateral flank spay in guinea pigs (Cavia porcellus)

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Nowadays, guinea pigs (GP) are rather popular pets. GP older than 2.5 yo have reproductive system pathologies – ovary and follicle cysts. To prevent this condition ovariohysterectomy (OHE) should be provided at the age of 5 months. Two methods of OHE can be used: midline or bilateral flank approach. We investigated two groups, which consisted of healthy female GP aged 6 mo to 6 years. First group: GP with ovary cysts less than 1.5 cm or without cysts, with bilateral flank OHE (n = 7). Second group: GP with cysts bigger than 1.5 cm, OHE with midline incision (n = 5). The premedication was Metoclopramide (1 mg/kg) to prevent GI-stasis and Ketofen 1% (2 mg/kg) to reduce the pain. General anesthesia was provided by isoflurane (4% for induction, 3% for maintenance). Flank spay approach was made to each ovary from each side. Anatomically, the left ovary is situated in 0.5 cm and the right ovary in 1.2 cm behind the last rib. The incision was made according to the ovaries location (dissecting skin, then pushing apart muscles, cutting the peritoneum). Ovarian and uterine blood vessels, ovarian ligament and uterine horn were ligated. After removal of the ovaries and uterine horns, the abdominal wall and skin was sutured. OHE through midline incision: A 4 cm incision was made on the white line of the abdomen because of the short ovarian ligaments. This method is favorable when cysts are bigger than 1.5 cm. According to our data, bilateral flank spay reduces postsurgical side effects, because of the size of the incision, and because contact with the GI tract is minimized. Furthermore, incision location provides a more convenient access to the ovaries. GP started to eat earlier after this method and recovered uneventfully. Flank approach is preferable if ovaries are not too large.

P93 | Seminal plasma affects the expression of the ABDH2 and VCAN genes in the sow internal genital tract

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The pig ejaculate induces gene expression changes in the female reproductive tract, either by spermatozoa or the seminal plasma (SP). We have studied the expression of ABDH2 (coding for the enzyme α/β hydrolase domain-containing protein 2, ABHD2; a key controller of sperm hyperactivation) and VCAN (coding for versican, indirectly binding Hyaluronan (HA) via CD44) in segments of the sow genital tract (cervix, distal/prox uterus, oviductal sperm reservoir (UTJ), isthmus, ampulla and infundibulum) surgically removed from 20 oestrus, preovulatory sows 24 h after mating (n = 4), or artificial insemination (AI) with P1-fraction (P1-AI, n = 4), the SP from either the whole ejaculate (SP-Ejac, n = 4) or solely from the sperm-peak fraction (SP-P1, n = 4). AI of BTS acted as negative control (Control, n = 4). RNA was Trizol-isolated for global transcript analysis with 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, $p < 0.05$ & False Discovery Rate (FDR $q < 0.05$)) identifying biological processes with PHANTER. ABDH2 was down-regulated in all tissues by mating, and in distal uterus by all experimental groups. VCAN was down-regulated in all tissues (mating), UTJ (SP-P1), or ProxUt, UTJ and Itshmus (P1-AI); but appeared up-regulated in isthmus by SP-Ejac. The general down-regulation of ABDH2 might relate to a delay of sperm hyperactivation during the preovulatory stage. VCAN would be increasing sperm-epithelial binding in regions where hyaluronan is present. Supported by The Swedish Research Council FORMAS (2017-00946), Stockholm, Sweden.

P94 | Protective extender medium may enhance seminal plasma tolerance in long-term stored spermatozoa of sensitive boars

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Recent studies showed that long-term exposure of sperm to 10 % of autologous seminal plasma (SP) in semen extended in Beltsville Thawing solution (BTS) caused a dramatic loss in sperm motility in some boars. The aim of this study was to examine if protective extenders are able to diminish the damaging effect of autologous SP during long-term semen storage. Semen (n = 8 boars) was

diluted split-sample to 18 × 10^6 sperm/ml with 10% autologous SP to 100 ml in three different extender media: BTS (Control), modified pH-stabilized BTS (mBTS) and pH-stabilized Androstar® Plus (APlus; Minitüb, Germany). Samples were stored for 144 h at 17°C. Data were tested for normal distribution (Shapiro-Wilk test) followed by pair-wise comparisons using Student t-test (PROC UNIVARIATE) with SAS Enterprise Guide 7.1. Spermatozoa extended in BTS from two boars reacted sensitive to their own SP as seen in low motility (13.1%, 7.2%) assessed with AndroVision® and a low proportion of viable sperm with high mitochondria membrane potential (hMMP) assessed by flow cytometry with propidium iodide and JC-1 (60.3%, 27.4%). The other six boars maintained high sperm quality in BTS-extended semen during storage (motility: 74.0 ± 3.8 %, hMMP: 87.6 ± 4.8%). Semen of the two SP-sensitive boars extended in APlus maintained high motility (74.1%, 65.1%) and hMMP (97.7%, 84.9%), whereas mBTS-extended samples showed similar low values for both sperm traits (motility: 4.3%, 10.9%; hMMP: 19.2%, 36.5%) as samples extended in BTS. In conclusion, in boars with an enhanced sensitivity of sperm to autologous SP, the protective long-term extender medium APlus can counteract the detrimental effect on sperm during long-term storage. This effect is independent of pH-stabilization in the sample.

P95 | Stimulation of follicle maturation in the parent flock of hens

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During the oviposition period, the ovaries contain from 500 to 3500 follicles of varying degrees of maturity. The purpose of our research was to study the process of stimulating the maturation of follicles in the parent flock of hens using a betulin (birch extract) nanoemulsion. To conduct the study two groups were formed: control and experimental (betulin emulsion 1 ml per head daily for 30 days). The research methods are biochemical and morphometric. As a result of the anatomic cutting, it was recorded that the test group of hens had uniform formation of secondary follicles (45–30 mm diameter), 4–5 follicles, which corresponds to the norm for high-laying hens with daily ovulation. Betulin accelerates the processes of metabolism and lipid excretion from the body, including through the egg, which confirms the data on the content of vitamin A (retinyl acetate) in the yolk, 6634 IU/kg in the experimental group and 4885 IU/kg in the control. Betulin contributed to the inhibition of cholesterol catabolism and inhibition of lipid synthesis in the liver: a decrease in abdominal fat deposition by 35% was observed (in the experimental group, 75.8 g and in the control 116.8 g); a decrease in liver mass by 29% (51 g in the experimental and 72 g in the control group). The control group showed the following results – 1/3 of the livestock had 8 – 9 secondary follicles and 2/3 of the livestock 1–3 secondary follicles. These figures indicate uneven maturation of secondary follicles. Indicators in live body weight confirmed the effect of betulin; in the experimental group, the rate was lower on average by 3.2%. It seems that the use of the betulin nanoemulsion has contributed to the stimulation of follicle formation due to the redistribution of lipid deposits in the body.

P96 | Calcification in organs of the reproductive system of broiler parent flocks

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Failure of the reproductive system of the breeding bird leads to a decrease in egg production and significant losses. The purpose of this study was to establish the causes of calculus and calcifications in the reproductive organs of the parent broiler flock and also to reduce the percentage of their occurrence. In the study, 2 groups of 8000 heads were formed: control (basic) and experimental (the bird additionally received probiotic based on Bacillus subtilis bacteria at the rate of 2 kg/1 ton of feed). Morphological, histological, biochemical and electron microscopic methods were used. Numerous pathological changes in the reproductive organs were recorded in the control group of hens and roosters. Calcifications detected in the ovaries and oviducts in 7% of the population led to stagnation in the reproductive organs, chronic inflammation and created the prerequisites of infection. In 10% of the population, it was revealed that productivity was reduced by 4.7% due to violation of egg formation. Ultrastructural changes in the permeability of mitochondrial membranes of spermatozoa in the form of calculus were established. A decrease in the quality of sperm was found in 50% of cases, the culling of the livestock of roosters was 35%. In the experimental group of hens and roosters receiving Bacillus subtilis, pathological changes due to calcification have not been identified. As a result, the main reasons for the occurrence of calculus and calcinate are a violation of the feeding schemes, the incorrect selection of calcium-containing additives for age and sex groups and the use of water with a high content of carbonates. To preserve the reproductive capacity of the birds it is necessary to observe feeding options and use of probiotics.

P97 | The influence of Bacillus subtilis on reproductive indicators of chickens in the second phase of productivity

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In breeding poultry, forced molting is used to extend the life of hens. The purpose of this research was to increase the reproduction rate of kross Hisex-Brown hens in the second phase of productivity after forced molting. After the first phase of productivity, the hens were forced to molt. Two groups were formed: the control group (CONTR, n = 1070) and the experimental group (EXP, n = 1068). The feeding and housing conditions were the same, except that hens in EXP were fed an additional probioticin, based on Bacillus subtilis in the amount of 0.3% of weight of food. The experiment lasted for 67–82 weeks. Egg production for laying hen over the period was 80.2 pcs. in CONTR and 82.7 pcs in EXP. The average productivity in CONTR was 67.7% and in EXP 68.9%. It was found that a number of parameters improved in the experimental group: clean eggs by 1.8%, the content of vitamin A in the yolk of the egg by 16%, fertilization by 1.0%, hatching by 1.4%, hatchability by 1.22% compared with CONTR. Feed consumption in EXP decreased by 4%. The increase in productive and reproductive indicators of laying hens was associated with the use of probiotic based on Bacillus subtilis in the second phase of productivity. The number of microorganisms/g in the large intestine increased in EXP compared to CONTR: Lactobacillus 100'000 in EXP and 1'000 in CONTR, Bifidobacterium 1'000'000'000 in EXP and 1'000'000 in CONTR, respectively. Improving reproductive performance of laying hens in the parent flock in the second phase of productivity is possible by feeding probiotics based on Bacillus subtilis.

P98 | Chemotactic activity in the follicular and oviductal fluid is independent on progesterone level in porcine

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Chemotaxis has been associated mainly to progesterone (P4). However, follicular fluid (FF) and oviductal fluids (OF) have several components (cyclic guanosine monophosphate, cyclic adenosine monophosphate, atrial natriuretic peptide) with chemotactic ability. The aim was to investigate the higher chemotactic activity by serial dilution curves of FF and OF in sperm. Periovulatory OF and FF were collected and P4 (ng/ml) concentration was measured. The serial dilution curves were: FF (%): 0.13%(P4:0.58), 0.25(1.1), 0.5(2.18), 1(4.36) and 1.5(6.5); OF(%): 0.13(0.007), 0.25(0.013), 0.5(0.025), 1(0.051) and 1.5(0.08). The best dilution curve from each fluid was selected and its effect was studied. A chemotaxis system consists of two wells (A and B). Six wells (A) were filled with fresh washed sperm (20 × 106/ml diluted in 500 µl) from fertile boars (N = 3). The opposite wells (B) were filled with TALP (control group) and TALP supplemented with corresponding chemoattractants concentration (FF and OF). After 20 min of chemotaxis, the sperm from wells B were evaluated. The results show that 0.25% FF (7.6%) and 0.25% OF (8.4%), attracted a higher sperm percentage than their respective dilution curves and TALP (FF and OF: 0.13, 0.5, 1, 1.5 and TALP ($p < 0.05$)) that attracted around 5% of sperm in each curves and TALP. However, no difference was observed for the best dilutions curve: 0.25% FF: 6.7% and 0.25% OF: 7.1% ($p > 0.05$), even so, both fluids attracted more sperm vs TALP (5.8%) ($p < 0.05$). Concluding, other components in these biofluids could be more important than P4 in sperm attraction. Supported by Fundación Séneca, Saavedra Fajardo (20020/SF/16). MINECO-FEDER (AGL 2015-66341-R).

P99 | The effect of isoflurane anesthesia on the cardiac activity and contractility of pregnant rabbit uterine

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The aim of the study was to assess the effect of inhalation anesthesia on the cardiac activity of the female (F) and fetus and the contractility of the uterus. Isoflurane was used as anesthetic at a concentration of 2.0 vol% in an oxygen-air mixture at a gas flow rate of 1.5 l/min. The accuracy and stability of the dose of isoflurane was provided by the mini-evaporator “MINIVAP-20”. Pregnant Chinchilla rabbits were included (weight 3.0 – 3.8 kg). In all F on day 30 of pregnancy by single-stage infusion into the ear vein with 1 U of oxytocin. In 10 F of the experimental group (EG), 10 min after induction of labor, inhalation mask anesthesia with isoflurane was performed for 20 min. The 10 F of the control group (CG) did not undergo anesthesia after administration of oxytocin. Electrodes were implanted to all F the day before to simultaneously record the electro-myographic activity of the uterus, the electro-cardiography (ECG) of the F and the fetuses. Indicators were recorded every 10 min. The contractile activity of the uterus was assessed by the number and duration of one contraction. Induction of labor with oxytocin in the CG at the 10th min led to the most significant increase in the number of uterine contractions by 20 times and their duration by 8 times, as well as

to a 9% decrease in the female heart rate and 30% fetal heart rate (FHR). In the EG, at the 10th minute of anesthesia, the number of uterine contractions was minimal (0.10 ± 0.07 vs. 7.50 ± 0.88 in the CG). The heart rate of the fetus and the F in both groups, starting from the 20th min of the study returned to baseline values. The data obtained confirm the feasibility and safety of isoflurane for anesthesia of pregnant animals.

P100 | Comparison of three treatments for subclinical mastitis in cattle

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One of the factors that have a significant impact on the profitability of cows is mastitis. Many authors mention that mastitis increases the index of insemination, the intercalving interval, and increases costs for veterinary care of animals. In this regard, it is important to reduce the disease incidence of mastitis and, as a result, to increase the level of reproduction of the herd. The goal of our work was to find an effective treatment for mastitis. The research was conducted in AC Doroninskoe Novosibirsk region. We included Black-and-White cows suffering from a subclinical form of mastitis. To diagnose mastitis clinical mammary gland examinations of cows were done and somatic cell count (SCC) in milk tested using Cenotest. Cows with 1.0–5.0 million/ml SCC in milk (n = 150) were divided into 3 groups of n = 50 each: for the 1st group Mastijet Gold (containing the antibiotic cephapirin) was used, in the 2nd group Masti Veyxim (containing enzymes) was used, in the 3rd one Septogel (containing halogens) was used. The preparations were injected into each affected quarter via the teat canal 3 times with 12 h interval. 15 days after treatment, the clinical mammary gland examinations and SCC in milk were again performed; cows were considered healthy with < 350'000 SCC/ml. The significance level between the shares of values in groups was determined by Student's t-test. There was found highest share (92.0%) of recovered cows in the 3rd group vs the 1st (90.0%) and the 2nd groups (87.0%), least relapses of the disease in 2nd and 3rd (11.0 and 14.3%) vs 1st group (77.8%). The differences by relapses between 1st and 3rd, 1st and 2nd groups were 66% and 64% ($p < 0.001$). Thus, for reducing the number of relapses, the most effective drugs to use are Septogel and Masti Veyxim. The study was funded by Krasnoyarsk Regional Fund for Support of Scientific and Technical Activities within the framework in the conference "23th Annual Conference of the ESDAR".

P101 | Use of medical grade honey and L-Mesitran™ soft, to improve the quality of chilled-stored ram semen

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In sheep, the use of new seminal extenders to lengthen sperm viability and increase fertilization rates is of primordial importance on the improvement of chilled semen preservation. Our objective was to test different concentrations of gamma-irradiated medical grade honey (MGH) or commercial L-Mesitran soft (with 40% MGH), added to a skimmed milk based extender (SMbe) on the lifespan and fertilizing ability of sheep spermatozoa. After collection, ram ejaculates were evaluated and immediately divided into 5 groups: Control (SMbe without supplementation), 1% or 5% L-Mesitran (SMbe plus 1% or 5% L-Mesitran soft); 1% or 5% MGH (SMbe plus 1% or 5% MGH) and refrigerated at 4°C. Sperm morphology, vitality, concentration and motility (Visual and Computer Assisted Sperm Analysis) were assessed at 5 h and 48 h (exp 1, 12 replicates). In vitro fertilization (IVF) experiments were also conducted at 30 h (exp. 2, 6 replicates). Independently of treatments, the sperm motility and viability decreased over time ($p < 0.001$). The 5% MGH group had higher percentage of static spermatozoa similarly to 5% L-Mesitran but exceeded the vitality of the control and 1% MGH groups ($p \leq 0.05$). Data analysis was conducted with proc mixed and proc glimmix of SAS. The 1% and 5% L-Mesitran groups had higher ($p \leq 0.04$) fertilizations rates compared to Control and 5% MGH. Overall, the 1% L-Mesitran group achieved the highest fertilization rate. It was demonstrated for the first time that L-Mesitran added to the skimmed milk seminal extender, can extend the viability of spermatozoa, after 30 h of refrigeration, through its cryoprotectant and nutraceutical properties. Funding: Projects Go BovMais (PDR2020-101-03112), UID/CVT/276/2019 (CIISA) and ALT20-03-0246-FEDER-000021.

P102 | Motility characteristic, morphology and acrosomal membrane integrity of epididymal sperm of red deer (Cervus elaphus L.) stored in a liquid state at 5°C

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This study investigated how storage time affected motility, morphology and acrosomal membrane integrity of red deer epididymal sperm stored in a liquid state. The epididymal semen

was obtained post mortem from nine bucks shot during the rut. The semen was diluted with the commercial extender Bovidyl® (Minitüb GmbH, Germany) and stored for six days (D1-D6) at 5°C. Sperm analyses included an assay of motility parameters through the use of the CASA system, the percentage of sperm with normal morphology (MOR) and acrosomal integrity (NAR). Duncan's post hoc test showed that values of the analyzed parameters were dependent on the day of storage. Significant decreases ($p < 0.05$) were recorded on D3 for VAP and LIN (values on D1 and D3 for each of those parameters were 127.5 ± 9.3 μm/s, 58.6 ± 1.1% and 114.2 ± 6.1 μm/s, 54.4 ± 1.7%, respectively). A significant decrease for MOR was found on D5 (values on D1 and D5 were 86.7 ± 4.8% and 85.7 ± 6.5% respectively). A significant decrease for NAR was demonstrated on D6 (values on D1 and D6 were 91.8 ± 1.1% and 84.5 ± 1.9% respectively). The results of this study indicated that the red deer epididymal semen can be stored up to several days at 5°C in a liquid state. The parameters of motility of the epididymal sperm appear to be more sensitive to storage time at a reduced temperature than the changes in morphology and acrosomal membrane integrity. (Supported by a NCN project, Poland (2017/25/B/NZ9/02544)).

P103 | Treatment of cows with subclinical mastitis

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The therapeutic efficacy of "Alvesol" (herbal drug) and "Pharmoxidin"(dioxidine) was studied under the conditions of the dairy farm in the Pskov region, Russia. The cause of the udder disease might have been a long time between cleaning the udder and putting the teatcups, which reached up to 2 min. In cases of subclinical mastitis (number of somatic cells before treatment ranged from 602.2 ± 11.9 thousand to 1300.9 ± 44.2 thousand), we used "Alvesol" (Helvet, Russia) in the 1st group (n = 42) to treat cows. In the 2nd group, "Pharmoxidin" (MosAgrogen, Russia) was used (n = 43). Both drugs were administered to cows after milking, "Alvesol" intramuscularly at a dose of 10 ml, once a day. "Pharmoxidine" was injected intracisternally at a dose of 20 ml into the affected quarter, once a day. Recovery was defined as the number of somatic cells in milk in the range from 140 to 200 thousand/cm3. After a double administration of "Alvesol", with an interval of 24 hrs, n = 32 cows recovered. With a negative California Mastitis Test (CMT), they were withdrawn from treatment. The remaining 10 cows were administered "Alvesol" after 24 hrs for the 3rd time, of which 8 cows recovered. After double administration of "Pharmoxidin" in the 2nd group with an interval of 24 hrs, a negative CMT was detected in n = 27 cows. In the remaining 16 cows, "Pharmoxidin" was injected for the 3rd time after 24 hrs. After that, 11 cows recovered. After 2 applications of "Pharmoxidine" with an interval of 24 hrs, recovery occurred in 63% of the cows and after triple use 88.6% of the cows recovered compared to a total of 95.2% in the "Alvesol" group. We can recommend both drugs for the treatment of subclinical mastitis (no significant differences between the groups; Fisher's test).

P104 | Is there a role for P2Y2 receptors in bovine oocyte cryopreservation?

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The knowledge of the mechanisms leading to the maintenance of cryopreserved oocyte viability is imperative to answer to the worldwide demand for this technology. Our goal was to test if it was possible to modulate the cumulus oocyte complexes (COC) purinergic G-protein P2Y2 receptors (P2Y2R) whose stimulation leads to calcium (Ca2 +) release, probably implicated in Ca2 + misbalances induced by cryopreservation. Firstly, RNA was extracted from bovine mature oocytes and cumulus cells (n = 273) and real-time PCR was performed to quantify P2Y2R transcripts (exp.1). Then the role of P2Y2R was assessed by measuring changes in intracellular Ca2 + concentration [Ca2 +]i of mature COC (n = 63; exp.2) induced by using cryoprotectants (CPA), UTP (P2Y2R stimulator, 100 μM) and/or suramin (P2Y2R inhibitor, 100 and 300 μM) and by analyzing whether this modulation of P2Y2R prior and during oocyte exposure to CPA leads to changes in oocyte viability (exp.3). Mature COC (n = 986) were randomly distributed into groups, either exposed to CPA and P2Y2R modulators or not (control). Oocytes' viability and competence for development were assessed. Data were analyzed by proc mixed and proc glimmix of SAS. Results showed that P2Y2R were expressed in both oocytes and cumulus cells and that [Ca2 +]i was increased by UTP and CPA stimulus. Suramin, totally or partially blocked the response elicited by UTP and CPA, respectively. Oocyte exposure to CPA and UTP reduced both cleavage and Day 7/8 embryo rates compared to control and Suramin 100 μM ($p \leq 0.04$). In conclusion, the inhibition of P2Y2R during CPA exposure along the cryopreservation process, improves the competence of bovine oocytes throughout their development. Funded by PTDC/CVT/2863/2012, UID/CVT/276/2019, PDR2020-101-03112 and ALT20-03-0246-FEDER000021.

P105 | Metabolic monitoring of adaptation processes at pregnancy final stage in different breeds of imported cattle in the Central Black Soil Region

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The aim of the study was to examine how well the imported heifers during their final stage of pregnancy adapt to the Central Black Soil Region climatic zone. There were 7 jersey heifers (group 1) and 7 monbellyards heifers (group 2) included in the study. Clinical analysis of the animals did not reveal any special abnormalities. In their blood, lipid, carbohydrate, and protein metabolism rates were analyzed at 1 month and 2 weeks prior to the expected parturition date and 3–5 days after parturition. The most obvious changes occurred in lipid and carbohydrate metabolism rates. A month before calving, the level of cholesterol in heifers' blood of both groups was 2.50–4.00 mmol/l, the glucose content 2.22–3.88 mmol/l, lactate 1.06 – 1.38 mmol/l, and the level of pyruvate was 110–190 mmol/l. Two weeks prior to the delivery, the glucose level increased by 30.5–32.6% ($p < 0.001$), lactate increased twice ($p < 0.001$), compared to the initial rates. After calving, cholesterol content decreased more, by 19.7–21.5%, glucose 4.4–8.5%, lactate by 7.6–7.9% ($p < 0.01$–0.001). Pyruvate content in cows of both groups increased by 9.4–11.7% ($p < 0.001$). Median samples were compared using the non-parametric Wilcoxon test. Such changes of rates within the studied parameters may be a result of the general adaptation of the imported animals, emphasizing the resumption of the involution mechanisms in their bodies associated with the postpartum period. It can, therefore, be concluded, that these breeds adapted well to the Central Black Soil Region climatic zone within the final dry period and the first days post partum.

P106 | Motility, mitochondrial membrane potential and antioxidant status of epididymal sperm of red deer (Cervus elaphus L.) stored in a liquid state at 5˚C

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This study aimed to investigate the total motility (TMOT), mitochondrial membrane potential (MMP) and selected antioxidant parameters (activity of enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as well as lipid peroxidation (LPO)) of stored red deer epididymal spermatozoa. The cauda epididymal semen was harvested post mortem from nine male red deer during the rut and stored in Bovidyl® extender (Minitüb GmbH, Germany) at 5˚C for 6 days (D1–D6). All analyses were performed on D1, D3 and D6. Duncan's post hoc test showed that CASA – analyzed TMOT significantly decreased on D6 of storage (values on D1 and D3 were 83.5 ± 1.8%, 77.44 ± 2.4 % respectively while on D6 was 65.44 ± 3.8%). A significant decrease for MMP (JC-1/PI) was observed on D3 of storage and then on D6 of storage (the values on D1, D3 and D6 were respectively 92.8 ± 1.8%, 88.67 ± 1.3% and 82.06 ± 2.0%). There were no significant differences ($p > 0.05$) in the antioxidant activity of the enzymes (measured spectrophotometrically with the use of commercial kits) as well as in the level of LPO (measured as the production of malondialdehyde (μM MDA by 1 × 109 spermatozoa)) during the storage time. These results indicate that when the storage time of red deer epididymal sperm at 5˚C lengthens, the mitochondrial potential decreases. Storage up to six days did not significantly influence the level of the analysed antioxidant status, which indicates the efficient protection of these cells against harmful reactive oxygen species (ROS). (Supported by a NCN project, Poland (2017/25/B/NZ9/02544)).

P107 | First attempts to save local cow breeds using MOET in Latvia

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The purpose of the study was to evaluate the first results of multiple ovulation and embryo transfer (MOET) to save local cow breeds in Latvia. Thanks to ERAF project No. 1.1.1.1/16/A/025 MOET in cows it was reintroduced in Latvia after more than 35 years. The number of gene-fond cows is limited, and their status basically does not meet the criteria of good donor cow. Materials and methods: 14 cows were offered to induce MO using FSH (Stimufol). They were 2.5 – 15 years old (lactation 1–9). General clinical examination, blood morphological, biochemical parameters and milk recording data were analyzed. Cows were divided in two groups: ≤ 2 transferable embryos (TE) (group A) and ≥ 3 TE (group B). Results: n = 106 embryos were obtained (range 0–18; 8.8 ± 6.89 per cow) and 73 of them were assessed as TE (5.8 ± 5.43 per cow). N = 40 embryos were transferred (28 fresh, 12 thawed). Six pregnancies were confirmed (fresh embryos). Pregnancy has not been detected in 24 recipients because time was too short after ET. Estrus in the next cycle was not observed in 10 recipients after ET. No statistical differences regarding productivity, milk fat, protein and blood morphology were established ($p > 0.05$) between groups A and B. More TE and pregnancies were obtained from donors which had less than 100*3/ml somatic cell count (SCC) in milk ($p < 0.05$). Negative correlation between SCC and count of TE was found ($r = -0.66$; $p < 0.05$), and

between cholesterol in blood and count of TE (r = 0.65; $p < 0.05$). Cholesterol was lower in group B than in group A (4.2 ± 0.79; 5.2 ± 1.16 mmol/L, respectively). Conclusions: Despite of a small number of gene-fond animals, it seems not profitable to introduce MO in cows with elevated SCC, because fewer pregnancies resulted out of the transfer of their embryos.

P108 | Effect of iron and silver nanoparticles on boar semen kinetics

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The aim of the study was to examine the possible toxic effect of Fe3O4 and Ag/Fe spherical nanoparticles (NPs) as alternative antimicrobial agents on boar semen. The NPs' minimum inhibitory concentration was used after in vitro antimicrobial activity assessment. Nine ejaculates (3 boars; 3 ejaculates/boar) were extended in Beltsville Thawing Solution (BTS; without antibiotics) at a concentration of 30 × 106 spermatozoa/ml and divided in 3 aliquots [group C: control; group Fe: semen with Fe3O4 NPs of diameter 40 nm (0.192 mg/ml semen); group Ag: semen with Ag/Fe NPs of diameter 30 nm, consisted of Ag and a 5% of zero-valent Fe (0.128 mg/ml semen)]. The aliquots were incubated at 17°C for 30 min following NPs' removal through a magnetic field. All post treated samples were stored at 17°C for 48 h. Total motility (TM) and kinetics (progressive motility PM; rapid R; medium; slow; VCL; VSL; VAP; LIN; STR; WOB; ALH; BCF; hyperactivation) were evaluated by CASA at 0, 24 and 48 h post treatment. Data were analyzed with a repeated measures mixed model. Group Fe did not differ from group C at any time point. TM and PM were lower at 24 h of storage in group Ag compared to groups C and Fe (all $p < 0.001$). By 48 h sperm aliquots of group Ag were dead and thus excluded from analysis. The comparison within groups and between storage time points showed that TM, PM, VCL and ALH decreased after 24 h of storage in group Ag (all $p < 0.001$), but not in groups C and Fe. The remaining parameters did not differ significantly between successive time points within any group ($p > 0.05$). In conclusion, Ag/Fe NPs exerted a harmful effect on boar spermatozoa, while the used concentration of Fe3O4 NPs did not affect boar sperm enhancing further research about their application on semen processing.

P109 | Indicators of protein metabolism and serum transferase activity in Holstein breed cows in the dynamics of the postpartum period and lactation

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The aim of the study was to identify changes of protein level in cows' blood, the final products of its metabolism, urea, creatinine, mean molecular peptides (MMP) and enzymes alanine aminotransferase (ALT), aspartate aminotransferase (ASAT), gamma-glutamyltransferase (GGT) activity when restoring ovarian cyclicity and during the folliculogenesis suppression in ovaries. Blood for the research was obtained from animals (n = 16) on days 6, 12, 19, 40, and 68 after calving. Median samples were compared by Wilcoxon test. It was established that the total protein content in the blood serum of cows with restored ovarian cyclicity and ovulation (n = 8) increased from 74.2 ± 1.16 g/L at the beginning of lactation to 87.3 ± 1.36 g/l (by 17.6%) by day 68, urea from 4.01 ± 0.35 mmol/L to 5.43 ± 0.26 mmol/L (by 35.4%), MMP from 0.723 ± 0.33 units to 1.104 ± 0.063 units (by 58.2%), and creatinine decreased from 77.4 ± 4.94 μmol/L to 61.6 ± 2.73 μmol/L (by 20.4%). ALT activity was at the level of 16.1 ± 2.12–16.6 ± 2.68 U/L until the 19th day of lactation, by day 40 it increased by 27.7% and by day 68 by 65.7%. GGT activity increased by 69.5% (from 14.6 ± 1.26 to 23.5 ± 2.02 U/L). In ASAT, there were no significant changes. The same dynamics of the studied blood parameters were detected in cows with suppression of folliculogenesis in the ovaries (n = 8). But in the first 40 days, it was characterized by a significant ($p < 0.05$) increase in the urea content (by 14.4–15.2%), creatinine (by 13.3–16.7%), GGT activity (by 16.5–25.7%) and ALT (by 11.1–16.6%), which reflects the increased activity of tissue catabolism of proteins, accompanied by the accumulation of ketogenic amino acids and pituitary-adrenal system pressure. This may be one of the factors blocking the generative function of the gonads.

P110 | Effect of sodium hyaluronate on postthaw sperm quality and fertility of frozen-thawed (FT) boar semen

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The objective of the study was to evaluate the effect of sodium hyaluronate (HA) on the quality and fertility of frozen-thawed (FT) boar spermatozoa. Only ejaculates with progressive motility (PM)

and normal sperm morphology > 80% were used for experiments. In Exp. 1, the sperm-rich ejaculate fractions collected from 4 Polish Landrace boars (n = 16) were cryopreserved in egg-yolk lactose extender (LEYG) supplemented with either 0 (E0, control), 1% (E1) or 2% HA (E2) according to the methodology previously described by (Trzcińska et al., Theriogenology, 2015,83:307–13). Post-thaw quality of semen was verified by PM % (CASA); viable sperm with intact acrosome (PNA-/PI-) and live sperm without translocation of phosphatidylserine (AnV+/PI-) analyzed by flow cytometer. The quality of FT boar semen was analyzed by ANOVA followed by Duncan's test ($p \leq 0.05$). In Exp. 2, the semen cryopreserved in E0 and E1 were selected for intrauterine artificial insemination. Each uterine horn of 15 synchronized gilts (5 for E0, 10 for E1) was inseminated with 1 x10*9 spermatozoa. Data were analyzed by the chi-square test ($p \leq 0.05$). The results showed that both treated groups (E1, E2) differ significantly with control (69.6 ± 3.2; 68.7 ± 2.5 vs 34.8 ± 2.3) in post-thaw motility. Post-thawing evaluation revealed statistically higher % of PNA-/PI- and lower AnV+/PI- in E1 (65.7 ± 3.2; 8.2 ± 1.4) compared with E0 (45.4 ± 2.6; 15.2 ± 0.9), E2 (63.7 ± 1.5; 10.4 ± 1.3). The highest farrowing rate 80.0% and litter size 10.2 ± 1.5 was observed in gilts inseminated with semen cryopreserved in extender supplemented with 1% HA. Our study demonstrates that 1% HA increased cryotolerance of boar semen during freezing and provided high sperm quality and fertility after freeze-thawing. Supported by BIOSTRATEG 2 No 297267/14/2016.

P111 | Nitric oxide levels in cows after parturition – influence on folliculogenesis

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One of the numerous biological effects of nitric oxide is the regulation of folliculogenesis in the ovaries of animals through its participation in the induction of secretion of a gonadotropin-releasing hormone and in the mechanisms of the ovulatory process. The aim of our study was to analyze the stable metabolites of the nitric oxide (NO.) content in the blood of Holstein cows (n = 16) after calving, when ovulatory function of the ovaries was restored or suppressed. The functional state of the ovaries was assessed during 68 days with an interval of 7 days using the ultrasound and the determination of progesterone and estradiol-17β concentrations in blood serum. According to the results of these studies, the animals were divided into 2 groups: cows with restored ovulatory functions (gr. I, n = 8) and cows with ovarian acyclia (gr. II, n = 8). It was determined that the concentration of NO • in the serum of group I cows in the preovulatory period was 13.1 ± 1.55 μmol/l with fluctuations of 6.0–21.2 μmol/l, during the period of ovulation and the formation of the corpus luteum it increased up to 38.9 ± 3.82 μmol/l ($p < 0.001$) with fluctuations of 26.7–60.8 μmol/l and during the involution of the corpus luteum decreased to 13.2 ± 0.31 μmol/l with fluctuations of 12.1–15.0 μmol/l. It was at the level of 5.6–27.8 μmol/l (average 14.4 ± 0.84 μmol/l) in cows of group II throughout the entire study period. Therefore, the inhibition of nitric oxide synthesis in the body of cows in the postpartum period is one of the components of the complex female reproductive system and is accompanied by a depression of folliculogenesis and ovulatory ovarian function.

P112 | Dynamics of sex hormones in cows with different milk production at the beginning of lactation

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Approximately two months after calving, fertility is restored in cows. Highly yielding cows prioritize lactation above reproductive functions. The goal of our research was to study the dynamics of sex hormones in cows with different productivity in the first month after calving. For the study, two groups of cows after calving were formed (n = 12, each). The first group included high-yielding cows (HY; over 8,000 kg), the second group low-yielding (LY) cows (less than 6,000 kg). In the blood serum of cows, the concentration of progesterone and estradiol was examined three times at 5–10; 20–25 and 35–40 days after calving. Statistical processing of the research results was carried out using Student's t-test. At 5–10 days after calving, there were no differences in the content of hormones. After 20–25 days, hormone concentrations in both groups increased. In the LY group, the level of progesterone was 10.9 ± 1.5 nmol/l, which is 1.8 times higher than in HY cows ($p < 0.05$). In cows of the HY and LY groups, the estradiol level was 294.1 ± 24.8 and 385.5 ± 22.7 pmol/l, respectively ($p < 0.05$). The opposite situation was found at 35–40 days of lactation. In HY cows, higher hormone values were found. The estradiol level was 343.6 ± 34.8 pmol/l, progesterone 4.8 ± 0.8 nmol/l, which was 31.6% and 51.0% increased compared to that of LY cows ($p < 0.05$) . The daily milk yield was 29.3 ± 1.7 for the HY cows and 22.2 ± 1.4 kg for the LY cows. The data obtained suggest that in cows with high milk yield, ovarian activity after calving resumes later than in low-yielding cows.

P113 | Artificial insemination (AI) with frozen-thawed boar semen

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The objective of the study was to determine the fertilizing capacity of frozen-thawed (FT) boar semen. Semen from three adult commercial hybrid boars (4 ejaculates/boar) with proven fertility was cryopreserved using a procedure developed in the Institute of Animal Production in Poland (patent no. PL 228 192 B1). Only ejaculates with 80% total motility (TM) and 80% morphologically normal spermatozoa were used in the experiment. The quality of FT semen was verified based on the motility (CASA: total motility TM%) and DNA fragmentation (TUNEL). Results were expressed as the means ± SD. Two types of insemination were used in the experiment: surgically insemination (SI) (10 synchronized gilts; 1 × 109 sperm/each uterine horn) and cervical artificial insemination (CAI) (10 gilts, double insemination with 2 x109 sperm/dose). The gilts were synchronized by the injection of 750 IU PMSG and 500 IU of hCG administered 72 h later with the FT semen. At the onset of estrus, 24 h after hCG, the gilts were subjected to SI. Significance of the differences between the means of sperm quality parameters were analysed by Student t-test ($p < 0.05$). Data of reproductive performance were evaluated by the chi-square test. High TM% and low-level of DNA fragmentation (79.6 ± 3.4 and 1.9 ± 0.6) was observed in boar semen after freeze-thawing. The results revealed high reproductive performance of both group of inseminated gilts (SI: farrowing rate: 80% and litter size: 12.4 ± 1.2; CAI: farrowing rate: 90% and litter size: 13.2 ± 0.8). Using FT semen for AI enhanced the similar reproduction success as achieved by conventional insemination with liquid stored boar semen. The results indicate the possibility of using FT semen for AI in commercial pig production. Supported by BIOSTRATEG 2 (297267/14/2016).

P114 | Mechanical activation of the bovine ovary in vivo

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Regular ovum pick up (OPU) procedures provide reasonable number of suitable quality oocytes for in vitro embryo production. However, it is not characterized how repeated OPU sessions influence antral follicle growth, oocyte retrieval and blastocyst rate. In the pilot experiment using 5 cycling, pubertal beef cows, five OPU sessions, every 6 days were performed. During each OPU session, we determined number of follicles on ovaries, presence of CL or DF, number of punctures, number of obtained oocytes and blastocyst rate. We found the lowest number of follicles (10 ± 6, vs 25 ± 9), oocytes (5 ± 4 vs 15 ± 6) and subsequent blastocyst rate (30 ± 5 vs 39 ± 6) during first OPU as compared to the rest sessions. The number of punctures did not differ between all OPU sessions. The number of oocytes and subsequent blastocyst rate increased from second OPU, but was stable till the end of the experiment. We noticed that quality of obtained blastocysts differed significantly and was positively correlated with presence of CL. We did not obtain any blastocysts, irrespective of the number of oocytes, during OPU session with DF present. We also reported dominant follicle presence on ovaries of two out of five cows in spite of continuous retrieval of follicles. Thus we suspect that 6 day interval is not the most sufficient for collecting oocytes of the best quality for IVP. To summarize, regular OPU sessions stimulate antral follicle growth, oocyte retrieval and blastocyst rate. However, there is no difference between obtained numbers of oocytes and blastocyst rates, but between blastocyst qualities. Moreover, the efficiency of IVP depends on the intervals between performed OPU sessions. To sum up, more replicates with different time brakes, on more animals are needed.

P115 | Platelet Factor 4 during culture does not improve the development and quality of porcine embryos produced in vitro

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Improvement of culture media is a main objective of in vitro embryo production systems. Platelet factor 4 (PF4) is a cytokine, which has been used on different stem cells lines showing enhancements related with differentiation and cell survival. The objective of this study was to evaluate if the addition of PF4 during culture of porcine in vitro-produced embryos improves embryo development and quality. A total of 3054 matured oocytes in 4 replicates were incubated for 5 h with 1000 thawed spermatozoa per oocyte. Then, they were sequentially cultured in NCSU-23 medium with 0.4 mg/ml BSA supplemented with 0.3 mM pyruvate and 4.5 mM lactate for 2 days and with 5.5 mM glucose for additional 5 days. After fertilization, presumptive zygotes were divided in 5 groups depending on the concentration of PF4 in the culture media: 0 ng/ml (control); 100 ng/ml; 200 ng/ml; 500 ng/ml or 1000 ng/ml. Differences among groups in embryo developmental parameters and total cell number were analyzed by ANOVA. Data are expressed as means ± SD. All groups displayed similar cleavage rate at day 2 (range: 65.0 ± 10.9% to 70.0 ± 5.8%), blastocyst rate at day 7 related to the cleavaged embryos (range: 46.6 ± 10.0% to 56.4 ± 8.2%) and total efficiency of blastocyst formation at day 7 (range: 32.3 ± 8.3% to 37.2 ± 7.3%). In addition, PF4 did not show any effect on the total cell number of day 7 blastocysts (range:

44.1 ± 23.2 to 50.5 ± 26.4). In conclusion, the supplementation of the culture media with these concentrations of PF4 failed to improve the in vitro porcine embryo culture. Supported by MINECO-FEDER (AGL2015-69735-R and BES-2016-077869) and SENECA (19892/GERM/15).

P116 | Methods of improving productivity of sows and of piglets

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The purpose of the study was to analyze the effect of supplementing basic ration with Vitamins (vit) on prolificacy of sows and the use of brooders on safety and growth of their litter. Pregnant sows (n = 120) were distributed into 4 groups (30 animals each). In the 1st (control) group they received the basic ration, in group 2 0.1 mg Biotin was added, in group 3 3.0 mg folic acid and in group 4 0.1 mg vit H and 3.0 mg Bc complex per 1 kg of dry matter, each. Statistical analyses were done with ANOVA. Addition of Biotin into the basic ration over the first 9 weeks of gestation increased the prolificacy of sows (piglets born) by 5.9% ($p \leq 0.05$) (10.27 ± 0.13 animals), folic acid by 8.5% ($p \leq 0.01$) (10.52 ± 0.15), vit H and Bc complex by 11.4% ($p \leq 0.001$) (10.81 ± 0.16) as compared to the animals in the control group (9.70 ± 0.15). After farrowing, piglets from each group were divided into 2 subgroups (sgr). For the piglets in the 1st sgr the source of heating was infrared lamps IKZK 220–250 or heated floor, for the piglets in the 2nd sgr brooders. As a result, the safety of weaned piglets / piglets born and their live weight at weaning in the 1st sgr was 8.91 ± 0.20 / 9.64 ± 0.22 animals and 7.78 ± 0.12 kg (group 1), 9.54 ± 0.24 / 10.31 ± 0.19 animals and 7.52 ± 0.11 kg (group 2), 9.75 ± 0.24 / 10.50 ± 0.22 animals and 7.45 ± 0.12 kg (group 3), 9.92 ± 0.26 / 10.77 ± 0.21 animals and 7.38 ± 0.14 kg (group 4). As for the 2nd sgr, the number of weaned piglets / piglets born and their live weight at weaning accounted for 9.36 ± 0.35 / 9.73 ± 0.26 animals and 8.47 ± 0.16 kg (group 1), 9.85 ± 0.23 / 10.23 ± 0.21 animals and 8.21 ± 0.15 kg (group 2), 10.17 ± 0.17 / 10.58 ± 0.23 animals and 8.14 ± 0.16 kg (group 3), 10.38 ± 0.20 / 10.85 ± 0.24 animals and 9.77 ± 0.15 kg (group 4). Microclimate in the 2nd sgr increased weight at weaning ($p \leq 0.01$).

P117 | Effects of stimulation with red-light upon motility parameters of liquid-stored donkey spermatozoa

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Previous studies carried out in other mammalian species have shown that red-light stimulation increases sperm motility and may also have an impact on fertilizing ability. The present work aimed to determine the effects of different stimulation patterns with red-light on the motility parameters of liquid-stored donkey semen. With this purpose, 7 ejaculates from 7 different donkeys were evaluated. Ejaculates were stored in Kenney extender at 4°C for 24 h, and light-stimulation was subsequently performed using 0.5-ml transparent straws. Straws (two per treatment) were randomly divided into control and eight treatments: three single periods of red-light exposure (1, 5 and 10 min), and 5 patterns of light-dark-light intervals (1-1-1, 3-3-3, 5-5-5, 10-10-10, and 15-15-15) Samples were stimulated with red-light (620–630 nm) using a triple LED system and sperm motility was then evaluated using a Computerized Semen Analysis System (CASA). Light-stimulation with the #3-3-3 pattern significantly increased ($p < 0.05$) some kinematic parameters when compared to the control. These parameters were: straight-line velocity (VSL, mean ± SEM; #3-3-3: 92.7 ± 2.9; control: 65.1 ± 2.3), average pathway velocity (VAP, #3-3-3: 102.1 ± 6.3; control: 78.9 ± 3.9), percentage of linearity (%LIN, #3-3-3: 76.6 ± 1.3; control: 64.4 ± 1.9), and percentage of oscillation (%WOB, #3-3-3: 86.7 ± 1.2; control: 78.5 ± 1.5). These results show, for the first time, that specific patterns of red-light stimulation increase some kinematic parameters of liquid-stored donkey spermatozoa. Further research, evaluating other sperm function parameters, such as mitochondrial activity, is warranted. This work was supported by MCIU, Spain (Grants: RYC-2014-15581 and AGL2017-88329-R).

P118 | Cytotoxic effect of silver nanoparticles (AgNPs) on frozen-thawed donkey sperm: preliminary results

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The aim of the present study was to investigate the cytotoxic effect of silver nanoparticles (AgNPs) on frozen-thawed donkey sperm parameters, prior to their inclusion as antibacterial agent in freezing

extenders. AgNPs were synthesized by reduction of silver nitrate with hydroxylamine hydrochloride. Five frozen-semen doses were thawed and incubated at 37°C for 2 h with different concentrations of AgNPs (0, 1.25, 2.5, 5, 12.5, 25 and 50 μg/ml). Sperm motility (CASA system), plasma membrane integrity (acridine orange/propidium iodide staining) and sperm morphology (Diff-Quik staining) were assessed at 0 and 2 h time points. Data were compared using ANOVA and Tukey's multiple comparisons test. Incubation time did not affect ($p < 0.05$) the sperm parameters assessed in all treatments, except at 25 mg/ml AgNPs in which all sperm parameters became worst ($p < 0.05$) at 2 h comparing to 0 h. In addition, when sperm was exposed to 50 μg/ml of AgNPs, all sperm parameters was decreased ($p < 0.05$) at both 0 h (Total motility: 15.1%; Progressive motility: 3.4%; Membrane integrity: 14.8%) and 2 h (Total motility: 7.1%; Progressive motility: 0.9%; Membrane integrity: 12.38%; Sperm abnormalities: 23%), in comparison to other concentrations. Taken together, our results revealed that AgNPs are toxic to frozen-thawed donkey sperm at higher concentrations (25 and 50 μg/ml), but biocompatible at lower ones, which could be used as antibacterial agents for donkey sperm cryopreservation. Supported by University of Cordoba PP. 2018 Mod.4.1.

P119 | The competence of wisent (Bison bonasus) sperm for in vitro fertilization

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The wisent (Bison bonasus) is a threatened species protected by international law. In vitro methods, such as cryopreservation of spermatozoa and in vitro embryo production are very important tools, which can be used to increase the safety of endangered species. Low fertilizing capacity of cryopreserved spermatozoa is one of the main problems. Therefore, the aim of this study was to determine the quality of wisent sperm after thawing and their capacitation status and suitability for in vitro fertilization. Semen from 3 wisent bulls was isolated post mortem and was frozen in TRIS- buffer extender. After thawing semen from all wisent bulls (Z1P, Z2P, Z3P) was tested in the aspects of vitality (BrightVit), morphology (Rapid SpermBlue), motility (inverted microscope) of spermatozoa and only motility was tested after capacitation and incubation in modified TALP medium for fertilization. The results were analysed using T-test with multiple range test LSD. After thawing, Z1P and Z3P bulls presented a similar high percentage of vitality (90%; 85%) and morphology (85%; 80%), while Z2L bull presented a lower quality (60%; 55%). Moreover, only Z1P bull showed a high level of progressive sperm motility (80%), while Z2L and Z3P bulls presented lower progressive sperm motility (35%; 45%). Furthermore, after capacitation, only one wisent bull maintained a high percentage of progressive motility: Z1P-80% vs Z2L-30%; Z3P-30% ($p < 0.05$). At the beginning of incubation in fertilisation medium, only one wisent bull showed high percentage of progressive motility: Z1P-70% vs Z2L-20% Z3P-30% ($p < 0.05$). Results from this study enable to choose the best wisent bull (Z1P) as a sperm donor for in vitro fertilization. Financed by the Forests Fund (Poland) – OR.271.3.10.2017.

P120 | Inadequate mare-foal bonding restored by adoption of her own foal – a case report

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Difficulties in mare-foal bonding are mostly consequences of an abnormal maternal behaviour. Despite current therapies usually based on restrain and sedation of the mare, the mare-foal bonding is not always created. However, adoption of foster foals and establishment of the maternal behaviour is often successful. Procedures of adoption mimic the transient increase of oxytocin during delivery and its effects by cervical and vaginal stimulation (Ferguson reflex), or by pharmacological simulation of the high concentrations of prostaglandins observed during parturition. Even if the mechanism is not yet well understood in mares, both hormones seem to have a direct effect on the brain control of maternal behaviour. A primiparous 7 years old mare was presented at the Equine Clinic showing aggressive behaviour towards her 4 days filly especially when the foal attempted to suckle. No evident cause of pain at suckling was found. A hormonal adoption procedure of her own foal was implemented after several attempts to restore the mare-foal bonding by restrain and sedation of the mare. After 3 h of fasting and isolation of the foal, the mare received an im injection of 750 μg of cloprostenol. The foal was carefully presented to the mare when the secondary effects of the prostaglandins were visible. The mare accepted the foal and allowed suckling rapidly. During the next 24 h under supervision, the mare showed a very normal maternal behaviour. After 3 days of normal bonding, mare and foal were discharged from the clinic. Protocol for adoption with the use of cloprostenol could be a valuable option in the treatment to establish or restore the normal bonding between a mare and her own foal.

P121 | Recovering sperm motility after thawing in gaur (bos gaurus) epididymal sperm

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The Gaur (Bos gaurus) is an Asian wild mammal which is listed as vulnerable species by the International Union for Conservation of Nature and Natural Resources. The aims of this study were to assess the freezability of Bos gaurus epididymal sperm and to evaluate whether the heterologous seminal plasma (SP) addition from bull (Bos taurus) could improve the motility, keeping a good physiological status after thawing. A sexually mature Gaur male dead after an acute course disease with a febrile process was used for this study. Sperm were diluted down in egg yolk-based extender and packaged in 0.25 ml straws to 100 × 106 sperm/ml. Before freezing (T0), total motility (TM), viability (V), caspase-3/7 activity (CA) and mitochondrial functionality (MF) were assessed. 4 straws were thawed (65°C 6s) and pooled. The pool was divided into 3 aliquots and supplemented (v/v) as follow: without SP (Control –C–), 10% (SP10) and 20% (SP20) of bull SP. The analyses were repeated immediately after thawing (T1), and after 2 (T2) and 4 h (T3) of incubation at room temperature. Sperm motility and physiological status worsened slightly after thawing compared to before freezing (T0: 9.1% TM, 77.5% V, 1.6% CA and 91.7% MF vs. T1: 7% TM, 66.3% V, 10.6% CA and 75.9% MF). Motility increased with incubation time and with seminal plasma addition (T2: 11.1% C, 17.4% SP10 and 15.4% SP20) (T3: 24.9% C, 18.4% SP10 and 29.2% SP20). The freezability of Gaur sperm seems to be good (85.5% for V and 76.9% for TM). After thawing, the effect of seminal plasma addition in this kind of samples looks like an effective tool to improve the spermatozoa motility keeping an optimal physiological status (Supported in part by Cantur SA.).

P122 | Influence of breed on serum progesterone concentration in the ovulatory period in the bitch

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Serum progesterone concentration (SPC) has been routinely used with success to determine the optimal time for mating in the bitch. Although it has been reported that SPC can be variable due to an individual variation, no information about a breed influence has been cited. The aim of this study was to compare the SPC obtained on 0, 2 and 3 days after LH peak in bitches belonging to 3 different breeds: American Bully (AB), English Bulldog (EB) and German Shepherd (GS) dogs. A total of 74 bitches have been used (15, 29 and 30 for AB, EB and GS, respectively). In all of them, a complete estrus control with vaginal cytology and SPC in alternative days from the 4–5 days of proestrus has been performed in order to time a natural mating or artificial insemination. All the bitches were confirmed as pregnant. SPC was determined by CLIA (Immulite®1000 system). Our results showed no differences between groups in SPC obtained on day 0 (LH peak) (2.56 ± 0.07, 2.27 ± 0.05 and 2.37 ± 0.08 ng/ml for AB, EB and GS, respectively). On day 2, no significant differences were obtained in SPC (5.54 ± 0.68, 6.88 ± 0.66 and 5.56 ± 0.37 ng/ml for AB, EB and GS, respectively) although we observed a high value in EB. However, in SPC on day 3, we found significant differences ($p < 0.05$) between groups. A greater value of SPC was obtained in EB group (11.65 ± 1.25 ng/ml) compared to the AB and GS groups (7.15 ± 0.63 and 7.57 ± 1.19 ng/ml, respectively). In conclusion, our preliminary results indicate that the breed influences SPC obtained in the ovulatory period. For that, further studies with a higher number of breeds are necessary (Supported by University Clinic Veterinary Foundation).

P123 | A preliminary study to calculate ram testicular volume using caliber and different formulas

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Sexual behavior and semen characteristics are the main parameters limiting male reproductive efficiency, and both of them are influenced by testicular size among other factors. The aims of this trial were to compare the testicular volume calculated by different formulas with the accurate testicular volume, and to determine the best formula for establishing the testicular volume under field conditions in order to estimate daily sperm output. Length (L), width (W) and height (H) of testes from 3 sexually mature Assaf ram in the non-breeding season were measured by caliber. The measures were performed in triplicate with the ram standing. Testicular volume was calculated using two formulas: the formula for an ellipsoid L × W × H × 0.52 (F1) used in stallions (Pozor et al. 2014, J Eq Vet Sci 34:38–39; Hedia et al. 2019, Theriogenology 123:68–73) and the empiric formula of Lambert L × W × H × 0.71 (F2) used in rams (2). The accurate testicular volume was determined after males' death. Spermatic cord and epididymides were removed and the accurate testicular volume was measured by the overflow of water. The data were analyzed using SAS 9.1 (GLM Procedure). The accurate testicular volume mean was 175.3 ± 17.2 cm3. Non significant differences were obtained between the accurate testicular volume mean and the testicular volume mean calculated using F1 (196.4 ± 13.2 cm3) ($p > 0.05$). However, there were significant differences between the

accurate testicular volume mean and the testicular volume mean calculated using F2 (228.7 ± 10.8 cm3) ($p < 0.05$). In conclusion, F1 seems to be the most suitable formula, but further investigations should be carried out. Supported partially by AGL2017-83098-R.

P124 | Evaluation of the immune status of mares in the first 2 months of pregnancy

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The aim of the work was to study the immune status of mares in the first months of pregnancy. Blood samples were taken from 22 Arabian mares. All mares were healthy. The concentration of alpha-1-globulins, alpha-2-globulins, beta-globulins and gamma-globulins was determined on SAS-1 Plus / SAS-2 (Helena Biosciences Europe, United Kingdom) by agarose gel electrophoresis. IgA, IgM, IgG were determined on a biochemical analyzer AU 680 (Beckman Coulter, USA). The data were processed by cluster analysis using the K-means in STATISTICA 10. As a result, the data were divided into 3 clusters. The first cluster (C1) with mares 45–60 days of gestation (n = 8), the second cluster (C2) with non-pregnant mares (n = 9) and the third cluster (C3) with mares 15–30 days of gestation (n = 5). C2 and C3 did not differ in all studied parameters, except for beta-globulins. Beta globulins were increased in pregnant mares compared to non-pregnant mares 12.9 ± 1.17% (mean ± SD) (C1) and 12.8 ± 2.69% (C3) vs 11.3 ± 1.87% (C2). An increased concentration of alpha-2-globulins (12.3 ± 2.11%), IgA (0.03 ± 0.023 g/L) and IgM (0.6 ± 0.27 g/L) was observed in C1 compared with C2 and C3 (11.0 ± 1.23 and 10.8 ± 1.21%, 0.02 ± 0.013 and 0.02 ± 0.020 g/L, 0.5 ± 0.19 and 0.5 ± 0.22 g/L, respectively). Thus, starting from the second month of pregnancy, activation of the mares' immune system was observed. Project № AAAA-A18-118021990006-9.

P125 | No evidence of transmission of bovine viral diarrhea virus by artificial insemination in gilts

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Introduction: Pestivirus infections can promote reproductive problems, such as the birth of low-viability piglets, the occurrence of mummification, and abortion. However, only reproductive disorders caused by classical swine fever virus (CSFV) are proven to be associated with artificial insemination transmission in pigs. The objective of this study was to evaluate whether bovine viral diarrhea virus (BVDV) can be transmitted to gilts by artificial insemination using BVDV contaminated semen. Material and Methods: In order to mimic BVDV transmission by AI, eight 180 days old BVDV-free gilts were divided into two groups, the challenged (n = 6) received two doses of contaminated semen, and the control group (n = 2) was inseminated with two doses of BVDV-free semen. A total of 3 ml of BVDV-2 strain LVB 16557/15 with a titer of 105.5 TCID50 was added to the semen doses instants before insemination. Blood samples were taken every four days up to 60th dpi for whole blood and serum samples submitted to RT-PCR and virus neutralization test, respectively. Results: The pregnancy of all females was confirmed and none of them returned to oestrus or aborted. Neither viral RNA nor antibody titres against BVDV-2 were detected in the blood and serum from both groups. In addition, no clinical signs indicative of viremia were observed. Conclusion: Differently from studies with BVDV in bovine and classical swine fever in pigs, the insemination of gilts with semen experimentally contaminated semen containing BVDV-2 did not result in transmission of the viral agent, which is supported by the absence of detection, typical clinical signs and neutralizing antibodies against BVDV.

P126 | Effect of stallion age on the ultrastructural integrity of spermatozoa during cryopreservation

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The purpose was to study the effect of stallion age on progressive motility and ultrastructural integrity of spermatozoa after cryopreservation. Ejaculates from 35 stallions aged 4 to 22 years were collected during the breeding season (February-May). Progressive motility after freezing-thawing was assessed by visual assessment. The structural integrity of the spermatozoa in the samples of frozen semen was studied on a Hitachi 700 electron microscope. To determine the relationship between the parameters Spearman's nonparametric correlation analysis was used. A negative correlation between stallion age and progressive sperm motility in cryopreserved sperm was established ($r = -0.34$; $p < 0.05$). A correlation between the stallion's age and the number of spermatozoa with acrosome hypoplasia in cryopreserved sperm ($r = 0.34$; $p < 0.05$) and the number of spermatozoa with disrupted chromatin of the nucleus ($r = 0.34$; $p < 0.05$) in cryoconserved sperm

was found. It has been suggested that the structural integrity of spermatozoa during cryopreservation is directly related to the aging of the stallions. Authors acknowledge financial support from Russian Science Foundation, Grant No: 18-16-00071 (Data analysis, development program of Bioresource collections "Cryobank of genetic recourses the All-Russian Research Institute for Horse Breeding".

P127 | The cryoresistance of sperm from poultry

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Cryopreservation of germplasm is the most effective method for the conservation and use of genetic resources. In contrast to farm animals, the use bird sperm cryopreservation is local, which is due to their biological peculiarity. The fertility of frozen and thawed sperm of birds varies widely from 20% to 60%, which limits the use of cryopreservation technology in this industry. The aim of research was to study the cryoresistance of sperm from different species of poultry. To collect semen from poultry used the massage technique. Ejaculates were diluted 1:1. The macro- and microscopic parameters of the obtained ejaculates were evaluated. In the experiments, roosters (n = 5), quails (n = 5), guinea fowls (n = 8), drakes (n = 7), ganders (n = 3) and turkeys (n = 4) were used. The ratio of sperm motility in a 6% glucose solution at different temperature conditions was used to evaluate the cryoresistance by the formula Rx=a1/a2 (Rx-cryoresistance, a1-sperm motility at the temperature 1°C, a2-at 37°C). To determine the cryoresistance of spermatozoa, a drop of the suspension was introduced into test tubes with a 6% glucose solution of different temperatures: cooled to 1–2°C and heated to 30–40°C. After 5 minutes, sperm motility was determined using the CASA system. The cryoresistance of bird spermatozoa varied depending on the species and individual characteristics of the males. High rates of cryoresistance were set for turkey, guinea fowl and roosters 0.40 ± 0.04, 0.38 ± 0.02 and 0.42 ± 0.02, respectively. Sperm of ganders and drakes were characterized by low cryoresistance of 0.25 ± 0.05 and 0.27 ± 0.05. The intraspecific variability of this indicator was 23% for roosters, 18% for quails, 26% for guinea fowls, 29% for ganders, 25% for drakes, and 26% for turkeys. Supported by RSF №16-16-04104.

P128 | Abstract withdrawn

P129 | The concentration of toxic elements in the sperm plasma in stallions with high and low cryostability of sperm

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The aim of the work was to study the concentration of toxic elements in the sperm plasma from stallions with different sperm cryostability. Sperm was collected from 39 Arabian stallions, aged 5 to 15 years. Motility was assessed by eye. Semen was centrifuged at 600 g for 15 min to get seminal plasma free from spermatozoa. The concentration of the toxic elements: aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), tin (Sn) and mercury (Hg) was determined by atomic emission and mass spectrometry using a mass spectrometer Elan 9000 (Perkin Elmer, USA) and Optima 2000 V atomic emission spectrometer (Perkin Elmer, USA). Stallions were divided into two groups: the first group (n = 28) motility after freezing/thawing ≥ 25% (28.5 ± 1.32%), second group (n = 11) motility after freezing/thawing ≤ 20% (13.6 ± 2.04%) (Mean ± SE, general statistics, data processed in Excel 2013). There was a large individual variability in concentration of tin, mercury, cadmium and arsenic in the sperm plasma in different stallions. There were (but not significantly) higher concentrations of AL (0.364 ± 0.045 μg/g) and As (0.0028 ± 0.0005 μg/g) in group 2, compared with group 1 (0.346 ± 0.028 and 0.0026 ± 0.0004 respectively). The concentration of mercury in the concentration from 0.00018 to 0.0063 μg/g, tin in the concentration from 0.00025 to 0.0261 μg/g and cadmium from 0.000024 to 0.00079 μg/g did not differ between the groups. Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01109, development program of Bioresource collections "Cryobank of genetic recourses the All-Russian Research Institute for Horse Breeding.

P130 | Morphological and morphometric features of the testes argali and its hybrids

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One of the main problems of hybridization is reproductive isolation between species. Interspecific hybrids especially males are sterile or have reduced fertility. The structure and development of reproductive organs has a species peculiarity. The study of the developmental stages, morphology and biometric parameters of the testis is very important for the characterization of the species peculiarities

and the development of assisted reproductive technologies. The aim of the research is to study morphological and morphometric parameters of the testes argali and its hybrids. The collection of biomaterial from argali (n = 5) was carried out postmortem. Selection of testes from hybrids was carried out after castration at the age of 12 months. The testes weight and morphometric parameters were determined. To fix the testicular tissue samples, they were kept in Bouin's solution for 48 h. Statistical analysis was performed using SPSS v.15.0. ANOVA and comparison between groups using T-test were performed. The testis mass in hybrids was 129.5 ± 10.4 g and in argali males 115.7 ± 9.8 g. The diameter of the seminiferous tubules in hybrids was 227.09 ± 5.89 microns and in argali 216.20 ± 4.79 microns. The area of seminiferous tubules in hybrids was 11.9% higher than in argali. Hybrids exceeded argali in the number of spermatogenic cells in the seminiferous tubule, being the difference between the groups 64.7% ($p < 0.01$). The obtained results might indicate the influence of the specific males' peculiarity on the testes morphology, including the number of spermatogenic cells in the seminiferous tubule. Supported by RSF No. 18-16-00079.

P131 | Relationship of boar semen proteins and characteristics with field fertility

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The aim of this study was to investigate the relation between pig field fertility and various in vitro tests of semen and seminal plasma analysis. Sixty five ejaculates from 18 boars were collected throughout a year, were diluted by a commercial extender and were used to inseminate 472 multiparous sows. An insemination dose of each ejaculate was transferred and analyzed to the lab. Motility/kinetics (computer assisted semen analysis-CASA), morphology (sperm blue), vitality (PI-Calcein), mitochondrial membrane potential (rhodamine), DNA fragmentation (acridine orange), sperm membrane biochemical activity (HOSt), selected sperm (HSP90, GPX5) and seminal plasma (Osteopontin-OPN) proteins (western blot) were assessed and related to field fertility. Data were analyzed by simple and multiple regression. In simple regression analysis immotile spermatozoa, head morphology, HOSt and mitochondrial membrane potential explained 5.1%, 7.4%, 23.1% and 11.5% of the variation in the number of live born piglets (LBP), respectively ($p < 0.05$). However, HOSt was the only variable that explained variation in multiple regression. Regarding the proportion of farrowings with ≥ 12 LBP, simple regression showed that immotile spermatozoa, HOSt, OPN-70 and OPN- 12 explained 5.0%, 6.1%, 5.0% and 7.4% of variation, respectively ($p < 0.05$). In multiple regression, the combination of immotile spermatozoa, HOSt and OPN-12 explained 19.3% of variation ($p < 0.05$). Sperm protein GPX5 explained 5.3% of the variation in farrowing rate ($p = 0.04$). In conclusion, the estimation of parameters like immotile sperm, morphology, membrane biochemical activity, mitochondrial membrane potential, OPN and GPX5 can provide useful information about field fertility of boars used for artificial insemination.

P132 | The concentration of essential elements in the sperm plasma of stallions

V Kalashnikov[1]; M Atroshchenko[1]; A Zaitcev[1]; S Miroshnikov[2]; O Zavjalov[2]; A Frolov[2]; A Krutikova[3]; A Musidray[3]; K Plemyashov[3]

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The aim the study was to determine the concentration of essential elements in the sperm plasma of stallions the effect of elements on the quality of sperm. Sperm was collected from 39 Arabian stallions, aged 5 to 15 years. Motility was assessed subjectively. Semen was centrifuged (600 g/15 min) to get seminal plasma free from spermatozoa. Concentration of boron (B), lithium (Li), nickel (Ni), silicon (Si), strontium (Sr) and vanadium (V) in the plasma were determined by atomic emission and mass spectrometry (AES-ICP and MS-ICP) using an mass spectrometer Elan 9000 (Perkin Elmer, USA) and an atomic emission spectrometer Optima 2000V (Perkin Elmer, USA). The highest concentration of essential elements in the sperm plasma was set for Si (15.36 μg/g). The concentration of Sr was 0.22 μg/g, Li – 0.12 μg/g, B – 0.02 μg/g, Ni – 0.013 μg/g, V – 0.0005 μg/g. A significant negative correlation was established between the concentration of Li and the volume of ejaculate ($r = -0.39$; $p = 0.02$). A positive correlation between the concentration of Li and the concentration of spermatozoa ($r = 0.33$; $p = 0.05$), significant negative correlation between the concentration of Si and motility ($r = -0.36$; $p = 0.03$), a positive correlation between the stallion's age and the concentration of Ni ($r = 0.38$; $p = 0.02$) and Si ($r = 0.35$; $p = 0.04$) were established. The study of the influence of the elemental status of the organism on the quality and cryostability of sperm is one of the promising areas during a set of measures for the preservation of the genetic material of farm animals. Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01109, development program of Bioresource collections "Cryobank of genetic recourses the All-Russian Research Institute for Horse Breeding.

P133 | Genetic transformation of quails primordial germ cells in vitro

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One of the promising methods for obtaining genetically modified individuals is the use the precursors of primary germ cells as target cells, for example, primordial germ cells – PGCs. In vitro experiments on the genetic transformation of quails, PGCs were performed using different gene delivery systems, such as lipophilic transfection and electroporation, and their effectiveness was studied. The isolation of PGCs was carried out from 5-day quail embryos. The plasmid ZsGreen1-N1 (Addgene, # 54702) with the ZsGreen gene under the CMV promoter was used for lipophilic transfection and electroporation. Electroporation was performed using the Neon transfection system (Invitrogen, # MPK5000) in 10 μl capillaries. Electroporation conditions were chosen by voltage (1200, 1350, 1400, 1500, 1650 V), pulse exposure time (10, 20, 30 μs), and number of breakdowns (1, 2, 3 pulses). Each experiment was performed in 5 replicates. Lipofectamine 3000 Reagent Kit (Invitrogen, # L3000001) was used for lipofection. The concentration of DNA, the amount of lipofectamine and the time of cell processing were selected experimentally. The effectiveness of transfection of the PGC with the lipophilic agent did not exceed 1%. Optimization of lipofection conditions was ineffective. The optimal electroporation parameters were 1400 V and 10 μs and 2 pulses. The percentage of cells transformed reached 12.7 ± 1.2%. When using other parameters, this indicator was lower by 3–10% ($p < 0.05$). Based on the data obtained, the use of electroporation can be recommended for the efficient transfection of the quails PGCs in vitro. Supported by RFBR No. 18-29-07079.

P134 | Biochemical status of cows in the I and II phases of the dry period due to reproductive capacity and milk production

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The aim of the study was to compare the biochemical profile of Holstein cows in I and II phase of the dry period, before the onset of the negative energy balance (NEB) stage, due to the length of the interval from calving to insemination and milk production. The average yield for 305 days of the last lactation was 9946 ± 497 kg of milk. The biochemical status was determined twice: 60–44 days (phase I) and 30–14 days before calving (phase II) ($n = 9$). The serum concentrations were determined for total protein, glucose (GL), triglycerides (TG), total cholesterol (TH), aspartate aminotransferase, alanine aminotransferase and their ratio. Body condition assessment (BCS) was performed. The obtained data were processed using the Sigma Plot program. GL concentration increased by 4.2% (3.75 ± 0.06 mmol /L and 3.91 ± 0.07 mmol /L, $p < 0.01$) in phase II compared to phase I. The remaining parameters had no significant differences. BCS was 3.81 ± 0.09 points and 3.81 ± 0.06 points, respectively. Correlation analysis showed that the interval from calving to insemination in subsequent lactation is related to the concentration of TG ($r = 0.851$, $p < 0.01$), TH ($r = -0.791$, $p < 0.05$) and the ratio of aminotransferase ($r = 0.741$, $p < 0.05$) in phase I. Milk productivity for 100 days of subsequent lactation was also associated with the concentration of TG and TH ($r = -0.721$ and $r = 0.693$, respectively, $p < 0.05$), in phase II. In the first phase of the dry period, the components of lipid metabolism had a correlation with the subsequent reproduction I, in the second phase of the dry period (before the onset of NEB) they had a connection with milk productivity for 100 days of the subsequent lactation. Project № AAAA-A18-118021990006-9.

P135 | Creating a system for genome editing of chicken embryonic cells

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A significant proportion of birds' body resources is going to the development of feathers. At the same time in modern conditions of poultry keeping the plumage does not give them and production advantage. To develop a method for creating chickens lacking plumage, the FGF20 and HR (hairless) genes associated with the development and growth of hair (feathers) in mammals and birds were selected as targets for inactivation. Inactivation of FGF20 and HR genes was performed in the region of the third exon of each gene, taking into account the analysis of their structure. To create genetic constructs for cutting the chicken genome in the regions encoding FGF20 and HR, the vector pX458 was selected (Ran F.A., et al., Nat Protoc. 2013,8:2281–2308). The efficiency of chicken genome engineering was studied in experiments on chicken embryonic cells. Efficiency of electroporation (Neon transfection system, Invitrogen) was evaluated on a high-performance fluorescent cell sorter BD FacsAria III by expression of the eGFP marker gene located on the same plasmid with the genes coding for the components of the CRISPR/Cas9 system. Using the electroporation parameters 1350 V and 30 μs and 1 pulse, the percentages of cells transformed with the plasmids for knockout of the FGF20 and HR genes were 5.4 ± 0.9% and 2.3 ± 0.7%, respectively. From the selected population of transformed cells, DNA was isolated and used as a template for amplification of the edited FGF20 and HR regions. Sequencing

of the resulting amplicons revealed multiple events of insertion/deletion mutagenesis. The data obtained indicate the success of the creation knockout system of FGF20 and HR genes in chickens using genetic constructs based on the pX458 vector. Supported by RFBR (18-29-07079).

P136 | The influence of the female body weight on selected features of the first and second eggs and the weight of the Wroclaw Pigeon's chicks

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The Wroclaw Pigeon is a Polish meat breed. Every year, the females lay approximately 10 pairs of eggs. In every litter the female lays the first egg 7–10 days after coupling, whereas the second about 48 h later. Incubation of the eggs lasts 16–19 days. The aim of this study was to estimate the impact of body weight of a female on selected features of eggs and body weight of the first and the second chick in the litter. It was carried out on n = 9 females from which there were received 2 eggs / 2 chicks in one litter. Birds were held in pairs, in cages in a room with a 12/12 light cycle and changing environmental conditions (temperature, humidity). They were fed daily and had water ad libitum. Eggs and chicks were weighed with an accuracy to 0.1 g. Eggs' length and width (shape index) were determined. The correlation (Spearman) between selected eggs' features and female and chicks body weight, and the Student's t-test significance was estimated using SPSS Statistics 23 PL. It was found that the average weight of the first egg (22.0 ± 1.65 g) was lower than the average weight of the second egg (23.4 ± 1.62 g). Similarly, the average weight of the first chick (23.39 ± 3.81 g) was 3.4 g lower than the average weight of the second chick (26.78 ± 4.14 g). No correlation was found between these characteristics. There were no significant correlations between the weight of the female and the length, width and weight of the egg, as well as between the weight of the egg and the weight of the chick. There were significant correlations between egg weight and length (r = 0.780) and width (r = 0.809), p = 0.01. A negative correlation was observed between the female body weight and the chick's mass (r = −0.473), p = 0.05. The results should be confirmed by repeating the study on a larger group.

P137 | Relationship between polymorphism c.1607C>T in the PRLR gene and growth indicators and body weight of cows and calves

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In recent times many authors focused on the analysis of prolactin receptor gene polymorphism with respect to reproductive characteristics of pigs and sheep. However, the state of knowledge on the impact of the PRLR gene on bovine reproductive traits is still limited. The aim of the study was to determine the relationship between c.1607C>T polymorphism in the prolactin receptor gene and fertility and body mass indexes of Polish Holstein-Friesian cows and calves. The research covered 250 cows kept in the stall barn and fed using a TMR (Total Mixed Ration) system. DNA from peripheral vein was used for DNA isolation. Analysis of genotypes of individual cows was carried out using the PCR-RFLP method. Statistical calculations were performed in the Statistica® 10 PL program (StatSoft, Inc. 2012). The statistical analysis carried out showed the existence of statistically significant associations. Cows with a homozygous CC genotype were characterised by the lowest value of the insemination index (1.07), the highest age at the first insemination (870 days) and the highest cow weight (650.2 kg) and calves of both sexes (males – 38.2 kg and females – 36.5 kg). The cows with the genotype CC differed ($p < 0.01$) from the cows with other genotypes, body weight after calving, and the calves born by them had the birth weight significantly ($p < 0.01$) higher than the female offspring of cows with the CT and CC genotypes. Animals with the genotype CC were also characterised by a significantly ($p < 0.05$) lower insemination index compared with cows having other genotypes. It was shown that the heifer with CC and CT genotype and male calves originating from CC homozygotes and CT heterozygotes differed ($p < 0.05$) among themselves: age at first insemination and birth weight.

P138 | The effects of the vitamin-mineral drug "Antimiopatik" use in cows during the dry period on postnatal growth and health of the offspring

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The study aimed at identifying the effects of vitamin-mineral drug "Antimiopatik" in cows during the dry period on postnatal growth and health of the offspring. There were two groups of calves formed with n = 30 each: the control group (CG) was getting the basic diet only, and consisted of 19 males and 11

females, while the experimental group (EG; n = 18 males and n = 12 females) consisted of cows that were, in addition, injected with "Antimiopatik" intramuscularly three times, 60, 40 and 20 days prior to the presumed due date in a single dose of 10 ml, containing vitamin E 400 mg, vitamin A 300,000 IU, selenium 8.0 mg, manganese 4.0 mg, copper 1.0 mg, cobalt 0.2 mg, zinc 2.0 mg. The calves were observed daily during two months, and on day 1, 30, and 60 after birth, body mass was determined. The vitality of newborn calves, assessed by VIGOR score, was not statistically different between the groups. In the EG, 1 (3.3%) case of omphalitis and 3 (10.0%) cases of diarrhea were registered, in the CG 6 (20.0%) and 13 (43.3%) cases, respectively. In the EG, respiratory syndrome (WI clinical score ≥ 5) was registered in 3 (10.0%) calves, bronchopneumonia in 2 (6.7%) calves, in the CG in 9 (30.0%) and 7 (23.3%) calves, respectively. The average daily body weight gain in the first month of life in EG was 557.4 ± 17.37 g, while in CG it was 461.7 ± 12.18 g, which was 17.2% less ($p < 0.01$). The second month of life the daily body weight gain in the EG averaged at 418.5 ± 9.80 g, and in the CG 387.3 ± 6.60 g, which was 9.6% less ($p < 0.05$). The data obtained lead to the conclusion, that Offspring of cows supplemented with "Antimiopatik" in the dry period showed better growth intensity and resistance to neonatal diseases.

P139 | Influence of vitamin-mineral drug "Antimiopatik" on reproductive characteristic in cows

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The aim of the research was to study the influence of the vitamin-mineral drug "Antimiopatik" on the reproductive parameters of cows in the Republic of Belarus (Mogilev region, Glussky district), where there is a lack of selenium, manganese, copper, cobalt and zinc in soils. Two groups of cows were formed with n = 30 each: the control group (CG) was getting the basic diet only, while the experimental group (EG) was injected with "Antimiopatik" intramuscularly three times, 60, 40 and 20 days prior to the presumed due date of calving in a single dose of 10 ml, containing vitamin E 400 mg, vitamin A 300,000 IU, selenium 8.0 mg, manganese 4.0 mg, copper 1.0 mg, cobalt 0.2 mg, zinc 2.0 mg. Groups of cows were selected based on the analogs; there were no significant differences between the groups at the beginning of the experiment. Median samples were compared using the non-parametric Wilcoxon test. In the EG, 3 cases of retained placenta were recorded, in CG 12 cases. In the EG, acute postpartum endometritis was recorded in 2 cows (6.7%), mastitis (≥300,000 SCC) in 3 cows (10.0%), in the CG in 9 cows (30.0%) and 8 cows (26.7%) respectively. The period from calving to the first insemination in experimental animals averaged 63.3 ± 3.70 days, while in animals from the CG it averaged 79.6 ± 4.45 days, which was 16.3 days ($p < 0.01$) less. The service period in the EG averaged 79.0 ± 3.15 days, and in the CG 92.2 ± 3.92 days, ($p < 0.05$). The duration of the inter-calving period in the EG was 355.1 ± 3.40 days, compared to CG with 384.7 ± 5.11 days, which was 29.6 days ($p < 0.05$) more. The data obtained lead to the conclusion, that the animals injected with "Antimiopatik" demonstrated better reproductive parameters by preventing diseases in the postpartum period.

P140 | Analyzing the level of heterozygosity in the Polish hunting spaniel population

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In the 1980s, Krzywiński started a breeding program aiming to recreate the polish hunting spaniel population based on dogs of the old Polish hunting spaniel type. In 2016, a preliminary breed standard was introduced and the Initial Breeding Book was opened thanks to the efforts of the breeders and The Polish Kennel Club (PKC). The goal of this study was to determine the average degree of kinship and inbreeding among the Polish hunting spaniel. Pedigree information of 130 individuals (PKC database) registered in the Initial Breeding Book of the breed was used for statistical analyses (Endog 4.8 application). Alarmingly, it was discovered that a single sire was used in 56% of matings. Average inbreeding ratio of the studied population amounted to 8.66%, while average kinship ratio amounted to 15.96%. Bearing in mind that pedigrees of 33 individuals were incomplete, the final results might be slightly underestimated. The number of matings between full siblings (1.23%), half-siblings (19.14%) and parent-offspring (12.35%) was relatively high. These results suggest that the population of the polish hunting spaniel has low genetic variability. It is therefore necessary to conduct further research at the molecular level (by using short tandem repeats). The results would help to create a long-term breeding programme (mating plan) for breeders, which would ensure proper genetic variability in the population of the Polish hunting spaniel and thus make it possible to register the breed at FCI.

P141 | Onset of oviposition by honey bee (Apis mellifera) queens inseminated at different age

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Many factors regarding onset of oviposition by instrumentally inseminated honey bee queens have been studied. One of these

factors is age of queens at insemination. However, obtained results are contradictory (Chuda-Mickiewicz, Pszczeln. Zesz. Nauk. 1993,37:32–31; Skowronek, J. Apic. Sci. 2002,46:85–95). The purpose of the study was to investigate the effect of age of queens at insemination on the beginning of egg laying. Altogether, 72 queens were included. They were reared from less than one-day-old larvae. Queens were emerged from their queen cells in the mating nuclei which contained about a thousand worker bees. To prevent natural mating, queen excluders were placed at the entrances of the nuclei. Three groups were created: queens inseminated at the age of 7, 10 and 13 days. All of the queens were treated with CO2 for three minutes two days before insemination and inseminated with 8 μl of semen. Nuclei were inspected daily or every other day for egg laying. Queens inseminated at the age of 7, 10, and 13 days started to lay eggs (on average) 10.2, 8.2, and 7.6 days, respectively, after insemination. The range was 4–20, 4–17, and 2–21 days, respectively. The differences were not statistically significant (Kruskal-Wallis test: p = 0.117). Of all queens, 25, 50 and 75% started to lay eggs within 6, 8 and 10 days, respectively, after insemination. It was found that age of honey bee queens during instrumental insemination does not affect the time from insemination to the onset of oviposition. However, taking into account the number of days from emergence to oviposition, queens inseminated at the age of 7 days started to lay eggs significantly earlier than those inseminated at the age of 13 days.

P142 | Hatching results of Japanese quail (Coturnix japonica) related to age of females

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Japanese quails are used for commercial meat and egg production but also for experimental purposes where reproduction results may be of great importance, especially the time of hatchability alignment (narrow hatch window), chicks' uniformity in weight and feed intake. Improvement in hatching results is far more advanced in domestic chicken than in other poultry species. However, Japanese quails compete in terms of rapid growth and early maturation (6–7 weeks), high egg production (up to 280–300 eggs/bird in the first year), short egg incubation and embryo development (16–18 days). The aim of this study was to investigate the differences in reproduction traits associated with age. The study was conducted on a total of 218 eggs of laying type Japanese quails. The eggs for incubation were collected in two different periods of the quails' life (both sexes at the same age of 3.5 and 7 months). Eggs were incubated in 37.8–37.9 C degrees until hatching (18 days). The results demonstrated higher egg weight in the second age period (10.75 g vs 10.55 g) and lower fertility (93.6% vs 95.5%) and hatchability from fertilized eggs (84.9% vs 87.5%) as compared with the first age period of the birds. Time of hatching was similar for both age classes and the hatching peak was in the middle of 17th day of incubation. Mean body weight of chicks after hatching changed with the females' age from 7.13 g to 7.34 g, while shank length remained at the same level (16.3 mm). Correlation coefficients calculated between each trait of eggs and chicks after hatching in both age classes were high, positive and highly significant in most cases. There are no contraindications to hatching the chicks from older hens for the herd renovation but this requires consideration for other purposes.

P143 | Inheritance of cock's sperm cryostability

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Sperm cryostability is important criterion for in vitro preservation of genetic resources. Formerly, we found a high correlation between the cryostability of rooster spermatozoa (activity after thawing) and its fertilizing capacity rs = 0.602 (P < 0.01). The goal of this research was to evaluate the influence of origin on cocks' sperm cryostability. The experiments were conducted on two generations of Rhode Island cocks (fathers n = 23, sons n = 26) at the age of 50/52 weeks. Ejaculates were diluted and frozen individually in pellets. Cryopreserved semen was thawed at 60°C (Tselutin K., 2015) and the activity of spermatozoa was analysed by Axio Imager. The results were statistically processed using MS Excel. The studies were carried out on a selected population, parametric methods of statistical processing can`t be applied. Therefore, we used the indicator of "frequency of occurrence" of individuals with a certain level of development of the trait. The frequency of occurrence (absolute frequency) is a number indicating how many times sign occurs in the aggregate or its interval (%). Roosters-fathers were divided into 2 groups according to the activity of frozen thawed spermatozoa. Animals in group 1 had sperm activity < 60% and in group 2 ≥ 60%. It was found that the frequency of occurrence of descendants with low activity of preserved sperm (< 60%) in group 1 was 73%; in group 2 – 36%. The frequency of occurrence of descendants with high activity of preserved sperm (≥ 60%) in group 1 was 27%; in group 2 – 64%. It can be argued that the cryostability of the sperm of roosters, estimated through the indicator of its activity after freezing/thawing, is a highly heritable sign. Research conducted on the topic AAAA-A18-118021590132-9; biomaterial from Genetic collection of the RRIFAGB.

P144 | Abstract withdrawn

P145 | Comparative andrological examinations of Charolais bulls with different quality of semen production

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Fertility is the key issue of beef cattle production. Bulls with low fertility have a critical role in decreased profit. Complex andrological examinations is an acceptable method for identify the bulls with low fertility. In the last 2 years, we examined 155 Charolais bulls (age ranged between 14 month – 5 years) before (January to April) and after (October to December) breeding season. Scrotal circumference (SC), thermographic (scrotal surface temperature (SST)-InfraCAM), ultrasonic (US-Draminski Iscan) examinations were used and after electro ejaculation semen motility and morphology were checked (mobile CASA-Ongo and differential interference contrast-DIC). Echotexture of reproductive organs (testis, epididymis) was measured on a 256 gray scale. Twenty-four of the 155 bulls were selected with different semen motility and morphology and 2 groups were formed (15 bulls with good /Group G/ and 9 with poor /Group P/ semen production) in order to compare the ultrasonic and thermographic findings. Semen motility data and rate of normal spermatozoa significantly differed in the two groups ($p < 0.001$). No significant difference was found in any other parameters (SC, SST, and testicular/epididymal echotexture). Although in 5 bulls with poor semen quality (in Group G) more connective tissue was detected by ultrasound in testes/epididymis the mean and standard deviation of echotexture did not differ statistically from the bulls with normal semen production. Although in our study with different methods no statistically significant different findings between good and poor semen producers could be observed, complex andrological examination can be useful to detect the reasons of some fertility problems in bulls and help to apply the appropriate treatment.

P146 | Seminal quality in Nellore bulls selected for low and high residual feed intake

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Residual feed intake (RFI) is calculated as the difference between observed consumption and estimated consumption. The selection of young animals with better food efficiency (low RFI) is very important for breeding programs in cattle. Thus, the selection of animals classified as low RFI may result in progenies that consume less food without impairing animal performance. The objective of this study was to evaluate the parameters related to the sperm quality of young Nelore bulls selected for RFI. Forty-eight young males were distributed into two groups: low RFI (more efficient) and high RFI (less efficient). The animals were evaluated for sperm quality at 24 months of age. Samples of fresh semen and post-thaw were evaluated for computer assisted sperm analyzer (CASA) and rapid thermoresistance test (TRT; 46°C for 30 minutes). The data were analyzed by PROC MIXED of SAS ($p < 0.05$). The RFI did not affect sperm quality (sperm concentration in the ejaculate, percentage of sperm defects). The thawed semen of bulls selected for low RFI exhibited better quality ($p < 0.05$) than high RFI bulls semen for the following parameters evaluated by CASA: average path velocity [VAP; μm / s: 87.38 vs. 82.76 post-thaw and 76.22 vs. 71.40 post TRT] and curvilinear velocity (VCL; μm / s: 144.71 vs. 135.04 post – thaw and 118.92 vs. 110.10 post – TRT). In conclusion, post-thawing sperm velocities were affected by the RFI effect.

P147 | Is the luteal vascularization percentage correlated with progesterone concentration and with different types of the luteal structures detected in superovulated sheep?

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The aim of the study was to correlate the luteal vascularization percentage (LV%) with serum progesterone concentration ([P4]) and with different types of the corpora lutea detected in superovulated sheep. Santa Inês ewes (n = 27) received intravaginal P4 devices (CIDR®) for nine days. On Day 0 and 8 the animals were injected i.m. d-cloprostenol (37.5 μg, Prolise®). The superovulatory treatment began on Day 6 and consisted in eight injections at 12-hr intervals in total doses of 100, 133 or 200 mg of pFSH (Folltropin V®) (n = 9 ewes/total dose). On Day 6, ewes also received 300 IU of eCG i.m. (Novormon®). B-mode and color Doppler ultrasound exams of ovaries were done on Day 15 using an equipment (MyLab™ Vet 30 Gold, Esoate) with a stiffened 7.5 Mz linear probe. Superovulatory responses were defined by laparoscopy, when the corpora lutea (CL) were classified as normal (healthy) or prematurely regressed (PR, ≤ 5 mm and grossly pale). Blood samples also were collected to determine serum [P4] by RIA. Ultrasound images were analyzed using a specific software (Adobe FireWorks® CS6 and Image J®) to define the LV%. Pearson correlations were determined between variables, independently of the group ($p < 0.05$). Numbers of total, normal and PR CLs were 15.3 ± 0.3, 11.8 ± 0.5 and 4.1 ± 0.4, respectively. The LV% and the [P4] were 59.2 ± 0.82% and 9.4 ± 0.6 ng/ml, respectively. The LV% were negatively correlated with [P4] ($r = -0.14$, $p = 0.02$), totals CL ($r = -0.13$, $p = 0.019$) and PRCL ($r = -0.19$, $p = 0.001$). In conclusion,

LV% observed at the embryo collection day is correlated with [P4], total CL and PRCL. Financial support: FAPESP (nº 2017/04193-9).

P148 | Spectral doppler ultrasonography during the last week of gestation of Santa Ines ewes: preliminary results

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The aim of this study was to determine the characteristics of placentome blood flow using spectral Doppler ultrasonography during the last week of gestation in ewes and to correlate the vascular indices obtained with the lambing date. Twenty five Santa Ines ewes were selected for this study and divided in two groups: NPG – animals with natural parturition (n = 15); and PPIG – animals subjected to a protocol of preterm labor at 135 days of gestation (n = 10). The animals were evaluated during the last gestational week twice a day until the moment of parturition. Placentome blood flow rate was compared between treatments, time and their interaction by ANOVA in an inter-randomized design in measurements repeated in the time and post-test of Tukey (mean ± SD, $p < 0.05$). No interaction or correlation between placentome resistance index (RI) and hours before parturition (HBP) were observed ($p = 0.377$; $p = 0.523$, respectively). However, an interaction between the peak systolic velocity (PSV) of the placentome and the HBP were observed ($p = 0.030$) and 72 to 36 HBP the PSV was higher in the NPG than PPIG ($p = 0.00$, 0.001, 0.00, 0.013, respectively). PVS did not show a correlation in the HBP ($p = 0.193$). The end diastolic velocity (EDV) of the placentome presented an interaction with HBP ($p = 0.003$) and 72 to 48 HBP, EDV was higher in ewes in the NPG than the PPIG ($p = 0.000$; 0.017; 0.000; respectively). Even though the blood flow rates (PSV and EDV) increased 48 to 72 HBP in the NPG compared to animals with PPIG. In conclusion, the variables evaluated are not effective in predicting the moment of parturition; therefore, they cannot be considered as parturition indicators.

P149 | The research of the toxic effects of antibiotic resistant S. aureus strains

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Staphylococcus aureus is the main causative agent of intramammary cattle infections. It has multiple resistances and is capable of producing enterotoxins. In this regard, the aim of the work was to study the S. aureus strains with resistance and its ability to produce enterotoxins, affecting the viability of udder cells. The extracted isolates were identified on the basis of cultural and biochemical properties. Four groups of antibiotics were tested in order to identify the sensitivity of S. aureus. All isolates were also analyzed for the presence of the sea, seb, sed, see, seg, seh, sei genes with the use of the polymerase chain reaction (PCR). The study of the extracted isolates showed that 53.6% were resistant to β-lactam, 80.4% to macrolides, 53.6% to lincosamides and fluoroquinolones. Of all the strains studied, 88% contained at least one of the enterotoxin genes under research, and in 5 samples both the seg and sei genes were present. The sea gene contained 53.3% of isolates, seb 3%, sec 50%, sed 4%, see 46%, seg 0%, sei 10%. The seh gene was not detected. The study of the effect of enterotoxins SEH, SEA, SEE, SEG on the viability of cow udder cells in culture in vitro showed that with the introduction of seh into the culture medium at a concentration of 10, 50, 100 and 250 μg/ml, there was a decrease in the proportion of viable cells amounted to 7, 8, 12 and 13%, respectively. When sea was added at the same concentrations, the differences accounted to 12, 16, 20 and 38%. The toxicity increased more than twice when studying seg and see. The research has shown that enterotoxins secreted by S. aureus cause apoptosis of epithelial cells in vitro, which can lead to regression of udder tissue. This work was supported by the Russian Science Foundation 15-16-00020.

P150 | The trace element status of lactating Holstein breed cows during the resumption and depression of ovarian ovulatory function after parturition

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The aim of the study was to identify and assess the microelemental status of Holstein breed cows after parturition during the resumption and depression of ovulatory ovarian function period. There were 16 cows kept on a balanced diet for all essential nutrients. On days 6, 12, 19, 40, 68 after calving, venous blood was obtained from the cows and the levels of Zn, Cu, Mn, I, Se, Co, Cr, Fe content were determined by inductively coupled plasma mass spectrometry (ME-ICP) using a Nixion 300D mass spectrometer. During the observation period, all cows showed a decrease in Zn concentration in the blood from 2.10 ± 0.09–2.39 ± 0.12 μg/ml to 1.89 ± 0.11–2.06 μg/ml (by 9.7–13.7%), a decrease in Cu from 0.90 ± 0.02–1.00 ± 0.03 to 0.77 ± 0.04–0.78 ± 0.04 μg/ml (14.6–21.9%), a decrease in Fe from 423.9 ± 13.0–439.7 ± 17.6 μg/

ml to 364.4 ± 26.2–381.9 ± 24.3 μg/ml (by 13.2–14.0%). As for Mn, I, Se, Co, Cr no significant differences were found over time. At the same time, in cows during the resumption of ovulatory function, the Cu content in the blood, compared to the animals with depression of folliculogenesis and ovulation was lower by 9.3–10.9% ($p < 0.01$), Mn – by 13.1–14.2% ($p < 0.05$), I – by 13.9–15.2% ($p < 0.05$) and Zn – by 11.8–14.0%. Meanwhile, an average blood concentration of Cr was higher by 19.7% (9.79 ± 0.13 μg/l versus 8.18 ± 0.23 μg/l, $p < 0.001$). Therefore, along with the main essential trace elements, the vital element Cr which is active in the regulation of energy processes in the body of animals, should be included into a nutritional plan and taken under overall control.

P151 | Determination of the influence of amino acid substitution on the spatial structure of the resulting protein in domestic pig

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Chondrodysplasia (chondrodystrophia) is a genetic disorder of epiphysial cartilage growth. The essence of this disorder is abnormal proliferation and maturation of cartilage cells, which leads to abnormal ossification on the cartilage substrate, which may result in limb bones and spine shortening (dwarfism). Previous studies carried out on individuals of the domestic pig species have shown a naturally occurring, dominant mutation that causes dwarfism. It has been found that the genetic background for chondrodysplasia in domestic pig is a mutation in a gene encoding an alpha collagen subunit of type X (Col10A1) causing the change of 590 amino acid glycine to arginine (G590A). However, previous studies have not shown how the identified mutation affects the spatial structure of protein and its functionality. The aim of this study was to perform an in silico analysis to demonstrate the effects of this mutation and to accurately determine the molecular background of the disease. The analysis was carried out using the COL10A1 (collagen type X alpha 1 chain) gene sequence deposited in the NCBI database GeneID: 448809. Bioinformatic programs such as InterPro v.72.0, ExPASy Translate, ExPASy Prosite and SWISS-MODEL were used for analysis. It was shown that mutation G590A does not affect the occurrence of C1q domain at the end of C of collagen molecule. However, changes in the spatial structure of this protein as a result of the identified mutation may cause its non-functionality. These changes, associated with a change in the side chain of amino acids, concern parameters such as clash score or Ramachander outliers. In silico analysis should be confirmed by comprehensive molecular studies. The results of the analysis are promising in terms of the possibility of targeted therapy.

P152 | Evaluation of progesterone measurement for the diagnosis pregnancy and embryonic mortality in dairy cows

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The aim of the study was to evaluate the effectiveness of measuring the concentration of progesterone (P4) in milk in lactating cows during different periods after insemination to diagnose pregnancy, infertility, ovarian pathology and embryonic mortality and to establish differences depending on the timing of progesterone measurement. To conduct the experiment, according to the breeding of the dairy enterprise, two groups of cows were formed (n1 = 37; n2 = 32). In their milk, the amount of P4 was measured by the ELISA method on days 21 and 28 (n1) and on days 35 and 45 (n2) after insemination, respectively. In the first group, 21.62% of cows were not pregnant (P4 < 5 ng/ml on the 21st day), 78.38% were pregnant (P4 > 5 ng/ml on the 21st day), but among the pregnant ones, 20.69% P4 decreased on the 28th day. In the second group, only 12.5% of pregnant cows were detected, the number of cows with probable fetal mortality was 15.63% and 56.25% with low activity of the corpus luteum. The study found that the number of pregnant cows in the second group was 49.66% less than in the first group. It can be noted that in the second group, the number of animals with fetal mortality (which was established when the P4 level fell below 5 ng/ml) and the likely ovarian hypofunction was also higher by 45.78% than in the first group. The results indicate the need to measure the concentration of P4 in the dynamics from 21 to 45 days. As a result of assessing the biochemical status of infertile cows of the studied groups, there was a violation of protein and mineral exchanges, as well as a low content of carotene (0.32 ± 0.06 mg/%). The correlation (Pearson linear correlation coefficient) between the maximum concentration of P4 and the concentration of carotene in cows was 0.79.

P153 | Prepartum metabolic status of dairy cows with different ovarian activities during first lactation

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Fertility of dairy cows may depend on the pattern of prepartum (PRP) metabolism. The goal of the present study was to compare PRP biochemical and thyroid profiles in primiparous dairy cows

with different postpartum (PP) ovarian activities. Blood samples from 58 Russian Black Pied cows were taken 2–8 weeks before and 2–7 weeks after calving. Animals were divided into 3 groups: (1) cycling cows (CY, n = 31), (2) cows with a low ovarian activity (no corpus luteum and large follicles; LA, n = 13), and (3) cows with inactive ovaries (no corpus luteum and large and medium follicles; IO, n = 14). The ovarian activity was assessed by rectal palpation, ultrasonography, and blood progesterone levels. Biochemical indices were measured in serum using a biochemical analyzer; hormonal levels were determined by ELISA. Variations in most biochemical indices, except the activity of alanine transaminase (ALT), throughout the PRP period were not different among the groups. The activity of ALT reduced 1.3–1.6 times ($p < 0.05$) by the 4th week PRP in CY and IO cows but remained unchanged in LA cows. Besides, the activity was higher ($p < 0.05$) in the CY group than LA group 8 and 6 weeks before calving. Thyroxine (T4) levels did not alter notably during the PRP period in all groups. Serum concentrations of triiodothyronine (T3) were maximum ($p < 0.05$) from the 2nd week PRP till the 2nd week PP in CY cows, did not change in LA cows, and declined 1.7 fold between the 2nd week before and the 2nd week after calving in IO cows. The T4/T3 ratio in the CY and LA groups correlated with the ALT activity ($r = 0.18$, $p < 0.05$) during the PRP period. Thus, prepartum thyroid and ALT profiles might influence the ovarian activity of dairy cows during first lactation. The study was supported by RFBR (18-016-00227).

P154 | The effect of the age on sperm quality in reindeer (Rangifer tarandus)

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The analysis of the effect of age on sperm quality in reindeer (Rangifer tarandus) was carried out. Sperm was collected during the rut at autumn 2017, 2018. Total and progressive motility, concentration and morphology were measured by CASA. Results are depicted as means±SDs and ranges. Sperm was not found in caudae epididymidae in males at the age of six months. The sperm was collected only from caudae epididymidae from males aged 1.5 and 2.5 years. The sperm concentration was 0.21 ± 0.90 billion/ml and 0.25 ± 0.042 billion/ml, respectively. Total and progressive sperm motility was low, both in 1.5-year-old males 23.0 ± 7.8 and 11.0 ± 2.00%, and in 2.5-year-olds 49.5 ± 12.89 and 25.5 ± 8.62%. However, the sperm of young males was highly cryostable. The motility after freezing was almost unchanged and remained at the same level. Sperm can be collected both from the testes and by the electric ejaculation in older males. The average volume of ejaculate increased from 0.3 ± 0.09 ml at the age of 3.5 years, to 0.7 ± 0.09 ml at the age of 5.5 years and older. The average concentration of sperm in the ejaculate increased by 2 times from males aged 3.5 years (0.33 ± 0.07 billion/m) to 5.5 years (0.65 ± 0.09 billion/ml). High individual variability of total (from 30 to 94%) and progressive (from 13 to 79%) sperm motility before freezing and after freezing (from 22 to 64% and from 13 to 45%, respectively) was noted. No significant effect of age on the morphological parameters of the quality of reindeer sperm was observed. Authors acknowledge financial support from Russian Science Foundation, Grant No: 17-16-01023.

P155 | Traditional gonadectomy vs gonadectomy by Ligasure® in grey squirrels (Sciurus carolinensis): Comparing surgical techniques

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The grey squirrel (Sciurus carolinensis) population has been spreading in the city of Perugia (Umbria, Italy) due to accidental releases in the early 2000s. The LIFE13/BIO/IT/000204 U-SAVEREDS (http://usavereds.eu) project aims to preserve European red squirrels (Sciurus vulgaris) in Apennines, thus preventing biodiversity loss. A method to limit spread of the grey squirrel consisted in the release of gonadectomised animals. The aim of this study is to compare two different surgical techniques: traditional gonadectomy (TG) using ligatures (3/0 polydioxanone); and gonadectomy using a Ligasure® (Electrothermal bipolar tissue sealing system) device (LG), preventing intraoperative complications such as hemorrhage and ligature dehiscence through cauterization and dissection. The squirrels (n = 59) were taken to the University Veterinary Hospital in Perugia. Anaesthesia was performed by ketamine and dexmedetomidine (im), and maintained with sevofluorane and O2. Two different surgical accesses were used: a) orchiectomy with a medial scrotal access for post-pubertal adult males with scrotal gonads; b) gonadectomy with a medial ventral celiotomic approach for female and pre-pubertal males with intra-abdominal gonads. The time needed to perform the gonadectomies was faster (Mann-Whitney test and logistic regression: $p < 0.05$) in gonadectomy by Ligasure®. Right gonad: 51.79 ± 3.95 sec (group TG) vs 22.19 ± 4.53 sec (group LG); left gonad: 46.83 ± 3.25 sec (group TG) vs 15.31 ± 2.37 sec (group LG). In conclusion, this new procedure is safer and requires a shorter period of anaesthesia with lower intraoperative complications.

P156 | Endometrial expression of Prostaglandin-Endoperoxide Synthase 1 and 2 in healthy, subclinical and clinical endometritis in dairy cows fourth week postpartum

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The study aimed to analyse the mRNA expression of Prostaglandin-Endoperoxide Synthase 1 (PTGS1) and 2 (PTGS2) in uterine biopsy samples by Real-Time PCR. Cows were examined between 21 and 28 days post partum. Vaginoscopy and cytology were performed to assign cows to three groups: clinical endometritis (C) – > 50% of pus in the vagina (n = 9), subclinical endometritis (S) – no signs of clinical endometritis and more than 5% of neutrophils in cytology samples obtained by cytobrush (n = 9), healthy cows (H) – no signs of clinical endometritis and less than 5% of neutrophils in cytology samples obtained by cytobrush (n = 11). A biopsy was collected from space between the uterine body and right horn using biopsy forceps. For statistical analysis, the Kruskal-Wallis test was performed using IBM SPSS Statistics 25. Expression of PTGS1 in the endometrium of cows from group C and S ($p = 0.031$ and $p = 0.005$) were higher than in a group H. There was no difference between groups C and S ($p = 0.524$). Expression of PTGS2 was the lowest in group S in comparison to group C ($p < 0.0001$) and group H ($p = 0.044$). Higher expression was observed in group C with contrast to group S ($p = 0.088$). In conclusion, cows with subclinical endometritis had the lowest expression of PTGS2 and not a different expression of PTGS1 as clinically sick cows. This finding shows that the expression of PTGS2 in the fourth week post partum significantly alters between healthy cows and cows with subclinical endometritis. Therefore differences in expression may be used as a potential diagnostic parameter.

P157 | Changes in somatic cell and total bacterial counts during the postpartum period in Damascus goats

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The aim of this study was to evaluate somatic cell count (SCC) and total bacterial count (TBC) using bacteriological isolations in milk samples from Damascus goats during the early lactation period. A total of 291 milk samples (milk could not be obtained from 3 teats = 9 samples) from each of the udder halves belonging to 50 goats were collected on days 10, 20 and 30 postpartum (Pp). The goats were randomly selected. Milk samples were examined through bacteriological isolations in a microbiology laboratory and all samples were analysed for TBC (BactoScan) and SCC (Fossomatic). Statistical evaluation was done by Chi-square-, Repeated Measures Define- and Pearson-Test. The most commonly isolated bacteria were Coagulase Negative Staphylococcus (CNS) with rates of 8.2%, 11.3% and 9.2% respectively (Pp days 10, 20 and 30). S. aureus was isolated at the rates of 2.0%, 4.1% and 2.0%, respectively. There were no statistically significant differences between Pp days ($p > 0.05$). The SCC rates > 1 million/ml in milk samples of udder halves decreased significantly ($p < 0.05$) however, the rates of SCC < 1 million/ml in milk samples of udder halves increased significantly on day 30 when compared to the other days ($p < 0.05$). The rates of TBC > 500 (*1000/ml) in milk samples of udder halves decreased significantly during the latter stages Pp (day 20 $p < 0.05$ and day 30 $p < 0.001$) whereas the rates of TBC < 500 (*1000/ml) in milk samples of udder halves were determined to increase significantly from 88.7% to 98.9% ($p < 0.001$). No correlation was found between SCC and TBC values ($r^2 = 0.426$, $p > 0.05$). In conclusion, CNS and S. aureus were isolated most frequently during the Pp period. It has been shown that > 1 million/ml SCC and TBC values of > 500 (*1000/ml) significantly decreased until 30 days post partum.

P158 | The qualitative composition of milk from cows with subclinical mastitis

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The aim of the study was to determine the quality of milk according to various rapid tests and to compare these parameters with the permissible levels of somatic cells (2 × 10*5/ml) of the technical regulations for milk and dairy products (No. 88-FL RF "Technical Regulations for Milk and Dairy Products"). All experimental animals underwent rapid diagnostics of udder health using the California mastitis test (CMT). Based on this result two groups of animals (n = 15, each) were formed. Animals of the first group had a + or ++ reaction in CMT, and the 2nd group of animals had +++ reaction. Organoleptic (color, smell, taste, texture), physico-chemical (acidity, density, fat, casein, lactose, rennet coagulability, free hydroxyproline, lactoperoxidase, lactoferrin, catalase activity), sanitary (somatic cells, total bacterial content, KMAFnM – the number of mesophilic aerobic and optional anaerobic microorganisms, pathogenic, mesophilic) characteristics of milk are determined by the methods described in the technical regulations № 88-FZ of the Russian Federation. The organoleptic dairy cows' milk characteristics of the first group corresponded standards provided by technical regulations No. 88-FL of the RF. Cows' milk of the 2nd group did not correspond to technical regulations. The total protein content in the 1st group was 3.23%,

of the 2nd group was 3.21%, while the content of casein was 2.5% in the first and 2.67% in the second group. Somatic cells in the 2nd group were 534'000/ml, and in the 1st group 278'000 ml. To conclude, the use of the CMT confirms the presence of an inflammatory reaction in the mammary gland and works as a fast diagnostic tool for subclinical Mastitis. Bibliography. Avdeenko et al., Biol Med (Aligarh).

P159 | Occurrence of cryptorchidism in male piglets

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Cryptorchidism (CR) is a pathological condition characterized by absence of one or both testes from the scrotum. Affected animals are known to have increased sexual potency and aggressive temper. Their meat has a distinct unpleasant smell and taste. We have studied the occurrence of CR among male piglets (n = 64'800) and its correlation to parity, number of piglets per litter and the season. Calculation of determination of reliability of differences by ANOVA was carried out. At the significance level α=0.05, the group averages differed significantly. First, we analysed the occurrence of CR in male piglets in relation to the parity of the sows. Replacement gilts had 27.7% (n = 3'412) CR piglets, animals in their 2nd litter 8.7% (n = 1'071), in 3rd 7.4% (n = 912), in 4th 3.1% (n = 381), in 5th 19.8% (n = 2'440) and in 6th 22.5% (n = 2'773). Sows in 1st, 5th, 6th (n = 8'625) litter had more CR offspring compared to 2nd, 3rd, and 4th (n = 2'364) by 72.6% ($p \leq 0.01$). The occurrence of CR was correlated to the litter size. The most affected animals were registered in the litters with 18 piglets (28.3%; n = 3'488), with 15 piglets (21.5%, n = 2'648), 12 piglets (19.8%, n = 2'440), 10 piglets (6.2%, n = 1'995) and 7 piglets (14.2%, n = 749). The season also had an effect on the occurrence of CR. It was determined that the most CR piglets were born in winter (32.7%; n = 21'190) and autumn (42.93%; n = 27'799) and less in summer (13.7%; n = 8'877) and spring (10.7%, n = 6'934). We can conclude that our results indicate an effect of parity, litter size and season on the occurrence of CR in piglets. Nevertheless, the most important preventive measure remains replacement of the breeding animals, producing offspring with higher incidence of cryptorchidism.

P160 | Semecarpus anacardium and Nigella sativa improve ovulation rate in dairy cows with silent heat 50–60 days after parturition

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The aim of this study was to evaluate the influence of Semecarpus anacardium (19 g/cow) and Nigella sativa (0.5 g/cow) herbs suspended in glycerol (380 g/cow) with sodium selenite (0.02 mg/cow) and vitaminum E (0.4 g/cow) on heat expression, ovulation and conception rates. The Holstein Friesian multiparous cows housed in a free stall barn being 50 to 60 days after parturition with no estrous signs were randomly divided into two groups. Cows from experimental group (n = 32) received herbal product per os in the first and seventh day of experiment and cows from control group (n = 30) received placebo. Ultrasound examination of the ovaries was performed three times in weekly intervals. Estrus and ovulation rates were calculated during six weeks after treatment. There was no difference in ovulation rate between experimental and control group during the first week after treatment (8/24 and 9/21 cows ovulated respectively, χ^2, p = 0.44). In the second week after medication, the ovulation rate in experimental group increased significantly (13/24 cows vs 4/21 cows, χ^2, p = 0.015). The estrus signs after treatment occurred 11 days earlier in the experimental group than in the control group (35.34 days vs 46.34 days); however, this difference was not significant (χ^2, p = 0.14). Conception rate in both groups was not different (21.9% vs 23.3%, χ^2, p = 0.89). We conclude that Semecarpus anacardium and Nigella sativa herbs improve ovulation rate but not heat expression and conception rate.

P161 | Variations in expression of boar sperm transcripts analyzed by RNA-Seq

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Transcriptome profiles were generated from total sperm RNA of six Polish large white boars using RNA-sequencing (RNA-Seq) technology. Total RNA was isolated from sperm of boars with good sperm freezability (GSF, n = 3) or poor sperm freezability (PSF, n = 3), according to post-thaw motility (56.6 ± 2.4% and 25.0 ± 2.3%), mitochondrial function (52.3 ± 1.9% and 34.3 ± 2.8%) and plasma membrane integrity (54.2 ± 1.8% and 37.7 ± 1.6%), respectively. RNA-Seq library preparation was used for paired-end sequencing on the Illumina platform (NextSeq 500). DESeq2, DESeq and EdgeR packages detected varying numbers of DE gene transcripts, which were annotated with the KEGG pathways and Gene Ontology (GO) terms. MAPK and Wnt signaling pathways are associated with cell proliferation, differentiation and migration,

whereas the pathway annotated to regulation of actin cytoskeleton is associated with cell motility. Most of the DE transcripts are involved in GO biological process and cellular component. Among the identified 28 DE gene transcripts commonly detected by the three packages, the expression of 22 transcripts was up-regulated (log2FC >1; q-value < 0.05) in spermatozoa from boars with PSF. The expression of 6 DE gene transcripts, FOS, EAF2, CPT2, CCDC85A, DTD2 and ND6 was verified by quantitative real-time PCR (qRT-PCR). qRT-PCR analysis confirmed RNA-Seq results indicating that the expression of FOS, EAF2, CPT2, CCDC85A and DTD2 were up-regulated in the PSF boars, whereas the expression of ND6 was down-regulated in the GSF boars. This study provides important insights in the functions of sperm-related transcripts associated with semen freezability. Supported by a NCN project in Poland (2015/19/B/NZ9/01333).

P162 | Post-thaw supplementation and proteomic analysis of fractionated seminal plasma from boars with different semen freezability

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This study investigated the effect of post-thaw (PT) supplementation of fractionated seminal plasma (SP) from boars with good and poor semen freezability (GSF and PSF, respectively) on the sperm characteristics. Two fractions, SP1 (> 40 kDa) and SP2 (< 40 kDa), were obtained by affinity chromatography of SP from four and three boars with GSF and PSF, respectively. Proteomic studies of the fractionated SP were performed using label-free quantitative LC-MS/MS analysis. PT semen was diluted with BTS, BTS+SP1 and BTS+SP2, and subjected to quality assessment. Newman-Keuls post hoc test indicated significantly higher CASA-analyzed motility characteristics, mitochondrial function, plasma membrane integrity and acrosome integrity in PT spermatozoa treated with SP1 or SP2 compared with BTS-treated spermatozoa harvested from boars with GSF. A large number of differentially expressed (DE) proteins were detected in SP1. The SP1 and SP2 from boars with GSF exhibited significantly high expression of FN1 protein, whereas SP2 was characterized by high expression of spermadhesins (PSP-1 and PSP-II). The KEGG pathway analysis showed that the oxytocin signaling pathway was predominant in SP1, whereas the apoptosis pathway was more prevalent in SP2. Gene ontology analysis showed that most of the DE proteins were related to protein binding, hydrolase activity, signal transduction and cellular response to stress. Western blotting confirmed the presence of FN1 and FCGBP proteins in the fractionated SP from boars of either freezability group. Results of this study indicated that components of fractionated SP from boars with good freezability ejaculates exerted beneficial effects on PT sperm survival. Supported by a NCN project (2016/21/N/NZ9/02289) and grant from UWM in Olsztyn (11.610.003-300).

P163 | The influence of extracellular matrix components on differentiation of porcine bone marrow, adipo- and myometrium-derived mesenchymal stem cells

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Cervical dysfunction in pigs may lead to contamination of the uterus, impaired fertilization or impeded pregnancy and parturition. Mesenchymal stem cells (MSC) seem to be a promising source for cell-based treatment in regenerative medicine. We hypothesize that MSC implantation into dysfunctional cervix may improve its function, as measured by electromyography. We have isolated and expanded MSC to clinically-relevant numbers from bone marrow, myometrium and subcutaneous fat. In an effort to create a protocol resulting in rapid and economical culture expansion, we cultured the cells in cell culture vessels, uncoated or coated with thin layer of gelatin, collagen or matrigel. Growth rates, total culture yield and differentiation capability at various stages of culture were assessed. When grown on collagen- or gelatin-coated surface, the cells, regardless of the source, exhibited morphology more consistent with MSC phenotype and grew more rapidly, with the myometrium-derived cells growing the slowest. Despite the accelerated growth, the cells still showed osteo, adipo- and chondro-differentiation capabilities when the culture was expanded to a total of 5*10^7 cells. When the cells were cultured in coated vessels or in 3D extracellular matrix (in case of chondrospheres) the differentiation progressed more readily, which demonstrates the importance of local microenvironment for proliferation and differentiation of MSC. In summary, we have generated a modified protocol that, at a negligible increase in cost, allows for more rapid generation of therapeutically-relevant numbers of mesenchymal stem cells. Funded by KNOW (Leading National Research Centre) Scientific Consortium “Healthy Animal – Safe Food”, decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

P164 | Presence and localization of the apelin system in the queen placenta at different stages of pregnancy

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Apelin is mainly produced by adipose tissue under the regulation of nutritional status. The expression of apelin (APLN) and of its receptor (APLNR) has been reported in the uterus, ovaries and placenta of different mammalian species (Briana et al, Ann N Y Acad Sci. 2010, 1205:82–7). For its role in vessel formation (Mughal et al, Pharmacol Ther. 2018 190:139–47), we can hypothesize a potential function of APLN in the regulation of pregnancy hemodynamic changes. The placenta is a tissue where angiogenesis is extremely important to ensure normal embryonic and foetal development. The aim of this study was to investigate the presence and distribution of the APLN system in the placenta from queens, at 30 and 60 days of pregnancy, by IHC and RT-PCR. Six adult half-breed queens were divided into two groups, based on the stage of pregnancy, and subsequently subjected to ovariectomy (day 30) or caesarean section (day 60). The immunoreaction was visualized using two primary rabbit polyclonal antibodies (anti-APLN and anti-APLNR) and the DAB as the chromogen. No positivity for APLN was observed in middle-pregnancy placentae. However, APLN appears evident on syncytiotrophoblasts and trophoblasts placenta of day 60 of pregnancy. Receptor positivity was always evident within the two groups, though without appreciable differences in uterine glands, endometrial epithelium, syncytia and trophoblasts. APLN transcript was detected in the placentae examined. In conclusion, the presence of an APLN system in the feline placenta allows us to hypothesize that APLN is involved in the control of placenta functionality, probably by a paracrine action, via APLNR.

P165 | Reproductive performance of bulls Kalmyk breed depending on the methods of mating

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The organization of reproduction in beef cattle has its own characteristics, as for this industry it is important to obtain offspring from each cow. A significant factor affecting the reproductive performance of the herd is the fertility of the bulls used. The purpose of this study was to assess the reproductive qualities of the Kalmyk breeding bulls (n = 11) with different breeding methods. Bulls were used in free mating, together with 25 cows in a common herd. In the second experiment, the herd was divided into several groups, consisting of 35 cows, each of which was assigned to one bull. In the third experiment, the number of cows per bull was 100. In the fourth experiment, artificial insemination of cows was used. To assess the quality of sperm the CASA system was used. Statistical analysis of the data was performed using Microsoft Office Excel 2007 (ANOVA; t-test). The ejaculate volume was 4.50 ± 0.22 ml, the spermatozoa concentration was 1.53 ± 0.08 billion/ml. The content of progressively motile spermatozoa in ejaculate was 93.33 ± 3.33%. Number of calves / 100 cows, depending on the breeding method, was: with free mating, n = 89 ± 2; in the experiment, 35 cows were divided into groups n = 90 ± 2; when securing one bull for a herd consisting of cows up to 100 heads n = 98 ± 2 ($p < 0.01$); with artificial insemination n = 72 ± 3. When using one bull in a herd, the lack of competition creates a dominant sexual behavior that allows the bull to reach more cows without stress, unlike free mating, when a bull is dominated by a competing behavioral response and not fully realize its genetic potential.

P166 | Rearing of honey bee (Apis mellifera) queens from larvae treated with amitraz

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Quality of queens is the main factor affecting the strength and productivity of honey bee colonies. Infestation of brood by Varroa destructor causes decreasing of weight and lifespan, and developmental deformations. Infestation of queen cells by the mites occurs very seldom. However, it happens in heavily infested colonies or those contained no brood. Fumigation with Apiwarol, containing amitraz, is a treatment used to control the parasite. The aim of this study was to investigate whether fumigation with amitraz affects the survival rate of queen larvae and their adult body weight. Larvae less than one day old were introduced to 13 rearing colonies, 39 in each. Altogether, 268 larvae were accepted and investigated. Experimental groups were made as follows: 4 rearing colonies fumigated when developing queen larvae were 2 days old, 4 colonies when larvae were 4 days old, and 5 control colonies not fumigated. One day after sealing, queen cells were put into the incubator at 34.5°C. The number of sealed queen cells was compared with the number of larvae initially accepted. Queens emerged in incubator. They were weighted within 8 h after emergence. Mortality of larvae, to the stage of sealed cells, treated at the age of 2 days, 4 days, and untreated was 5.7, 11.5, and 15.1%, respectively, and did not differ significantly (χ^2 test: $p = 0.888$). Queens did not emerge from 8.4, 7.8, and 12.7% queen cells, respectively (χ^2 test: $p = 0.597$). Mean body weight of queens from larvae fumigated at the age of 2 days, 4 days, and non-fumigated was 206, 212, and 208 mg, respectively, and also did not differ significantly (ANOVA: $p = 0.257$). Amitraz fumigation of honey bee colonies was found not to affect the survival rate of queen larvae and the body weight of obtained queens.

P167 | Double ovulations and twin pregnancies in mares

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The aim of the work was studying characteristics and repeatability of double ovulations in mares, pregnancy rate and results of manual reduction of twins by ultrasound control. The frequency of single and double ovulations (n = 2207) was calculated during the years 2013–2016 in mares of different breeds and statistically analyzed by Student-Fischer t-test. The average frequency of double ovulations in mares per year was 9.0%, and there was no significant difference between breeds: Russian Trotter 8.2% (n = 672), French Trotter 11.9% (n = 244), Standardbred 12.7% (n = 181) and others (draft, saddle) 8.6% (n = 152). The number of double ovulations per month during breeding season was significantly lower ($p < 0.05$) in February (3.2%), March (4.9%) and August (5.1%) than in April-July (9.9–11.3%). The number of multiple ovulations gradually decreased from young (4–8 yo, n = 66/556, 11.9%) to old (>14 yo, n = 10/164, 6.1%) mares ($p < 0.05$). It was established that 35.8% of all mares with poliovulations tended to repeat this. Synchronous ovulations were detected twice more often (68.34 %, n = 82) than asynchronous ovulations (31.66 %, n = 38) ($p < 0.001$). 45.0% of all synchronous ovulations occurred in one ovary ($p < 0.001$). Asynchronous ovulations in one or two ovaries were distributed equally (15.83%). Ultrasound examination on day 12–14 of 102 mating mares with double ovulation resulted in 35.0% of twin embryos and 28.1% of single embryos. The success rate of manual reduction of the second embryo during mobile phase (n = 34) was 85.3%. In conclusion: it is advisable not to miss the estrus cycle of mares having double ovulation and to use transrectal manual crushing of the excess embryo on days 12–15.

P169 | VTIQ™ (Virtual Touch Tissue Imaging Quantification) elastography of placental structure of brachycephalic bitches – Preliminary Results

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The objective of this study was to determine shear wave velocity (SWV) of placental structure of bitches during gestational period by ARFI (Acoustic Radiation Force Impulse) ultrasonography using VTIQ™ software. Three French bulldogs (6 total placental units, two placental units per animal), weighing 10.5 ± 3.3 kg, after conducting preliminary tests and checking their healthiness, were evaluated daily from day 15 of gestation until parturition, using ultrasound equipment ACUSON S2000/SIEMENS and 9.0 MHz transducer. Using the technique described, six samples were obtained in each evaluated portion (dorsal, lateral and ventral) to obtain the SWV mean. The parameters were compared between the regions and repetitions by the ANOVA test. The SWV averages were correlated with gestational days by Spearman test and tested adaptation to regression models. The animals did not present clinical and obstetric changes during gestation and delivery. Repeated measures of each structure were not different from each other ($p > 0.05$). Ventral SWV (IC = 2.5 ± 0.3 m/s) was higher ($p < 0.001$) than dorsal (CI = 2.1 ± 0.3 m/s) and lateral (CI = 0.3 m/s). Only ventral SWV had a significant correlation ($p < 0.001$; $r = 0.32$) with gestational age. Behavior of ventral SWV adapts to a quadratic regression model (Ventral SWV = 3.879–0.07720 DG + 0.00981 DG2; $r^2 = 0.29$), the values decreased between days 20 and 40 of gestation and increased from day 40 to 60. The elastography proved to be applicable as a non-invasive method of evaluating placental tissue and correlated with gestational development. These results are promising in the evaluation of normal gestational process and provide a basis for studies involving pathological processes in different species.

P170 | The effect of different cosmophysical activity on successful insemination in cattle

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The results of artificial insemination of 18,140 black pied cows were analyzed in breeding farms in the Moscow region of the Russian Federation from 1986–2018. The 22nd, 23rd and 24th 11-year cycles of solar activity were observed during this period. Correlation between cow's fertilization and parameters of cosmophysical activity, such as: solar activity – sunspot numbers or Wolf number (W) and solar radio flux F10.7 on the wave length 10.7sm (2800 MHz) were studied. Successful insemination of cows was evaluated after single and double (2 times within 2 days) inseminations. The conditions of keeping and feeding of animals were according to the norms adopted in the farms. One-way ANOVA in SPSS Statistics was used. Different animal responses to cosmophysical activity in even and odd 11-year solar cycles were found. In even cycles, an increase in solar activity promoted successful insemination of cows. During this period, an average of 88.1% of the cows got pregnant. In odd cycles, an increase in solar activity reduced the successful insemination of cows to 48.3% ($p < 0.001$). Within 2–3 years at the lower peak of solar activity, and 2–3 years at the upper peak of solar activity minimal and unreliable correlations are observed ($-0.02 < r < +0.14$). The most significant correlations were observed in the middle of an ascent (3–5 years) or a descent (3–5 years) of solar activity and radio flux ($-0.18 < r < +0.68$, $p < 0.01$). Over the entire study period, the number of sunspots varied from 3.4 to 173.7, and the solar radio flux F10.7 ranged from 694 to 2633. This study showed that cows'

successful insemination might be influenced also by cosmophysical activity parameters. The publication was prepared with the support of the RUDN University Program "5-100".

P171 | Association between serum testosterone concentration and cholesterol content in bulls

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The purpose of this study was to evaluate the association between the changes in blood serum concentrations of testosterone and serum levels of cholesterol in bulls of different breeds. The breed composition of the included bulls was: 4 black-and-white, 4 Auliekol, 6 Angus, 2 Holstein and 2 white-headed Kazakh. The testosterone concentration was determined in the Ekvi-Lab laboratory by the ImmunoChem 2100 enzyme immunoassay analyzer using the Hema commercial kit, and the cholesterol content was determined using the «Vital kit» on the Biochem SA biochemical analyzer. The average concentration of testosterone and cholesterol in bulls (n = 18) was 55.59 nmol/L and 2.94 g/L, respectively. Testosterone concentrations in black-and-white bulls aged 8–18 months ranged from 12.39 to 89.78 nmol/L, in Auliyekol bulls 12 months of age from 11.30 to 71.26 nmol/L, in Angus bulls aged 12–48 months within 24.93 to 111.58 nmol/L. The results showed that an increase in the cholesterol content of the serum of the bulls was accompanied by an increase in the concentration of testosterone. Statistical analysis of the results was carried out using the AtteStat program. The results of our study suggest that determination of cholesterol in the serum of bulls can potentially be used as a proxy for the sexual activity of the animals, since there is a positive correlation (r = 0.87; p = 0.01) between the cholesterol and testosterone concentrations in the serum of bulls.

P172 | Effects of highly dispersed silica nanoparticles on the cryoresistance of porcine cumulus-oocyte complexes

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Creation of an effective model of vitrification of oocyte-cumulus complexes (COCs) is an important task for cryo-and embryotechnologists. Highly dispersed silica nanoparticles (HDSn) can be proposed as potential components of cryoprotectants and IVM medium. The aim of the present study was to determine the effect of HDSn on chromatin status of porcine COCs during IVM. Vitrification was performed by equilibration of COCs in CPA (Cryoprotective Additive) – 1:0.7 M dimethyl sulphoxide (Me2SO) + 0.9 M ethylene glycol (EG) (30 sec); CPA-2:1.4 M Me2SO + 1.8 M EG(30 sec); CPA-3:2.8 M Me2SO + 3.6 M EG + 0.65 M trehalose(20 sec) and loading into straws. After thawing, COCs were washed in 0.25 M,0.19 M, 0.125 M trehalose in TCM-199 and finally in NCSU-23. COCs were cultured in NCSU-23 with 10% (v:v) follicle fluid, 0.1 mg/ml cysteine, 10 IU/ml eCG and 10 IU/ml hCG. COCs were cultured with pieces of follicular wall (600–900 μm in length) 44 h. IVM medium and CPA of experimental groups were added with 0.001% HDSn (Chuiko Institute of Surface Chemistry, Ukraine). Data were analyzed by χ^2 test. Chromatin status of 267 oocytes was evaluated by Hoechst 33342 staining. Nuclei of 14800 cumulus cells were tested (Tarkowski A., 1966, Cytogen. 5:394–400). Level of apoptotic cumulus cells of 260 oocytes was evaluated by TUNEL-test. Addition of HDSn to IVM medium and CPA decreased the percentage of picnotic cumulus cells (47% vs. 29%, p < 0.05). Using of HDSn in the steps of the COCs vitrification procedure decreased the level of apoptotic cumulus cells (54% vs. 35%, p < 0.05) and increased the level of matured devitrified oocytes (33% vs. 59%, p < 0.001). Funded by Minobrnauka, project #181180215901329.

P173 | Isolation of the guinea fowl spermatogonia

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The spermatogonia are valuable biomaterial for creating cryobanks in order to preserve and maintain the bird gene pool. These cells, when transplanted into the testes of male recipients, are able, in the process of differentiation, to give rise to a significant population of germ cells. The aim of our study was to optimize the conditions for obtaining a culture of guinea fowl spermatogonia. Initially, histological studies of testes were carried out in males of different ages in order to determine the age optimal for obtaining a culture of spermatogonia. The population of spermatogenic cells in the seminiferous tubules of males aged 1 week to 4 months was studied. Disaggregation of testis tissue to obtain a culture of spermatogonia was performed using 0.25% trypsin and 0.1% collagenase solution. The duration and conditions for spermatogenic cells cultivation were selected experimentally. Spermatogonia colonies were identified using SSEA-1 antibodies. A significant proportion of spermatogonia from the total number of spermatogenic cells in the seminiferous tubules of guinea fowls testes was observed at the age of 1 to 6 weeks. This indicator reached 72 ± 2%. Given this finding, the testes obtained from

3-week-old males were used to obtain a culture of spermatogonia. The cell culture with a high proportion of spermatogonia (81 ± 3%) was obtained using the method of separating different types of spermatogenic cells by transfer non-adherent cells to a new plate after 24 h of cultivation. It was shown that the optimal feeder layer for cultivation of spermatogonia of guinea fowls are their own Sertoli cells. The formation of colonies of spermatogonia was observed at 5–6 days of cultivation. Supported by RSF (16-16-04104).

P174 | Effect of hatching egg storage period on hatchability and post-embryonic development of broiler chickens

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In a small group of breeding poultry, one of the main limiting factors in continuous production of poultry meat is the long period required to collect batches of eggs for setting in the hatchery. At the same time, an increase in egg storage period deteriorates egg quality. The studies of the post-embryonic development of broiler chickens were carried out in the hatchery of the Center for Genetics and Selection "Zagorskoye EPH". The material for the study was the Cobb 500 cross eggs. Three groups were formed: the first group was control (I) – eggs were stored for 3 days until incubation at t + 20–21 °C, the second group was experimental (II) – eggs were stored for 7 days at t + 15–16 °C, the third group was experimental (III) – eggs were stored for 15 days at t + 15 °C. The relative humidity in egg storage room was 60%. T-test for statistical analysis was used. It was established that the number of infertile eggs in the control group was 1.7%; in group II it was 1.2%, and in group III it was 2.3%*; the number of eggs with blood ring in group I was 0%, in group II – 2.3%, in group III – 3.4%*; dead-in-shell – 2.3; 1.2 and 1.7%; addled eggs – 1.1; 0.6 and 3.4%* accordingly. The hatching rate was, %: – 92.2; 90.4 and 85.9*, and hatchability, % – 93.2; 92.4 and 86.9* accordingly by groups. By 38 days of age, the live weight of broilers in the control group (1) was, g: 2410 ± 33.1; group II – 2274 ± 31.5; group III – 2138 ± 28.6; the weight of carcass, g: I – 1738 ± 20.3; II – 1643 ± 17.7; group III – 1544 ± 18.6; weight of muscles, g :I – 1131 ± 13.9, II- 1067 ± 16.4 and group III – 1003 ± 21.8; weight of bones, g: I – 299 ± 7.8; II – 281 ± 6.9 and group III – 264 ± 6.2 respectively. Thus, we recommend storing the hatching eggs of meat-type chickens for at most 7 days. Note: * P < 0.05.

P175 | Identification of the Hcrtr2 gene mutation – in silico analysis

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Narcolepsy occurs in many species of animals, including more than 15 breeds of the domestic dog. Many features or disorders, such as: sleep apnea, depression, periodic movements of the limbs during sleep, sexual dysfunctions, including decreased libido, obesity, hypothyroidism are associated with narcolepsy. The cause of hereditary narcolepsy in domestic dog is the mutation of the Hcrtr2 gene (Orexin receptor type 2) located in the chromosome 12. It is an autosomal recessive disease with full penetration in Labrador Retriever and Doberman Pinscher breeds. The aim of this study was to analyze the similarity of the Hcrtr2 gene sequence in selected mammalian species, to predict functional partner genes, and to propose a molecular test to identify mutations in Labrador Retriever and Doberman Pinscher. The following bioinformatics programs were used in in silico analyzes: BioEdit, MUSCLE (for sequence comparison), WebCutter and NebCutter (to find restriction sites), as well as UniProt and String (for gene identification related to Hcrtr2 gene). It was found that the protein encoded by the Hcrtr2 gene is a conserved protein. The similarity of the nucleotide sequence of the domestic dog Hcrtr2 gene ranged from 83% to domestic sheep, 92% to human and 94% to domestic cat and horse. We predicted the functional partners genes of Hcrtr2 gene, such as: HCRT, NPS, NTS, EDN1, AVP, GNG: 1, 2, 3, 4, 13. In the molecular test to identify the mutation of the Hcrtr2 gene in the Labrador Retriever gene, the use of two restriction enzymes: ApoI or MluCI was proposed. It was found that the insertion of 226 bp in intron 3 of the Hcrtr2 gene in Doberman Pinscher can be recognized using electrophoretic techniques. The above in silico results can be used in puppies diagnostic test.

P176 | Short-term clinical effects of GnRH analogue deslorelin in bitches during late prepubertal period – preliminary results

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The aim of this study was to determine the clinical (flare-up effect, behavioural and physiological oestrus findings) and hormonal (serum P4 and E2) changes in female dogs treated with deslorelin implants (Suprelorin, Virbac, F) during the late pre-pubertal period. Eight clinically healthy, Kangal cross-breed, pre-pubertal bitches aged 7.4 ± 0.2 months with a body weight of 23.4 ± 1.4 kg

were used. Implants containing 4.7 mg deslorelin acetate, were administered subcutaneously in the interscapular region using a single-use applicator. The signs of oestrus were monitored once daily by assessing physical changes (vulvar appearance and swelling, serosanguinous vaginal discharge), vaginal cytology and sexual behavioural changes, until the occurrence of the first oestrus. Serum P4 and E2 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) test every other day. Data were expressed as mean ± SEM. Half of the deslorelin-treated bitches (E group, n = 4) showed proestrus 8.5 ± 1.0 days after implant insertion. The duration of the clinical proestrus and estrus was 8.0 ± 1.4 and 3.5 ± 1.2 days, respectively. The mean serum concentrations of P4 and E2 were 3.32 ± 0.99 ng/ml and 77.42 ± 19.76 pg/ml in the E group at the beginning of clinical signs. Examination of vaginal cytology indicated proestrus and estrus in the E group, but revealed a gradual increase in the intermediate and superficial cell index in all non-estrus bitches (NE group; n = 4). Our results indicate that deslorelin acetate applied to late prepubertal female dogs induces oestrus accompanied by a strong increase in E2 but in some bitches only vaginal cytological changes associated with hormonal changings. (Support: Kafkas University Scientific Research Projects Coordination Unit, Turkey, project nr 2018-TS-52).

P177 | Transmissible venereal tumor and rhabdomyosarcoma in a dog – a case report

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This report aimed to describe a case of transmissible venereal tumor (TVT) and rhabdomyosarcoma in a 4-year-old crossbreed dog with an overgrowth around the penis, bleeding in the back region, skin nodules, and a mass on the eyelids. A purulent discharge was seen in the penis, prepuce, and nasal cavity. Clinical findings such as depression, cachexia, reluctance to move, and pain were also reported. A complete blood count was performed, neutropenia and lymphocytosis (2.63 × 10*3 µl and 11.71 × 10*3 µl, respectively) were detected. Smear samples were taken from the prepuce, penis, and eyelid masses and stained with Diff-Quick for detecting a TVT. In addition, biopsy specimens were collected from the eyelids, neck, and skin and sent to the Veterinary Faculty Pathology Laboratory for analysis. The internal organs were radiographically examined, but no metastasis was found. The mass was examined histopathologically and immunohistochemically and diagnosed to be a mixed tumor. The TVT was detected in the eyelids, and rhabdomyosarcoma was detected in other biopsy materials. The patient was treated with vincristine sulfate (0.025 mg/kg, intravenously, once a week) in saline for 7 weeks and cyclophosphamide (1 mg/kg, orally, once a day) for 10 days. In addition, two doses of Tarantula cubensis extract (Theranekron®, 2 ml/animal, subcutaneously) were repeated at an interval of 7 days. After the treatment, tumor masses were dissolved and an improvement was observed. The masses in the nasal cavities, on the skin, and around the penis disappeared. The general health condition of the dog was very good. Hence, it was concluded that a combination of vincristine sulfate, cyclophosphamide, and Theranekron® could be successfully used to treat mixed tumors in dogs.

P178 | Expression of selected genes of the uterine sows and their association with reproductive traits

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The reproductive traits have a polygenic character and genes involved in these processes can be divided into several groups due to the location and functions of coded proteins. We examined the level of expression of selected transcripts (AREG, FABP3, IL1A, IL1B, ITGβ3, OPN, S100A1, SELL) obtained from RNA-seq in the luteal (LP; n = 46) and follicular (FP; n = 25) phases of Polish Large White (WBP) sows. Next, the relationship between level of transcripts and reproductive traits related to the size of the litter was performed. Sows were slaughtered 10 days after weaning of piglets and classified on the basis of the morphological changes in the ovary and the corpus luteum. Tissues from the uterine horns were harvested and gene expression analysis was conducted using the qRT-PCR method. A significant higher level of AREG, FABP3, IL1A, ITGβ3 and OPN mRNA was observed in the LP. In contrast, higher expression in the FP showed IL1B, S100A8 and SELL. Among the selected genes OPN and AREG expression showed a significant correlation with traits related to the size of litter. Significant negative correlation between OPN, AREG and SELL expression in the LP with the number of live-born and weaned piglets was observed and a similar relationship also occurred for OPN and AREG expression in the FP. These results provide molecular insights into the genes underlying pig fecundity. (Study was funded by grant NCN, no. 2013/09/N/NZ9/03135 and supported by KNOW, Leading National Research Centre-Scientific Consortium Healthy Animal – Safe Food No. 05-1/KNOW2/2015).

P179 | Udder pathogen species in subclinical mastitis cases in dairy cows in Rwanda

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The objective was to evaluate the prevalence of subclinical mastitis (SCM) and identify udder pathogens in dairy cows in herds linked to milk collection centers (MCC) in Rwanda. Screening with California Mastitis Test (CMT) was done in 572 cows from 404 herds linked to eight MCCs in eastern, northern, western and southern provinces in Rwanda. Udder quarters with CMT ≥ 3 (scale 1–5) were milk sampled for bacteriological analyses, first with traditional culture and final identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The overall prevalence of SCM was 37.3% at quarter level and 62% at cow level based on CMT. There was significant ($p \leq 0.05$) difference of SCM prevalence among MCCs, with those in the north showing the highest prevalence. Bacteria were isolated in 66.3% of the cultured milk samples, whereas culture-negative and contaminated samples were 30.7% and 3.0% respectively. Contagious bacteria, namely non-aureus staphylococci (NAS) and Staphylococcus (S.) aureus were the most prevalent causative udder pathogens, representing more than half of all bacteriological results in SCM cases in dairy cows in all MCCs. Staphylococcus chromogenes and S. epidermidis were the most prevalent NAS identified. Beta-lactamase production was more prevalent in S. aureus (78.9%) than in NAS (54.8%). Sanitization, biosecurity and mastitis control plans are required to limit the spread and lower the prevalence of SCM in Rwanda, with possibilities to increase milk yield and quality.

P180 | Effect of different two-step IVM protocols on the developmental competence of matured bovine oocytes

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Our study was aimed at assessing the effects of different conditions during the terminal phase of IVM on maturation of bovine oocytes and their developmental competence in vitro. Bovine cumulus-enclosed oocytes (CEO) were cultured for 16 h in TCM-199 supplemented with 10% fetal calf serum (FCS), 10 μg/ml porcine FSH, and 10 μg/ml ovine LH (standard medium). Then CEO were transferred to and cultured for additional 8 h in one of three experimental media: (1) TCM-199 containing 10% FCS (group 1), (2) TCM-199 containing 3 mg/ml BSA (group 2), or (3) Fert-TALP medium supplemented with 6 mg/ml BSA (group 3). After IVM, a part of oocytes was used for cytogenetic analysis of nuclear maturation. Another part of matured oocytes underwent IVF and IVC procedures. Embryo development was evaluated at Days 2 and 7 for cleavage and blastocyst rates. The obtained blastocysts were fixed, and the total cell number and the level of apoptosis were determined using DAPI and TUNEL staining. The data from 3 to 5 replicates (66–159 oocytes per treatment) were analyzed by ANOVA. The rate of M-II oocytes did not differ between culture systems, ranging from 82.7 to 93.4%. Furthermore, no effects of the used two-step protocols on the cleavage rate (65.4–71.7%), the total cell number (63.0–78.8 cells per blastocyst) or the proportion of apoptotic nuclei in blastocysts (3.3–5.3%) was observed. By contrast, the blastocyst yield in groups 2 and 3 (26.2 ± 2.7 and 30.0 ± 2.9 %, respectively) was higher ($p < 0.05$) than in group 1 (17.4 ± 0.4%). The findings indicate that replacement of serum with BSA at the terminal stage of oocyte maturation exerts a beneficial effect on the oocyte developmental capacity but does not affect the blastocyst quality. The study was supported by RFBR (No. 17-29-08035).

P181 | The effect of culture medium renewal on the in vitro development of feline embryos

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The aim of this study was to analyse the influence of culture medium renewal on feline embryo development after ICSI. The gonads were obtained from cats intended for routine castration procedures. The oocytes were collected by the scarification of ovarian cortex (1). After in vitro maturation for 24 h (Waurich et al., Reproduction. 2010;140:531–40) oocytes with polar body (n = 172) were microinjected with the frozen-thawed epididymal spermatozoa. The embryos were cultured in groups in 50 μl droplets of single step commercial medium (Continuous Single Culture supplemented with 10% Serum Substitute Supplement, Irvine Scientific, Newtownmountkennedy, Co. Wicklow, Ireland) at 37°C and 5% CO_2 for 7 days. The experiment was performed in 30 replicates. Each embryo culture was subjected to either no medium renewal (N) or medium replacement on the Day 3 (D3), 4 (D4) or 5 (D5). The number of cleaved oocytes, the number of embryos at morula stage and the number of embryos at blastocyst stage were evaluated 24 h, 6 days and 7 days after ICSI, respectively. The results were summed up within experimental groups and analysed statistically with chi-square test. The results were considered significant at $p < 0.05$. Results: No statistically significant differences were observed between experimental groups. Cleavage rate ranged from 42.4% (D3) to 64.5% (N),

morula rate ranges from 45.5% (D4) to 78.9% (D5) and blastocyst rate varied from 7.1% (D3) to 32.5% (N). We can conclude that medium renewal has no influence on the development of feline embryos and the embryos can be cultured continuously in the same medium up to 7 days. The study was financed from NCBiR PBS3/B8/16/2015.

P182 | Relationship between subclinical ketosis in postnatal period and days open period in dairy cows

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Hyperketonemia (HYK) is metabolic disorder in high-producing dairy cows and its diagnosis is based on beta-hydroxybutyrate (BHB) concentration in blood. Ketosis type I normally occurs during the fourth week of the lactation. The aim of the present study was to determine whether and to which extend can subclinical ketosis in postpartum period effect the length of days open period (DO) in Holstein Friesian dairy cows. In this abstract, we present preliminary results from ongoing research. Only cows with no postpartum treatment were included and the samples were randomly collected throughout the year. Average age of dairy cows in research was 5 years and there were no heifers included. Blood analysis of beta-hydroxybutyrate (BHB) was performed between day 28 to 35 after calving, using RX Daytona (Randox Laboratories Ltd., VB). The cows were divided into group of high and low BHB, with the BHB threshold set at 1.20 mmol/L. Differences between the groups were compared by one-way Anova. Cows in the high group had a mean BHB concentration of 1.51 mmol/L compared to the low BHB group's mean of 0.77 mmol/L ($p < 0.001$). Before calving, all animals had an average body condition score (BCS) of 3.4 and it decreased to 3.0 one month after calving, with no significant difference between the groups. The animals were inseminated and the pregnancy was confirmed with ultrasound examination around day 50 post insemination (SonoSite, M Turbo). Cows in the high BHB Group had significantly longer DO period (177.6 day) compared to cows with low BHB (100 days) ($p = 0.003$). These preliminary results clearly suggest that the days open period is prolonged in cows with hyperketonemia, diagnosed by increased BHB concentrations one month after calving.

P183 | Abstract withdrawn

P184 | Embryonic stem cells generated from haploid mouse embryos are prone to self-diploidization.

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The embryonic stem cells (ESC) have potential to generate all cell types in the body. The diploid genome of model species limits genetic approaches. The haploid embryonic stem cells (haESC) seems to be the solution. The aim of the study was to establish haploid embryonic stem cell (haESC) lines from haploid mouse embryos. We derived haploid mouse embryos by activation of unfertilized oocytes using strontium chloride (parthenogenetic) and by removal of the male pronucleus from mouse zygotes (gynogenetic). After in vitro culture, 13 gynogenetic and 24 parthenogenetic morulae/blastocysts were selected and transferred into separate well on feeders cells in ESGRO-2i medium. Afterwards 4 gynogenetic and 6 parthenogenetic embryos formed colonies and after few passages eight cell lines were established (22%). The alkaline phosphatase assay gave a positive result for all cell lines. Individual ESC colonies were validated by Mouse Embryonic Cell Marker Panel that contains antibody for Oct4, Nanog, SOX2 and SSEA1. Immunofluorescence imaging detected markers of mouse ESC in all colonies but single cells in colonies had no signal for Oct4 in their nuclei. The karyological analysis showed that most of the cells in all ESC lines were diploid and no more than 16 % were haploid. Our results revealed that haploid cells tend to spontaneously convert into diploid cells. The haploid cell enrichment in ESC lines generated from haploid mouse embryos is critical from the beginning. (Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015).

P185 | The effect of cumulus morphology on progression of in vitro maturation and parthenogenetic development of porcine oocytes

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The aim was to study effects of the different somatic compartment of cumulus-oocyte complexes (COCs) and duration of in vitro culture on nuclear and cytoplasmic maturation of the pre-pubertal and pubertal oocytes. Three types of COCs (n = 3963) (COC-I: cumulus cells of 4 or more layers, COC-II: cumulus cells of 2 to 3 layers, COC-III: cumulus cells of 1 layer) and granulose-cumulus-oocyte complexes (GCOCs) were cultured in TCM 199 supplemented with

3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 4 mg/ml BSA and 10% porcine follicular fluid for 40–48 h (the first 22 h with and then without hormones). After IVM, oocytes with first polar body were activated by treatment with ionomycin and 6-DMAP and then cultured in NCSU-23 for analyzing the cleavage and blastocyst rates. The data from 5 replicates (42–176 oocytes per group (depending on the morphology of COCs)) were analyzed by ANOVA. The nuclear maturation was shown to be activated in pre-pubertal oocytes on 2 h later (after 44 h) than in pubertal oocytes and did not change up to 48 h of culture. The IVM rate of oocytes from different morphological groups both physiological types of cells was similar, except for pubertal COC-III that was lower than other COCs at 40–44 h (at mean $p < 0.05$) IVM. The cleavage and blastocyst formation rates of three types of COCs did not differ at each time point, but they were higher than in groups of GCOC (at mean $p < 0.05$). The highest blastocyst development rate for pubertal COCs was at 44–46 h (ranging from 34.2% to 41.8%) and from pre-pubertal COCs at 46 h (ranging from 25.9% to 30.8%). Thus, pre-pubertal and pubertal oocytes have different dynamic of maturation and subsequent embryo development that do not depend on cumulus-oocyte morphology.

P186 | Expression of connexin 37 and connexin 43 throughout follicular development in canines

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Connexins 37 (Cx37) and 43 (Cx43) are necessary for the regulation of follicle development and the oocyte meiotic resumption in many species. This study aimed to evaluate the canine Cx37 and Cx43 expressions in the ovaries and oocyte cumulus complexes (COCs) over the estrous cycle. Around 3,000 COCs were recovered from four types of follicles: preantral, small antral, medium antral and large antral from 76 bitches at different phases of the estrous cycle and after ovulation from oviduct of bitches at estrus. These connexins were assessed by immunohistochemical (10 ovaries), immunofluorescence (600 COCs) and western blot (2,400). Western blot data were evaluated by ANOVA. Both Cxs were localized in all follicular stages over the estrous cycle, Cx43 was mainly in the granulosa cell, and Cx37 in cumulus and oocyte complexes (COCs). Immunofluorescence staining from isolated COCs showed that Cx37 and Cx43 remained without change at preovulatory stage, suggesting that, in contrast to other mammals, the communication between the oocyte and follicular cells is maintained previous ovulation. But, Cx37 protein abruptly dropped in ovulated COCs, whereas Cx43 did not have changes at this level. By western blot, a strong immunoreaction for Cx37 protein was present in preantral follicles in anestrus, decreasing significantly at the onset of antral formation, while no changes in Cx43 were seen during this phase. At diestrus, the expression of Cx43 was high and increased ($p < 0.05$) with antral follicular size. In conclusion, these two connexin were differentially expressed during the follicular development and the maintenance of these Cxs at the pre-ovulatory period observed herein, may be involved in the prolonged meiotic arrest in this species. FONDECYT 1171670.

P187 | Abstract withdrawn

P188 | Injecting the cows with prostaglandin (PGF2α) on day 8 instead on day 7 in an Ovsynch protocol did not increase the luteolysis rate–preliminary study

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Failure to accomplish a complete luteolysis, after single PGF2α (PG) injection on d7 in an Ovsynch protocol (GnRH1–7d–PGF2α–56 h–GnRH2–16 h–AI), especially in cows that ovulated after GnRH1 (G1), still represents a limiting factor for achieving a high pregnancy rate. Therefore, many daily operators, in order to ensure a complete luteolysis, have included a second PG treatment 24 h after the first PG injection (d8) in an Ovsynch protocol. Nevertheless, the latter require additional manipulation of the cows and it is a time consuming. Therefore, the aim of the present study was to investigate if single PG treatment on d8 instead of d7 can increase the luteolysis rate (LR), increase progesterone (P4) concentration before, and decrease P4 concentration 72 h after PG administration. Seventy dairy cows from two dairy farms were pre-synchronized using PG-3-G protocol and 7 days later submitted to an Ovsynch protocol for first AI. Cows were allocated into two groups, Ovsynch 7 (OV-7) ($n = 5$) with an injection of PG on d7 and Ovsynch 8 (OV-8) ($n = 35$) with an injection of PG on d8 after G1, respectively. Ovaries of the cows were scanned on the day of G1 and on d7 and d8 in OV-7 and OV-8, respectively, and blood samples collected on the same days and at AI to measure P4. Overall, more cows in OV-8 (24/35) ovulated after G1 than OV-7 (22/35). The P4 concentrations at G1 and at PG administration on d7 and d8 did not differ between the groups ($p > 0.05$). The LR differed between the groups with more cows having a complete luteolysis in OV-8 group than the OV-7 group ($p < 0.05$). Nevertheless, the P4 at PG on d7 and d8 and the LR in cows that ovulated from G1 did not differ between the groups ($p > 0.05$). In conclusion, injecting the cows with PG on d8 did not increase the luteolysis rate.

P189 | Membrane progestin receptor (mPR) δ and ε expression and localization throughout the estrous cycle in the bovine uterus

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The membrane progesterone receptors (mPR) which belong to the progestin and adipoQ receptor family (PAQR), are known to mediate rapid nongenomic progesterone (P4) functions in different cell types, including uterine cells. Recently, we found the expression of mPRα, β and γ receptors (also called PAQR7, 8, 5) in bovine uterus. However, there were limited data describing the expression and localization of new mPR isoforms: mPRδ (PAQR6) and mPRε (PAQR9) in the bovine uterus. Therefore, in this study the profile of mRNA expression (real time PCR) and protein localization (immunohistochemical analysis) of mPRδ and mPRε receptors were determined in the bovine endometrium and myometrium on days 2–5, 6–10, 11–16 and 17–20 of the estrous cycle (n = 5/each period).The results were statistically analysed by one-way ANOVA followed by the Bonferroni test (Graph Pad Prism 7.0). The highest ($p < 0.05$) mPRδ mRNA expression was in endometrium on days 11–16 of the estrous cycle, compared to the other stages of the estrous cycle, while expression of mPRε was on the similar level during the estrous cycle. No changes ($p > 0.05$) in mPRδ and mPRε mRNA expression in myometrium were observed. These receptors were localized in luminal and glandular epithelial cells of endometrium and in the endothelial cells of the blood vessels in uterus. The results showed the presence of mPRδ and mPRε mRNA in endometrial and myometrial tissues, but only mRNA expression of mPRδ changed throughout the estrous cycle, especially during the luteal phases. This suggests that, the mPRδ expression in the bovine endometrium depend on ovarian steroids. Supported by Grant NCN 2017/27/B/NZ4/02973.

P190 | Reproductive ability of sows with vitamin H feeding

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The purpose of the research was to substantiate the introduction of biotin additive into the sows' ration. Two experiments were carried out. In the first experiment young sows (n = 75) and in the second experiment adult sows (n = 75) of the Belarusian Large White breed were divided into five groups of 15 heads each, two first groups being control and two second, two third, two fourth, two fifth ones being experimental. Basic ration (mixed pig feed SK) containing 0.13-0.20 mg of biotin per 1 kg of dry matter was fed to the sows of the first control groups. During the first nine weeks of pregnancy in addition to the basic ration biotin at a dose of 0.05, 0.1, 0.2, 0.3 mg per 1 kg of dietary dry matter was fed to the sows of the second, third, fourth and fifth experimental groups, respectively. It was found that in the first experiment young sows farrowed in the first group (n = 11), in the 2nd and in the 4th groups (n = 12 in each), in the 3rd and in the 5th groups (n = 13 in each); as for the second experiment, adult sows farrowed in the 1st, 2nd and 5th groups (12 per group), in the 3rd and 5th groups 13 per group. The number of piglets born alive per litter of young sows in the first control groups was 8.82 ± 0.18 and that of adult sows 9.67 ± 0.15. Supplementary biotin fed to pregnant young and adult sows of the experimental groups increased the number of piglets born alive per litter in comparison to the control groups: in the second groups by 4.0 % (9.17 ± 0.16) and 2.6 % (9.92 ± 0.12), in the third groups by 7.3 % ($P \leq 0.05$) (9.46 ± 0.20) and 5.8 % ($P \leq 0.05$) (10.23 ± 0.17), in the fourth groups by 6.8 % ($P \leq 0.05$) (9.42 ± 0.17) and 5.0 % (10.15 ± 0.19), in the fifth groups by 6.3 % ($P \leq 0.05$) (9.38 ± 0.18) and 5.2 % ($P \leq 0.05$) (10.17 ± 0.16), respectively (ANOVA).

P190 - [Correction added on 8 November 2019, after first online publication: Abstract P190 has been added.]