

P220 | Study of the activity of cysteine cathepsins in the blood serum and seminal plasma of stallions of different age groups

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Cathepsins B, L and H are lysosomal cysteine proteases widely expressed in various tissues. Their activity was found also in seminal plasma and blood of stallions. They are involved in protein degradation, but also in a number of processes, such as cancer, neurodegenerative diseases, arthritis, ageing. The aim of this work was to identify differences in the activity of cathepsins B, L and H in the blood serum and seminal plasma of stallions of 2 age groups: 5–15 years-old (group 1, $n = 12$) and 16–21 years-old (group 2, $n = 8$). The blood and seminal plasma of 20 stallions of the Arabian breed was collected in 2020 year during the breeding season (February-May). The activity of cathepsins was studied by the spectrofluorometric method (System 3 Scanning Spectrofluorometry, Optical technology devices, inc. Elmstord, NewYork, 10523) by Barrett&Kirschke. Mann-Whitney *U*-test was used to compare differences in the studied groups. It was found that the activity of cathepsin L in the seminal plasma of stallions from group 1 was statistically significantly higher than in the seminal plasma of stallions from group 2 ($P < 0.01$). A decrease in the activity of cathepsin L in seminal plasma of aged stallions could be due to the accumulation of undigested material during the aging process, which can inhibit the activity of cathepsin L. Also free radical processes in the body increase with age and cysteine in the active center of thiol cathepsins is a target for the action of free radicals. These factors could lead to a decrease in the activity of cathepsin L in the seminal plasma of aged stallions. The studies were supported by grant No. 20-16-00101 from Russian Science Foundation.

P221 | Informative use of hair as a diagnostic tool to assess the levels of chemical elements in the seminal fluid of Holstein bulls

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The mineral composition of the seminal fluid affects the quality characteristics of semen. Due to the fact that the collection of semen when using servicing bulls for mating is expensive, it seems relevant to research and identify an informative biosubstrate for assessing the mineral composition of seminal fluid. The aim of this study was to identify correlations between the concentration of chemical elements in hair and seminal fluid (earned by centrifugation of semen). The studies were performed on Holstein bulls aged 3–4 years ($n = 55$; from each bull hair and semen were sampled), located at a sperm production station. The elemental composition of hair and

seminal fluid was determined by 25 chemical elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn) with ICP-AES and ICP-MS methods. We used the Statistica 10.0 application package to process the data (Stat Soft Inc., USA). Spearman correlation coefficients were calculated. The concentration of elements in the hair positively correlated with its level in the seminal fluid for I ($r = 0.59$; $P \leq 0.05$), Cu ($r = 0.45$; $P \leq 0.05$), As ($r = 0.58$; $P \leq 0.05$), Pb ($r = 0.49$; $P \leq 0.05$). Hair was characterized by a much higher content of a chemical elements in: Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Si, Hg, Sr, V, Zn compared to seminal fluid. The results of the present study indicate the possibility of using hair as a diagnostic biosubstrate for assessing the content of I, Cu, As, Pb in bulls' semen. The studies were carried out in accordance with the research plan for 2019–2021. FSBSI Federal Scientific Center BST RAS (No. 0761-2019-0006).

P222 | Evaluation of the effect of oxidative stress in blood serum and endometrial cells in cows with metritis

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Postpartum (pp) dairy cows with metritis may experience greater oxidative stress (OS) and a decline in antioxidant concentrations than healthy. This study aims to assess the mitochondrial activity (MITO), intracellular ROS levels (iROS), and OS markers in pp cows, and their use as potential markers for metritis. A total of 55 endometrial and blood samples were weekly collected from 11 Holstein cows from 7 ± 3 to 35 ± 3 dpp. Six of these cows were diagnosed with metritis. Metritis was defined as fetid vaginal discharge and $>39.5^\circ\text{C}$ within 21 d pp. MITO and the iROS status of endometrial cells (collected via cytobrush cytology) were evaluated using probe MitoTracker Orange and dichlorodihydrofluorescein diacetate, respectively. Reactive oxygen metabolites (d-ROM), antioxidants (OXY), and oxidative status index (OSI) tests were analyzed in blood serum samples. Statistical analysis was done by ANOVA, accounting for repeated measurements. The MITO was higher in metritis than in healthy ($24,229 \pm 2,501$ and $11,722 \pm 678$ ADU, respectively; $P < 0.05$). However, there were no differences in iROS levels between metritis and healthy ($42,244 \pm 1,634$ and $37,143 \pm 2213$ ADU, respectively; $P > 0.05$). Serum concentrations of d-ROMs and OSI were greater in metritis than healthy (116 ± 28 vs 84 ± 23 Carratelli Units (UCarr); $P < 0.001$ and 0.42 ± 0.26 vs 0.18 ± 0.05 ; $P < 0.001$, respectively). OXY concentration was lower in metritis than healthy (345 ± 153 and 474 ± 115 $\mu\text{mol/L}$; $P < 0.001$, respectively). This study showed that diseased cows experience a greater OS and endometrial cell mitochondrial dysfunction in comparison to healthy cows. These findings provide new avenues for research for prevention and potential treatments for metritis via the utilization of antioxidants.

P223 | Morphological and histological analysis of uterus masculinus in Eurasian beaver

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Uterus masculinus (UM) is a remnant of the Müllerian tube in males, and it has been reported in several mammals. The development of UM seems to be involved with reproductive status, and, in dogs, the enlargement of UM results in clinical symptoms. Whereas UM was reported in European beavers, it is unclear if the presence of this organ is a result of pathological or physiological processes. In the present study, we aimed to clarify the structural characteristics of UM, and we compared the structures between male UM and the female uterus in European beavers. We collected samples from 1 adult male and 3 females (2 juvenile and 1 adult) harvested as a nuisance control under the permission of the Regional Direction of Environment Protection in Warsaw (WPN-I.6401.72.2020.AJC.). Uterus masculinus was found in the dorsal side of the ampulla of the deferent duct. The structure (the major axis was 4 cm) was shaped like a bicornuate uterus, composed of 2 horns and a body, and the lumen ended up in a blind tube. Histological analysis revealed a luminal structure enclosed by a monolayer of columnar epithelial cells, and the wall was composed of 3 layers; perimetrium, myometrium and endometrium, similar to the female uterus. The glandular structure of UM was less developed compared with those of females. Sperm was present in the cauda epididymis, suggesting that the male was fertile. Furthermore, since there was no pathological finding including inflammatory cell infiltration, the presence of UM in the male beaver was not considered a pathological phenomenon. This is the first report of a morphological and histological examination of UM in the European beaver. This research was funded by Polish National Science Center, grant MINATURA (G77).

P224 | Subclinical mastitis and health of the newborn calves

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This study aimed to assess newborn calves' health from cows with subclinical mastitis during the dry-off period. A total of $n = 40$ healthy cows was included which responded positively to the Californian Mastitis Test (CMT) without clinical signs of mastitis. Cows from the 1st group ($n = 20$) were administered intracisternally by the DC series's drug (Orbenin DC; cloxacillin) into all the udder quarters. Cows from the 2nd group ($n = 20$) served as the negative control without therapy before drying off. From the newborn calves, venous blood was sampled within 1 hr after birth. Total protein and its fractions, total immunoglobulins (Ig), bactericidal

and lysozyme activity of blood serum (BABS and LABS) were determined. Newborn calves' clinical state was assessed 1.5–2 hr after birth (temperature, heart and respiratory rate, the emergence of a confident standing posture and sucking reflex). The experimental data was carried out with Student's *t*-test. In calves born from cows of the 2nd group, in comparison with 1st group, the Ig content was significantly ($P < 0.05$) decreased by 17.3% (7.6 ± 0.5 g/L), γ -globulins by 26.5% (13.9 ± 1.2), BABS by 18.5% (37.1 ± 2.8), LABS by 44.6% (0.048 ± 0.011). The time for the appearance of a confident standing posture occurred 15.5 min later (45.3 ± 1.7), and the sucking reflex 30.2 min (70.6 ± 1.5) later. Examined parameters of newborn calves born out of cows suffering from subclinical mastitis without therapy at drying off was assessed as lower. The present study demonstrates the advantage of the intracisternal administration of Orbenin DC in cows with subclinical mastitis at the end of lactation. This work was carried out with financial support from the Russian Science Foundation (project No. 20-16-00085).

P225 | Effect of breed and donor on motile subpopulation structure in frozen-thawed ram sperm

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Computer-assisted sperm analysis (CASA) allows to detect different motile subpopulations (SP) in a sperm sample. To study the effect of donor and breed on motile SP structure in frozen-thawed sperm, 6 sessions of semen collection by artificial vagina ($n = 6$) were carried out from 4 Xisqueta and 4 Aranosa rams. In each session, 2 ejaculates by male were mixed, centrifuged and diluted in a Tris-based media with 15% powdered egg yolk and 5% glycerol. Next, sperm samples were refrigerated for 4 hr at 5°C before freezing in liquid N₂ vapours. After thawing, sperm kinetic parameters were assessed using a CASA system (ISAS, PROISER SL, Spain). A non-hierarchical cluster analysis (k-means) was performed using Euclidean distances to identify sperm SP and General Linear Model (GLM) to assess differences in its distribution. Potential Pearson's correlations were also analysed between the SP distribution and total (TM, %) and progressive motility (PM, %). Three sperm SP were identified by different kinetic patterns ($P < 0.05$). SP1 sperm (62.6%) was characterized by linear and very slow movement (VCL = 57.8 μ m/s; LIN = 47.6%). SP2 (29.8%) had intermediate speed trajectories with the highest linearity (VCL = 128.0 μ m/s; LIN = 51.3%). SP3 sperm (7.6%) showed the lowest linearity but the highest speed (VCL = 177.3 μ m/s; LIN = 40.9%). SP2 proportion varied between breeds ($P < 0.05$). No differences were found in SP distribution between donors. Moderate correlation coefficients were observed among PM and SP1 ($r = -0.3$; $P < 0.05$) as SP3 ($r = 0.4$; $P < 0.01$), although no correlation was found between TM and SP distribution. In conclusion, breed influences motile SP structure in frozen-thawed ram sperm, but not the donor. PM seems to affect some motile sperm subpopulations.