

(HHct) (32.0–36.9%) and mean haematocrit (MHct) (28.3–29.8%). At the end of breeding campaign and immediately after shearing, ewes were transported from the Institute farm (altitude 500m) to a mountain pasture (altitude 1440m). Blood samples were taken by jugular venepuncture at the following days: before transportation (baseline level), on day 7, 20 and 42 after the transport. NRBC count, NRBC (%), MCHC and Hct were measured via five diff VET haematology analyser. Students *t*-test and Pearson correlation were used. NRBC count declined significantly on day 7 in MHct group as compared with basal levels ( $p < 0.05$ ). On day 20, NRBC count increased significantly in LHct group as compared with basal levels ( $p < 0.05$ ). NRBC count increased significantly on d 20 in LHct ( $p < 0.001$ ), HHct ( $p < 0.01$ ) and MHct ( $p < 0.01$ ) ewes as compared with d 7. There was a slight but significant correlation ( $r = 0.470$ ,  $p < 0.05$ ) between MCHC and NRBC (%) on d 20. The observed similarity between these hematologic variables gives rise to the notion of a possible association between MCHC and NRBC appearance in peripheral blood caused by moderate altitude hypoxia. Acknowledgement: This work was financially supported by Bulgarian National Science Fund (grant KP-06-H26/2, 04.12.2018).

#### P38 | Effect of the frequency of semen collection on sperm characteristics by massage technique in *Chinchilla lanigera*

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In the present study, the optimal frequency of sperm collection by massage technique was investigated in chinchilla, as the frequency of semen collection can influence greatly the sperm parameters. The semen samples from 11 males/group were collected once (group 1), twice (group 2), three times (group 3) and five times a week (group 5) during 10 weeks. The sperm motility and concentration were determined with CASA (SCA®). The proportions of live, intact, morphologically abnormal and dead cells were determined in the aniline blue-eosin stained smears. For statistical analyses, ANOVA and Tukey's post-hoc tests were used. The ratio of successful semen collection in groups 1 and 2 was 48.6 and 40.7%, while in groups 3 and 5 it was less successful, only 23.9 and 24.9%. There were no significant differences among the various groups in motility parameters and concentration. In each group, the proportion of cells with rapid, progressive movement showed the optimal high rate. The group 5 had a significantly higher ( $p \leq 0.05$ ) rate of live, intact cells compared with the groups 1 and 2, however, the success of sperm collection was lower in the case of group 5. Morphological abnormalities were highest in group 1, while there were no differences in the ratios of dead cells among the groups. Examining the different types of abnormalities, the midpiece disorders were the highest in each group. Overall, two sperm collections/week seemed to be the most optimal

frequency in the case of chinchilla, due to the highest successfulness and fewer abnormal cells in semen.

#### P39 | Improving the efficiency of synchronization protocols by using teaser bulls

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The aim of the study was to find methods of additional stimulation of the sexual function of cows during synchronization of oestrus. There are reports that animals of the opposite sex stimulate reproductive function [1]. Cows were divided into six groups, where three main protocols of oestrus synchronization were used. In the first group, a protocol with prostaglandin (Estrophan); in the second – prostaglandin (Magestrophan) and a GnRH analogue (Surfagon), in the third – progestogen (CIDR). In groups 4–6, besides the three indicated protocols, the cows were exposed to a teaser bull (with penis stitching) in the morning and evening for 1 h. Animals of all groups were additionally treated with vitamin E and selenium. Pregnancy was diagnosed by gynaecological ultrasound examination. The degree of their influence on the efficiency of synchronization schemes was taken into account. The results were processed by ANOVA. Conception rate of the first group was  $62.5 \pm 1.94\%$ , of the second group  $64.7 \pm 1.97\%$ , of the third  $66.7 \pm 1.83\%$ . The differences between the groups were not significant. The use of teaser bulls significantly increased the efficiency of these protocols. So, in the first synchronization protocol (group 4), conception increased by 5.9% ( $68.4 \pm 2.03\%$ ), in the second (group 5) by 5.3% ( $70.0 \pm 2.05\%$ ) and in the third (group 6) by 5.6% ( $72.2 \pm 1.90\%$ ). All these changes were statistically significant compared with the indicators of groups 1–3 ( $p < 0.05$ ). To conclude, the inclusion of teaser bulls in oestrus synchronization stimulated sexual functions and increased conception rates. [1] Julanova N.2016/Acta Veterinaria Brno Vol.85, No.1, 63–69.

#### P40 | Point-of-care device for assessing male fertility in animals through measurement of sperm concentration and motility

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This work aims at realizing a low-cost and portable device to perform on-site reliable and quick semen quality analyses based on

spermatozoa concentration and motility expressed via different velocities such as average path velocity (VAP). The developed acquisition device includes only the essential optical, mechanical and electrical parts. It integrates an HD camera sensor with an inverted webcam lens and an LED-based illumination, allowing sufficient contrast for cell detection. It is compatible with different microscopy disposable chamber slides. The solution encloses a companion Android-based application that incorporates a user-friendly interface to guide the acquisition process and analyse the images locally on the connected smartphone/tablet. The illumination inhomogeneities are compensated with a locally adaptive threshold during the cell detection phase, and tracks connecting the cells between successive image frames are reconstructed to measure the respective motility. The validation was performed on data from two fresh bull semen samples at various dilution levels (10–50×) and five undiluted unfrozen samples. The same slides were processed by standard phase-contrast microscopy using QualiSperm (AKYmed Ltd.) for analysis and our system. Measured concentrations were similar between the two approaches ( $R^2 = 0.98$  for linear regression:  $0.83x + 0.96$ ). The resulting profiles of cell velocity at each trajectory percentile follow the QualiSperm percentage thresholds classifying the cells into fast, slow or immotile. Furthermore, the measurement error of mean VAP is not greater than the intrinsic QualiSperm uncertainty. Our solution fulfils the requirements for a portable semen analysis tool for veterinary use outside of laboratory conditions. (Project PACMan funded by HES-SO).

#### P41 | In vitro study on the effect of L-Carnitine on the efficiency of sperm freezing of Partridge-colour Hungarian roosters

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Avian spermatozoa are particularly sensitive to the stress during freezing procedure. It is known that L-Carnitine (LC) plays a role in antioxidant processes of the cells, therefore the effect of LC added to the drinking water (Experiment 1) or to the semen extender (Experiment 2) was assessed in the quality of frozen/thawed sperm of Partridge-colour Hungarian roosters. In Experiment 1, the drinking water of 10 individually placed 1-year-old roosters was supplemented with 200 mg LC/day/rooster, including a control group not receiving any supplementation. In Experiment 2, semen of 10 cockerels was collected twice a week and diluted (1:1) with Lake's extender. Half of the samples were supplemented with 2 mM LC/100 ml extender. The sperm samples were frozen in 0.25 ml French straws for 15 min at 5 cm above the surface of the liquid nitrogen (LN2) followed by 15 min at 1 cm over the LN2. Thawing was performed for 1 min in a 5°C water bath. Following the normality test, Kolmogorov–Smirnov two-sample test was used for statistical analyses. Sperm motility was evaluated by computer-assisted sperm analysis (CASA; SCA, Microptic, Spain). The sperm viability (live/dead) and the abnormal

cell ratio were determined by aniline blue-eosin staining. The penetrating ability of spermatozoa was examined with in vitro sperm–egg interaction assay. Although LC added to the drinking water or to the semen increased neither the proportion of sperm motility, nor the ratio of live intact spermatozoa, the penetration ability of spermatozoa was significantly ( $p \leq 0.05$ ) higher (37.1 vs. 44.8; 37.6 vs. 73.4 holes/mm<sup>2</sup>) in both Experiments 1 and 2. Further studies are needed including fertility trials.

#### P42 | Persistent heat in a 1.5-year-old Shiba-Inu: A case report

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Persistent heat (prolonged proestrus) is defined as a proestrus phase longer than 21 days. Prolonged proestrus is related to elevated oestrogen levels. High level of estrogens may be related to an anovulatory follicle, ovarian cyst or ovarian tumour [1]. A 1.5-year-old Shiba-Inu bitch was referred to the clinic due to persistent heat. The owner reported it was already the second heat of the bitch and that it started already 7 weeks ago. During 7 weeks, the bitch had bloody discharge and was attractive for males. She was in good condition and no other symptoms were observed. Vaginal cytology showed the presence of red blood cells, intermediate cells and superficial cells, which confirmed the late proestrus phase. The serum progesterone (P4) was low (0.7 ng/ml), and serum estradiol was quite high (35.41 pg/ml; normal value for proestrus). An ultrasound examination was performed and showed small anovulatory follicles on the left ovary. Hormonal treatment with HCG (three injections of 300 IU once per day for 3 days) was implemented. The bitch stopped the signs of the heat after the first injection and recovered very fast with no side effects. One week later, during control, level of P4 was 6.15 ng/ml and the cytology corresponded to diestrual phase. Prolonged proestrus is not a common problem in bitches, though it usually occurs in older bitches having follicular cysts. Yet, some data show that it may happen especially in the second cycle of young bitches. The cause of this finding remains unknown. Further research should be performed. [1] Risvanli et al., Abnormalities in sexual cycle of bitches. Intech, 2016.

#### P43 | Study on semen cryopreservation of indigenous Hungarian duck

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The aim of the work was to develop a freezing protocol that can effectively preserve the genes of indigenous Hungarian drake. Semen of