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Effectiveness of optidyl®, bioxcell® and egg yolk tris-based extenders to freeze Brown-Swiss and Holstein bull semen

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The aim of this study was to compare the effectiveness of egg yolk Tris-based extender and two commercial extenders (Bioxcell® and Optidyl®) to freeze bulls semen. Ejaculates from Holstein (n = 36) and Brown-Swiss (n = 36) were divided in three aliquots and diluted in Tris-based, Optidyl® and Bioxcell® extender, respectively. Thereafter they were frozen and thawed following a standard protocol. The effectiveness of freezing extenders was assessed according to post-thaw sperm motility evaluated by CASA, acrosomal and total abnormalities examined microscopically and plasma membrane integrity measured using the hypoosmotic swelling test. Regarding to Holstein bulls, the highest percentages of subjective (53.1 ± 1.8%, $p < 0.01$), CASA progressive (22.7 ± 1.5%, $p < 0.001$), and CASA toal motility (64.7 ± 0.8%, $p < 0.001$) were found in semen diluted in Optidyl®. Optidyl® extender also provided best protection to acrosome (4.1 ± 0.5%) and plasma membrane integrity (60.4 ± 1.7%) compared to other extenders ($p < 0.001$). Regarding to Brown-Swiss bull, the lowest percentages of post-thaw subjective (28.6 ± 1.6%, $p < 0.01$), CASA total motilities (36.2 ± 1.1%, $p < 0.001$) and membrane integrity (34.6 ± 1.2%, $p < 0.001$) were obtained in the semen samples diluted in Bioxcell®. The percentage of progressive motility was found to be higher in Optidyl® (17.7 ± 3.1%) than Bioxcell® (7.2 ± 1.1%) ($p < 0.01$). The highest percentages of acrosomal (11.2 ± 0.6%; 10.6 ± 1.3%) and total abnormalities (20.1 ± 1.4%; 16.8 ± 1.6%) were found when Bioxcell and Tris extender were used. In conclusion, Optidyl® extender could be used for successful cryopreservation of Holstein and Brown-Swiss bull's semen.

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Investigation of seasonal exocrine function of the testes in Hungarian black racka rams

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Hortobágyer Racka is a native Hungarian sheep breed, which is bred strictly seasonal. Aim of the study was to determine how seasons effect testicular exocrine function of Racka rams. Nine mature (18–20 months of age) Black Racka rams were included into the year long trial. Size of the testes was measured every 2 weeks, whilst semen was collected weekly. Ejaculate volume was noticed and motility was assessed under phase contrast microscope and classified between 1 and 5. Concentration of the semen was determined in Buerker-chamber. Morphology was evaluated after Cerovsky-staining. Circumference of the testes increased continuously from 22.58 ± 1.43 cm (winter) to 31.55 ± 1.16 cm (autumn). A significant correlation was found between testes circumference and day length ($r = 0.38$, $p < 0.001$), as well testes circumference and monthly average temperature ($r = 0.68$, $p < 0.001$). Maximum ejaculate volume was recorded in autumn (0.89 ± 0.34 ml), whilst minimum was measured in spring (0.52 ± 0.29 ml). The highest sperm concentration was detected in summer ($6.68 \pm 2.06 \times 10^9$/ml, the lowest one in winter ($5.07 \pm 1.64 \times 10^9$/ml, $p = 0.017$). Total sperm number was the highest in autumn (4.78×10^9). There was no significant difference in motility during the trial (4.81 ± 0.58%). The lowest morphological anomalies were detected in autumn compared to other seasons (8.73%, $p < 0.01$). In conclusion slight seasonal differences could be detected in testicular function of Racka rams. (Founded by OTKA-K 76371)

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Variations in iron-status in Spanish purebred mares during the estrous cycle

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In women during sexual cycle, there are fluctuations in fluid volume and significant blood loss. These changes are associated with variations in iron status (Kim, 1993, Am J Clin Nutr, 58, 705–709). The aim of the present research was to analyze the effect of estrous cycle on hemoglobin concentration (HB), packed cell volume (PCV), iron (FE) and ferritin (FERR) concentrations in Spanish mares. Venous blood samples were taken from 20 reproductive Spanish mares during follicular (FF; follicle diameter >3.5 cm) and luteal phases (LF, from the fifth day diestrous). HB and PCV were analyzed by Sysmex F-820 and microhematocrit, respectively. FE was determined by spectrophotometry (METROLAB 2300 Plus V3®) and FERR were analyzed by a turbidimetric method using reagents from Spinreact® with a coefficient of variation intra-assay of 5.0%. There were no differences in the HB (FF: 12.4 ± 1.6; LF: 12.4 ± 1.3 g/dl), PCV (FF: 35.0 ± 4.1; LF: 35.2 ± 3.3%), FE (FF: 164.3 ± 32.3; LF: 183.7 ± 47.5 μg/dl) or FERR (FF: 161.4 ± 77.2; LF: 156.7 ± 77.9 μg/dl) concentrations between both phases of cycle. The absence of changes throughout the estrous cycle suggests that examination of these parameters have no diagnostic value for reproduction in the mare.

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Evaluation of isolation and analysis of RNA quality and integrity for bovine placenta; including separated caruncle and cotyledon evaluation

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Acid guanidium phenol preparations such as Trizol allow the reproducible isolation of high-quality total RNA from various sources. In order to establish an economical and reproducible method for the high-quality RNA extraction from bovine placenta, total RNA was isolated with 1 ml acidic solution containing guanidinium thiocyanate, sodium acetate, phenol and chloroform, followed by centrifugation. Total RNA was precipitated with isopropanol and no purification kit was used. The RNA quality was determined by spectrophotometry using the optical density (OD) absorption ratio (OD260 nm/OD280 nm should be >1.7). The ratio was between 1.70 and 2.10. Integrity of the RNA was verified by agarose-gel electrophoresis. The results of electrophoresis showed three clear bands of 28s, 18s and 5s rRNA, respectively. First strand cDNAs were amplified with extracted RNA using a kit (GeneAmp Gold RNA PCR Core Kit), followed by basic PCR. The RT-PCR products were successfully derived from the extracted RNA. Total content and quality of isolated RNA tended to be lower in the cotyledon (3200.45 ± 1515.91 ng/μl) than in the caruncle (4055.65 ± 1692.80 ng/μl). This RNA extraction method represents an economical and reproducible method in obtaining high-quality RNA from fetal and maternal parts of bovine placenta with Trizol, without any purification method. Supported by TUBITAK-TOVAG/107O259.