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Influence of four freezing protocols on morphology and viability of stallion epididymal sperm

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We evaluated four freezing protocols of sperm from three segments (cranial: E7, middle: E8 and caudal: E9) of the cauda epididymidis obtained via mincing (E7, E8) or retrograde flush (E9) and compared pre-freeze and post-thaw sperm morphology and viability (non-parametric matched pair analysis; Wilcoxon signed rank test). Sperm cells were frozen at fast and slow cooling rates using either a programmable freezer [20°C (−1.0 vs. −0.1°C/min) 5°C (−60°C/min) −140°C; LN2 (liquid nitrogen)] or nitrogen vapour [step 1: 20 vs. 20 and 4°C for 150 min; step 2: 5 cm above LN2 surface for 20 min; step 3: plunging into LN2]. In raw sperm, there were more coiled and looped tails and less detached heads in E9 ($p < 0.05$). After freezing and thawing the percentage of midpiece anomalies (0–12% vs. 1–25%), bent (2–82% vs. 1–88%) and looped tails (0–5% vs. 0–25%) increased, and cytoplasmic droplets (4–67% vs. 0–60%) decreased in all segments ($p < 0.05$). Only in cranial segments (E7, E8), the amount of coiled tails increased and detached sperm heads declined ($p < 0.05$). Freezing did not influence sperm head anomalies ($p > 0.05$). Sperm morphology did not differ among freezing procedures, but the percentage of viable sperm decreased to a greater extent after the fast freezing process above LN2 surface compared to the other methods ($p < 0.05$). In conclusion, the method of harvesting sperm seems to influence the amount of detached heads. Membrane damage of spermatozoa differed depending on the epididymal area. Although the freezing procedure did not cause differences in sperm morphology, there was an effect on viability.

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Comparison of DNA fragmentation dynamics in frozen sperm of *Equus asinus* between uncentrifuged and SLC selected samples

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Single layer centrifugation (SLC) has been used to decrease the percentage of sperm DNA fragmentation of equine frozen semen. However, no studies on the effect of SLC on DNA fragmentation have been performed in donkeys. The aim of this study was to compare the rate of increase of DNA fragmentation expressed as the slope of the dynamic regression line of frozen donkey semen between unselected and SLC samples. Sperm DNA fragmentation (sDF) was assessed using an adaptation of the sperm chromatin dispersion test developed for stallions in five donkeys. SLC was performed after thawing using Androcoll-E in one aliquot of each ejaculate. Sperm samples were thawed and incubated at 37°C and sDF was assessed at 0, 3, 6 and 24 h of incubation. Comparison of the medium values of the slope of unselected and SLC groups were statistically compared by ANOVA. The medium slope of the regression line of sDF was significantly lower in the SLC group ($p < 0.05$) in comparison to unselected samples (0.64 ± 0.34 vs. 1.25 ± 0.36). A lower slope indicates higher DNA stability. In conclusion, SLC using Androcoll-E decreases the slope and velocity of DNA fragmentation of frozen-thawed donkey semen.

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Levels of zinc and selenium in seminal plasma of boar ejaculate fractions

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Concentrations of Zinc [Zn] and selenium [Se] are present in the ejaculates of mammalian species playing an important role in the regulation of sperm function in both a positive and negative sense depending of the concentration. Boar ejaculate shows three clear fractions that differ in the proportion of sperm and seminal plasma (SP). In this context, the sperm of the first 10 ml of sperm rich ejaculate fraction (SREF) sustain best handing *in vitro*, probably due to peculiarities of SP composition. This study aimed to determine putative differences in the [Se] and [Zn] among the different fractions of boar ejaculate. Five ejaculates from five boars were manually collected in three fractions: the first 10 ml of SREF (P1), rest of SREF (P2) and rest of ejaculate (P3). The semen samples were centrifuged twice (1500× ***g*** 10 min) immediately after ejaculation to obtain the SP, which was stored at −80°C until [Zn] and [Se] analysis. [Zn] and [Se] were determined using atomic absorption spectrometry of air-acetylene flame and graphite furnace, respectively. Levels of Zn and Se were quantified in the three-ejaculate fractions of the five ejaculates. Whereas [Zn] differed ($p < 0.001$) among the ejaculate fractions, being lower in P1 (7.0 ± 0.7 mg/l) than in P2 (28.5 ± 2.5 mg/l) and P3 (34.3 ± 2.8 mg/l), [Se] was similar in the three fractions (19.7 ± 2.1 μg/l, 21.5 ± 1.6 μg/l and 17.4 ± 2.5 μg/l, in P1, P2 and P3, respectively). In conclusion, the first 10 ml of SREF show lower [Zn] than the rest of ejaculate fractions. (Supported by MINECO-AGL2012-399903 and SENECA-04543GERM07.)

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Influence of concentration on equine fresh semen conservation

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Low volume deep horn insemination with highly concentrated fresh semen has become popular in the mare. However, only a few data are available about conservation of highly concentrated equine semen. Five stallions were collected four times. Concentration and motility were determined in raw semen. Volumes of semen containing 110, 440 and 880 millions spz were extended (1 v/4 v) with INRA96 and centrifuged (1000 ***g***, 20 min) with cushion medium and the sperm-rich pellet re-suspended in 1 ml of supernatant. Total (TM) and progressive motility (PM) were determined for each sample after 8 and 24 h at 20°C. As TM and PM in raw semen were stallion dependent, Percent of Conservation of Progressive (PCPM) or Total Motility (PCTM) were studied. Differences in the maintenance of motility were determined using Friedmann test and Dunn's post-test. Spermatozoa recovery rate (RR) after centrifugation were respectively $64.11 \pm 28.05\%$, $98.65 \pm 27.28\%$ and $99.90 \pm 27.37\%$. RR was lower in low concentration samples ($p < 0.001$). Mean final conservation concentrations were 70.45 ± 30.59, 434.82 ± 120.02 and 879.97 ± 241.15 millions/ml. PCTM was decreased in 880×10 millions/ml samples after 8 and 24 h of conservation ($p < 0.001$) when compared to other concentrations. PCPM was lower after 24 h within highest concentration sample ($p < 0.001$), when compared to lower concentrations. Our data show that semen conservation with high concentration is rapidly deleterious for total motility, whereas progressive motility decrease between 8 and 24 h when compared to non-concentrated semen. However, progressive motility is the only factor that has been associated to fertility, suggesting insemination with highly concentrated fresh semen should be performed within 8 h.