A181E Physiology of reproduction in male and semen technology

Sperm selection by density-gradient centrifugation of Merino ram semen cold-stored up to 48 h improves viability and membrane integrity

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Liquid ram semen stored at 5°C would be more competent than frozen/thawed for sheep crossbreeding programs. The aim was to evaluate the kinetics and membrane integrity of Merino ram semen cold-stored up to 48h at 5ºC before and after density-gradient centrifugation (DGC) selection. Pools of 3 normospermic Merino ram (2-7 years) ejaculates were collected by artificial vagina in fifteen sessions (45 ejaculates), diluted to $200x10^6$ spermatozoa/ml with skim milk-based extender contained 6% egg yolk and cold-stored up to 48h at 5ºC. Motile spermatozoa were separated by BoviPure® DGC (Galarza et al., 2018, Anim Reprod Sci 192: 261-270) using 250µl of fresh (n = 30) and cold-stored semen (24h: n = 10 and 48h: n = 10). The final pellet of 300µl was used to assess semen quality. The kinetic parameters were evaluated by computer-assisted sperm analysis (CASA) while plasma, acrosomal and mitochondrial membrane status was analyzed by PI/FITC PNA/Mitotracker fluorescence. The effects of storage time (fresh, 24 & 48h) and sperm selection process were analysed by univariant ANOVA and Bonferroni´s test ($P < 0.05$). In terms of sperm storage time, CASA analysis of non-selected semen samples showed a significant decrease after storage for 24 and 48h compared to fresh samples with regard to progressive motility [SPM (%): 52.30 ± 4.1 and 36.9 ± 5.5 vs 71.3 ± 1.6], straight line velocity [VSL (µm/sec): VSL 132.2 ± 6.1 and 109.7 ± 6.3 vs 176.7 ± 4.3], linearity [LIN (%): 69.2 ± 3.5 and 59.0 ± 5.0 vs 82.0 ± 1.2], and straightness [STR (%): 75.7 ± 3.3 and 66.0 ± 4.3 vs 86.9 ± 0.9], respectively. However, analysis of DGC-selected semen showed a decrease only at storage for 48h when compared to 24h or fresh samples with regards to SPM (35.6 ± 3.9 vs 56.1 ± 6.91 and 59.3 ± 2.6), VSL (83.5 ± 4.4 vs 105.3 ± 6.5 and 110 ± 2.0) and LIN (63.9 ± 3.4 vs 75.0 ± 3.7 and 80.7 ± 2.4), respectively. A comparison between DGC-selected and non-selected samples showed a significant lower total motility [TM (%): 94.4 ± 0.8 vs 85.4 ± 1.90], VSL (176.7 ± 4.2 vs 110.0 ± 2.0) and wobble [WOB (%): 94.2 ± 0.6 vs 88.5 ± 1.5] only for fresh semen. Fluorescence analysis evidenced a decrease only in 24h cold-stored non-selected compared with fresh semen with regard to plasma membrane integrity [PMI (%): 64.8 ± 2.9 vs 80.1 ± 1.7], high mitochondrial function [HMF (%): 88.2 ± 1.6 vs 93.9 ± 1.0] and total intact plasma/intact acrosome/high mitochondrial function [IPIAHM (%): 61.8 ± 3.1vs 78.7 ± 2.0]. In contrast, no differences were observed between fresh and cold-stored DGC-selected semen. A comparison between selected and non-selected semen showed a significant increase of PMI (64.8 ± 3.14 to 89.4 ± 2.32), HMF (88.2 ± 1.26 to 96.0 ± 1.26) and IPIAHM (61.8 ± 3.14 to 87.6 ± 2.04) only for 24h. These results suggest that kinetic activity of cold-stored and DGC-selected ram spermatozoa is maintained and the selection process results in improved viability and membrane integrity. Therefore, liquid storage combined with DGC-selection might become a good alternative to fresh or frozen non-selected semen to be used for artificial insemination in sheep crossbreeding programs.