Effects of a second freeze-thaw cycle on bighorn sheep (*Ovis canadensis canadensis*) semen motility and membrane integrity

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Only a fraction of a dose of frozen semen is needed when advanced reproductive biotechnologies are used. The ability to refreeze thawed semen would be a valuable tool to save germplasm from rare or endangered species. Big horn sheep are a threatened species that may benefit from these technologies. We hypothesized that refreezing will allow preservation of viability of a significant proportion of previously frozen-thawed big horn epididymal spermatozoa. The objective of this experiment was to study the effect of a second freeze-thaw cycle on big horn sheep epididymal sperm motility and membrane integrity.

Epididymal sperm were previously harvested and frozen from two big horn rams that died from pneumonia.¹ Semen from each ram (n=8 replicates per ram, 2 x 0.5 mL per replicate) was thawed in a water bath (37°C) for 30 seconds. Aliquots were taken from each replicate and evaluated for progressive motility, membrane integrity, and acrosome integrity. Motility was evaluated subjectively (400x). Membrane integrity was evaluated by hypoosmotic swelling test (HOST) (10 µL of sperm incubated in 190 µL of 100 mOsm sucrose solution).² Acrosome integrity was evaluated using the Spermac® (Minitube, Verona, WI) staining technique.³ Hypoosmotic swelling test and Spermac® evaluations were performed by evaluating 100 spermatozoa from each sample. The remaining frozen-thawed semen was repackaged in 0.5 mL straws and frozen in liquid nitrogen vapor as described previously.¹ Refrozen semen was thawed and evaluated one week later in the same manner as described above.

Data were analyzed by ANOVA. There was no significant ram effect; therefore the data were analyzed with refreezing as the main treatment. As expected, all sperm quality parameters were significantly lower after refreezing. Post-thaw sperm quality after refreezing decreased on average by 48%, 38%, and 28%, for acrosome integrity, progressive motility, and membrane integrity, respectively. The discrepancy observed in the quality of the sperm as assessed by HOST and Spermac® merits further evaluation. This study shows that refreezing of big horn epididymal sperm results in recovery of at least 50% of the initial spermatozoa. Further studies are in progress to optimize the refreezing process using different refreezing rates and to evaluate in vivo fertility of refrozen-thawed semen.

Keywords: Sperm, fertility, refreeze, cryopreservation, endangered species

References