Effects of thawing time on membrane integrity and motility of frozen-thawed canine spermatozoa using commercial semen extenders

S. du Bois, J.A. Len, J.M. Parlevliet, B.E. Eilts

Faculty of Veterinary Medicine Utrecht, The Netherlands; Deptartment of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA

Hypothesis

Our hypothesis was that a commercially available canine extender, compared with a human extender, using our current cryopreservation protocol would yield increased post-thaw motility and intact membranes when thawed at 50°C for 10 sec or at 37°C for 30 sec. The specific aims were to determine how two different thawing times and temperatures affected post-thaw motility of canine semen cryopreserved with two different commercial extenders.

Experimental methods

A single ejaculate was collected from 11 mature dogs of different breeds. Each ejaculate was prepared for cryopreservation with two commercial extenders, Irvine (Irvine Scientific, Santa Ana, CA; IRV) or Partner (Port Huron, MI; PAR). After a 10 min centrifugation at 900 x g and aspirating the supernatant, each extender was added and each sample was cooled 60 min before adding more extender containing 12% glycerol (yielding a final glycerol concentration of 6% and 50 x 10⁶ cells/ml). Each of the four aliquots was immediately loaded into 0.5 mL straws, placed on a boat 4 cm over liquid nitrogen for 10 min, and then plunged and maintained at -196°C for at least seven days before thawing. For each aliquot, two different thaw protocols were used; 50°C for 10 sec (50) or 37°C for 30 sec (37). Five minutes after thawing, each sample was assessed for total (TM) and progressive (PM) motility using a computer assisted sperm analyzer (SpermVision, Minitube, Verona, WI) and for membrane integrity (IM) using SYBR-14/PI (LIVE/DEAD® Sperm Viability Kit, Invitrogen™, Carlsbad, CA). A repeated measures analysis in an ANOVA of a 2 x 3 factorial arrangement of treatments with dog as a random effect in a mixed effects model using the SAS mixed procedure was performed. Pair-wise t-tests of least squares means were performed as follows, (IRV37, IRV50), (PAR37, PAR50), (IRV37, PAR37), and (IRV50, PAR50).

Results

For TM, the values (mean±SE) were 51.5±4.4, 55.9±3.4; 36.9±3.9; and 43.5±4.1 for the IRV37, IRV50, PAR37 and PAR50, respectively. For PM the values (mean±SE) were 45.2±5.0, 49.6±4.1, 31.8±4.5 and 36.7±4.6 for the IRV37, IRV50, PAR37 and PAR50, respectively. For IM the values (mean±SE 4.0) were 56.3±3.7, 51.8±3.2, 40.6±2.8 and 44.0±3.3 for the IRV37, IRV50, PAR37 and PAR50, respectively. The IRV50 had significantly greater TM (P = 0.0072) and PM (P = 0.0037) than the PAR50, but not the IRV37 (TM P = 0.330, PM P = 0.3112). The PAR50 did not differ significantly in TM (P = 0.1452) or PM (P =0.2546) than the PAR37. The IRV37 had significantly greater TM (P = 0.0017) and PM (P = 0.0026) than the PAR37. The IM were greater for the IRV37 than the PAR37 (P=0.0611), but there were no significant differences among the other IM comparisons.

Conclusions

There were no differences within extenders for TM, PM or IM, however the IRV had significantly greater values than the PAR for all parameters. Our hypothesis was rejected and changing the extender or thaw protocol offered no advantage over the current protocol.

Keywords: Canine; semen; cryopreservation; sperm; motility; membrane integrity