ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium
on
Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the

European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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

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INTRODUCTION: Cryopreservation of bovine spermatozoa has been successful for many years. More recently, cryopreservation of canine spermatozoa has been performed. Protocols using different extenders, different freeze rates and different thaw rates have been empirically derived in our laboratory over the years. The objective of this project was to compare the currently employed laboratory protocol with another protocol using different cooling times, and a different extender.

OBJECTIVES AND METHODS: Our hypothesis was that cryopreservation of canine spermatozoa using a new, commercially available canine extender using a shorter cooling time would yield increased post-thaw motility and more intact membranes than the currently employed laboratory protocol (Irvine extender, a 60 minute cool down and a 50° 10 second thaw).

The AAALAC guidelines were followed and the project was approved by the Louisiana State University IACUC and Clinical Protocol committees. A single ejaculate was collected from 11 mature dogs of different breeds. Each ejaculate was prepared for cryopreservation with two different commercial semen extenders, a human extender, Irvine (Irvine Scientific, Santa Ana, CA, 92705-5588) (IRV) or a canine extender, Partnar (3560 Pine Grove, Unit 227, Port Huron, MI 48060) (PAR). After adding each extender, each sample was centrifuged for 10 m at 900 x g, the supernatant removed and each sample was re-suspended to 200 x 10⁶ cells/ml with each extender. For each extender, two different cooling times were used, 30 (30m) and 60 (60m) minutes, before adding the corresponding extender containing 12% glycol (resulting in a final glycerol concentration of 6% and 100 x 10⁶ cells/ml), for a total of four treatments (IRV 60m, IRV 30m, PAR 60m, and PAR 30m). Aliquots of each extender and treatment were immediately loaded into 0.5 mL straws, placed on a boat 4 cm over liquid nitrogen for 10 minutes and then plunged and maintained at -196° C for at least seven days before thawing. Five minutes after thawing for 10 seconds at 50° C, each sample was assessed for total and progressive motility using a computer assisted sperm analyzer (SpermVision, Minitüb, 419 Venture Court, Verona, WI 53593, USA) and for membrane integrity using SYBR-14/PI (LIVE/DEAD® Sperm Viability Kit, by Invitrogen™, 5791 Van Allen Way Carlsbad, CA 92008 USA). Statistical analysis included a repeated measures analysis in an ANOVA of a 2 × 3 factorial arrangement of treatments with dog as a random effect in a mixed effects model using the SAS mixed procedure. Pair-wise t-tests of least squares means differences, (a,b) were IRV 60m, 49.6 a,1 + 4.6; PAR 30m, 46.3 b,3 + 4.8; with pair-wise comparisons showing the following statistical differences, (a,b)
P = 0.0150), (1,2
P = 0.0683), (1,2
P = 0.0175), and (3,4
P = 0.1905). Using the IRV extender resulted in significantly greater total motility compared to using the PAR extender when a 60 minute cool down was used, but not when a 30 minute cool down was used. Using the IRV extender resulted in significantly greater total motility when samples were cooled for 60 minutes than when cooled for 30 minutes in IRV, however when using the PAR extender total motility was not different for the two cooling times. Using the IRV extender resulted in significantly greater progressive motility compared to using the PAR extender when a 60 minute cool down was used, but not when a 30 minute cool down was used. Using the IRV extender resulted in significantly greater progressive motility when cooled for 60 minutes than when cooled for 30 minutes in IRV, however when using the PAR extender the progressive motility was not different at the two cooling times. There was no difference in the membrane integrity between the IRV and PAR extender comparing either a 30 or 60 minute cool down between extenders. There was no difference in the membrane integrity comparing 30 and 60 minute cool down times within the IRV or PAR extenders.

CONCLUSIONS: Our hypothesis was rejected and changing the extender or the cool down protocol offered no advantage over the currently employed laboratory protocol.