(31.0 ± 22.48%) concentration, (70.39 ± 32.1%) motility; Isolate® (49.67 ± 41.4%) concentration, (59.7 ± 21.4%) motility. PureCeption® had a significantly higher percent recovery of motile sperm cells compared to the other sperm separation media. In this preliminary study, PureCeption® was superior to the other density gradient centrifugation media for separation of viable, motile sperm from non-motile sperm and red blood cells.

Keywords: Spermatozoa; Motility; Blood; Centrifugation; Canine

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Effect of follicular fluid on the developmental competence of dromedary (*Camelus dromedarius*) oocytes obtained from small follicles

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We recently demonstrated that camel oocytes originating from large follicles were more competent than those collected from small follicles [1]. The objective of this study was to evaluate the ability of various concentrations (2.5, 5, and 10%) of follicular fluid from large follicles (LCFF) to support cytoplasmic maturation of camel oocytes derived from small follicles. Cumulus–oocyte complexes (COC) were obtained postmortem from ovaries by dissecting small and large follicles (3–6 and 7–10 mm, respectively). The LCFF was collected from follicles 7 to 10 mm in diameter, pooled, centrifuged (300 × *g* for 10 min), and the supernatant stored (−20 °C) until used for in vitro maturation (IVM). The quality of COC was evaluated after IVM, in vitro fertilization (IVF), and in vitro culture (IVC). All cultures were done in four replicates at 38.5 °C, under 5% CO_2 and > 95% humidity. The COC from small follicles were matured for 28 h in a basic IVM-medium (TCM-199 medium + 10 ng/mL EGF + 250 μm cysteamine; Sigma Chemical Co., St Louis, MO, USA), supplemented with various concentrations of LCFF or with 10% fetal calf serum (FCS; Sigma F24-42). The COC from large follicles (control) were matured with 10% FCS. Fresh semen (0.5×10^6/mL) in modified TALP-solution was used for IVF. Fertilized oocytes were cultured in mKSOMaa, under 5% O_2 and 90% N_2. Statistical analysis was done with Chi square and Fisher's Exact test.

Adding 2.5% LCFF during IVM supported in vitro development of camel oocytes from small follicles. We inferred that LCFF contained factors essential for acquiring developmental competence; however, that higher rates of LCFF suppressed development was unexpected, with no apparent explanation. Further studies are ongoing to determine if LCFF affects viability of hatched embryos transferred to recipients.

Treatment	No. oocytes	No. cleaved (%)	No. blastocysts (%)	No. blastocysts hatching (%)
0% LCFF	104	39 (38)[a]	11 (10)[a]	0 (0)[a]
2.5% LCFF	121	83 (69)[b]	45 (37)[b]	22 (49)[b]
5% LCFF	96	57 (59)[b]	24 (25)[b]	5 (21)[a]
10% LCFF	110	48 (44)[a]	8 (7)[a]	0 (0)[a]
10% FCS	92	51 (55)[b]	21 (23)[b]	6 (29)[ab]
Control	107	76 (71)[b]	36 (34)[b]	18 (50)[b]

[a,b]Within a column, values without a common letter differed ($P < 0.05$).

Reference

[1] Khatir H, Anouassi A, Tibary A. Effect of follicular size on in vitro developmental competence of oocytes and viability of embryos after transfer in the dromedary (*Camelus dromedarius*). Anim Reprod Sci 2007;99:413–20.

Keywords: Follicular fluid effect; IVM; IVF; IVC; Oocyte competence

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Effects of recovery technique, freezing extender and antioxidants on motility parameters of cryopreserved stallion epididymal sperm

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The objectives were to determine: (1) the best technique to recover sperm from the cauda epididymis; and (2) the effects of freezing extenders and presence of antioxidants on post-thaw motility of cryopreserved epididymal sperm. Testes and epididymides were obtained from six stallions (2–25 y old) presented for elective castration. Each cauda epididymis were isolated and randomly assigned to one of two recovery techniques: floating or retrograde flushing. Sperm were recovered using 20 mL of skim milk extender (EZ-Mixin "CST", Animal Reproduction Systems, Chino, CA,

USA) and diluted to 100 mL with the same media. Total number of sperm was determined using a hemacytometer, and motility was determined by CASA (Hamilton Thorne Biosciences, Beverly, MA, USA). Samples were pooled and centrifuged ($600 \times g$, 10 min). The supernatant was removed and sperm were diluted to 100×10^6 sperm/mL with either Lactose EDTA (LAC) or BotuCrio® (BC; BioTech-Botucatu, Brazil), containing 0, 2, or 6 mM of Tempol (Sigma Aldrich Co., St. Louis, MO, USA) or 0, 2, 6, or 18 mM of L-Ergothioneine (Oxis International Inc., Foster City, CA, USA). Samples were frozen in 0.5 mL straws using the IceCube® (Minitube of America, Verona, WI, USA). Motility was determined prior to cryopreservation and post-thaw (after 0, 30, 60, and 90 min at 37 °C). Sperm recovery data were analyzed by ANOVA and paired *t*-test, whereas motility data were evaluated by repeated measures ANOVA and Tukey's HSD test ($P < 0.05$ was significant). There was no difference between floating and flushing for mean (±S.E.M.) total number of sperm recovered ($8.4 \pm 3 \times 10^9$ and $8.9 \pm 3 \times 10^9$, respectively), or for percentage of progressively motile sperm (46 ± 4 vs $33 \pm 7\%$). Total motility tended ($P = 0.087$) to be higher for floating than flushing (86 ± 2 vs $77 \pm 4\%$). The addition of antioxidants did not affect sperm parameters. The interaction of extender*time was significant for total motility, and tended ($P = 0.093$) to be significant for progressive motility. Post-thaw total motility was higher for BC versus LAC at 0, 30, 60 and 90 min (67 ± 2 vs 35 ± 4; 58 ± 3 vs 19 ± 3; 55 ± 2 vs 21 ± 3; and 44 ± 3 vs 19 ± 3). Post-thaw progressive motility was also higher for BC versus LAC at 0, 30, and 60 min (41 ± 2 vs 12 ± 1; 35 ± 2 vs 8 ± 1; 32 ± 2 vs 9 ± 1), but was not significantly different at 90 min (24 ± 2 vs 10 ± 2). In summary, recovery of sperm from cauda epididymis by either floating or flushing resulted in similar numbers of sperm and similar motility. Antioxidants in the freezing media did not affect post-thaw motility. Cryopreservation of epididymal sperm in BC resulted in higher post-thaw motility than LAC.

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Keywords: Stallion; Epididymis; Sperm; Antioxidants; Cryopreservation

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Post-thaw quality of canine semen cryopreserved with commercial canine and equine semen extenders

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Two commercial canine semen extenders, CaniPRO (Minitube of America, Verona, WI, USA) and CanFreeze (Partnar Animal Health, Port Huron, MI, USA), and a one-step semen extender (LE, Animal Reproduction Systems, Chino, CA, USA), originally designed to cryopreserve equine semen, were used to cryopreserve canine semen. The main objective was to investigate whether simplified semen cryopreservation protocols (CanFreeze and LE) would be as efficacious as a proven protocol (CaniPRO). We hypothesized that post-thaw semen quality would not differ among extenders.

Ten pooled ejaculates were obtained from proven stud dogs. Concentration, motility, viability, morphology, and integrity of the sperm membrane and acrosome were determined, and the pooled semen was used to test the three cryopreservation media (using their respective protocols). Processed semen was loaded into 0.5 mL plastic straws and cryopreserved in liquid nitrogen vapor, 2 cm above the liquid nitrogen (LN) for 10 min, before being plunged into the LN. For each extender, one straw was randomly thawed by immersion in a water bath (37 °C for $\geq$ 30 s). Post-thaw semen samples cryopreserved with the two canine semen extenders were diluted with thaw media before analysis (as recommended by the manufacturer), whereas, samples cryopreserved with LE equine extender (one-step protocol) were not. An ANOVA ($P = 0.05$) was used to determine the effect of treatment groups.

For post-thaw semen, the mean (±S.E.M.) percentage of total motility for CanFreeze (74 ± 4.5), CaniPRO (71 ± 4.8), and LE (70.2 ± 5.1) did not differ significantly. However, post-thaw progressive motility of semen cryopreserved with LE extender (12.6 ± 3.4) was significantly lower than that of semen cryopreserved with CanFreeze (35.5 ± 2.7) or CaniPRO (31.7 ± 2.9). In contrast, the percentage of membrane integrity of post-thaw semen samples cryopreserved with LE extender (81.9 ± 1.3) was greater ($P = 0.018$) than that obtained with CanFreeze (74.3 ± 2.1) or CaniPRO (72.4 ± 3.2). Acrosome integrity and sperm viability did not differ among extenders ($P > 0.1$).

Overall, the three semen-freezing extenders successfully cryopreserved canine semen. The only differences