

( $31.0 \pm 22.48\%$ ) concentration, ( $70.39 \pm 32.1\%$ ) motility; Isolate<sup>®</sup> ( $49.67 \pm 41.4\%$ ) concentration, ( $59.7 \pm 21.4\%$ ) motility. PureCeption<sup>®</sup> had a significantly higher percent recovery of motile sperm cells compared to the other sperm separation media. In this preliminary study, PureCeption<sup>®</sup> 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

DOI: 10.1016/j.theriogenology.2008.05.015

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

H. Khatir<sup>a</sup>, A. Anouassi<sup>a</sup>, A. Tibary<sup>b</sup>

<sup>a</sup>Veterinary Research Center, Abu Dhabi, PO Box 44479, United Arab Emirates

<sup>b</sup>Veterinary Clinical Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA, USA

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 ( $300 \times g$  for 10 min), and the supernatant stored ( $-20^\circ\text{C}$ ) 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  $38.5^\circ\text{C}$ , under 5%  $\text{CO}_2$  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  $\mu\text{M}$  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 ( $0.5 \times 10^6/\text{mL}$ ) in modified TALP-solution was used for IVF. Fertilized oocytes were cultured in mKSOMaa, under 5%  $\text{O}_2$  and 90%  $\text{N}_2$ . 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) <sup>a</sup> | 11 (10) <sup>a</sup> | 0 (0) <sup>a</sup>           |
| 2.5% LCFF | 121         | 83 (69) <sup>b</sup> | 45 (37) <sup>b</sup> | 22 (49) <sup>b</sup>         |
| 5% LCFF   | 96          | 57 (59) <sup>b</sup> | 24 (25) <sup>b</sup> | 5 (21) <sup>a</sup>          |
| 10% LCFF  | 110         | 48 (44) <sup>a</sup> | 8 (7) <sup>a</sup>   | 0 (0) <sup>a</sup>           |
| 10% FCS   | 92          | 51 (55) <sup>b</sup> | 21 (23) <sup>b</sup> | 6 (29) <sup>ab</sup>         |
| Control   | 107         | 76 (71) <sup>b</sup> | 36 (34) <sup>b</sup> | 18 (50) <sup>b</sup>         |

<sup>a,b</sup>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

DOI: 10.1016/j.theriogenology.2008.05.016

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

A.E.M. Johnson, M.A. Coutinho da Silva

Department of Clinical Sciences, Cornell University, Ithaca, NY, USA

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,