10% FBS, 10% DMSO, and 0.07 M sucrose; (B) DMEM with 10% FBS and 10% DMSO; and (C) FBS with 10% DMSO. The vials were held at −80 °C for 24 h, and then transferred to liquid nitrogen (−196 °C). After 3 weeks, cells were thawed in a water bath at 38 °C for 2 min. After adding 2 mL of DMEM with 10% FBS, cells were centrifuged and resuspended to 0.5 mL in DMEM with 1% BSA. Cell viability was assessed by Trypan blue exclusion immediately after isolation (L_0), before adding the freezing medium (L_1), and after freezing and thawing (L_2). Survival rate (S) was defined as $L_2/L_1 \times 100$. The effect of treatment on L_2 or S was evaluated using one-way ANOVA. Cell viability after isolation, culture, and freezing and thawing (L_0, L_1 and L_2) was compared using ANOVA for repeated measurements (SAS Institute Inc., Cary, NC). There was no effect of treatment on L_2 or S. Overall mean values (±S.D.) of L_0, L_1 and L_2 were 99.1 ± 1.9, 78.5 ± 14.9 and $67.8 \pm 20.5\%$, respectively ($P < 0.05$). Overall survival rate was $86.4 \pm 21.5\%$. We concluded that the three freezing media tested were equally suited for freezing equine testicular cells. Enzymatic digestion yielded a cell suspension with excellent viability, which decreased after incubation and cryopreservation. Freezing equine testicular cells immediately after isolation may result in better post-thaw viability. However, functional assays are still required to positively identify SSCs among testicular cells, and to determine the ability of this cell population to survive freezing and thawing.

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Keywords: Spermatogonial stem cells; Spermatogonia; Freezing; Cryopreservation; Stallion

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Separation of viable, motile sperm from red blood cells and dead spermatozoa: A comparison of four density gradient centrifugation media in the dog

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Prior to using semen form AI or another reproductive technique, it is essential that ejaculates are devoid of factors such as blood, urine, bacteria, or debris, which could reduce fertility. Our aim was to compare different commercial media [Isolate®, Percoll®, PureCeption®, PureSperm®] for their ability to optimally separate viable, motile sperm from non-motile sperm and red blood cells (RBC). The sperm-rich second fractions of four dogs were pooled. The pooled semen was analyzed with Spermvision (Minitube of America, Verona, WI, USA) for concentration, motility, morphology, and diluted to 300×10^6/mL with CaniPro® chilled extender (Minitube of America). Canine whole blood (10%, v/v) was added to semen to mimic contamination. An aliquot (1 mL) of the blood/sperm admixture was pipetted over 4 mL of a double layered-column of the following gradients: Isolate® (Irvine Scientific, Santa Ana, CA, USA); Percoll® (Pharmacia, Uppsala, Sweden); PureCeption® (SAGE In-Vitro Fertilization, Inc., Trumbull, CT, USA); and PureSperm® (Nidacon International AB, Molndal, Sweden). After centrifugation at $400 \times g$ for 20 min at 23 °C, the gradients were divided into 1 mL fractions (Top, B, C, D, E, and Pellet). The 1-mL fractions were analyzed with Spermvision. The fractions were stained with Dif Quik (Webster Veterinary Supply, Sterling, MA, USA), and assessed with light microscopy (1000X). For the RBC:sperm ratio, 100 cells were counted in three fields in each slide (and the average calculated). The study was repeated three times over a 2-week interval. Data were expressed as means ± S.D. and analyzed with GraphPad Instat3 (GraphPad Software, Inc., San Diego, CA, USA) using one way-ANOVA to compare sperm parameters and efficiency of each media for separation of contaminants. Following $P < 0.05$, two-by-two comparisons were done with the Bonferroni multiple comparisons test. The fraction with the highest concentration of motile sperm and the lowest ratio of RBC for each media was as follows: PureCeption® pellet; PureSperm® E; Percoll® D, pellet; and Isolate® D, pellet. The percent recovery of sperm cells from the fraction with the highest concentration in each media was as follows: PureCeption® ($93.0 \pm 2.3\%$) concentration, ($72.0 \pm 13.0\%$) motility; PureSperm® ($44.8 \pm 25.9\%$) concentration, ($53.5 \pm 17.1\%$) motility; Percoll®

(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,