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

in post-thaw semen quality were the percentage of progressively motile sperm and membrane integrity, with LE extender having the lowest mean progressive motility, but greatest mean membrane integrity. Differences in thawing protocols resulted in LE samples being analyzed for motility at a relatively high concentration, which may have affected the accuracy of the computerized semen analyzer for assessing progressive motility. Unlike the CaniPRO, the CanFreeze and the LE protocols do not require the addition of fresh egg yolk. CanFreeze and LE are simplified freezing protocols that may be of benefit for semen freezing laboratories with limited technical assistance.

Keywords: Semen; Sperm; Cryopreservation; Extender; Dog

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Use of a vital fluorescent stain to identify apoptosis in bovine oocytes

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The objective was to determine if a vital fluorescent stain, Yo Pro-1 (Invitrogen, Carlsbad, CA, USA) could be used to identify early apoptosis in bovine oocytes. To accomplish this, bovine oocytes were stained with both Yo Pro-1 and Annexin V (Invitrogen), a fluorescent stain previously used to identify early apoptosis in oocytes.

Two shipments of cow oocytes were obtained from BoMed (Madison, WI, USA). Each shipment contained approximately 50 oocytes that were ''good'' quality, and 50 oocytes that were ''poor'' quality, as determined by the vendor. Oocytes were shipped overnight in an incubator in maturation medium at 39 °C. The oocytes were first exposed to Yo Pro-1, then to Annexin V. The two stains can be used together; Annexin V indicates phosphatidylserine translocation and uses a blue fluorescent marker, whereas the Yo Pro-1 indicates early loss of plasma membrane integrity and uses a green fluorescent marker. Propridium iodide (Invitrogen) was used to stain dead oocytes (red fluorescent stain). A total of 164 oocytes were stained and individually evaluated using a Zeiss Axiovert 200 fluorescent microscope with Axiovision software. The percentage of apoptotic oocytes identified by the two fluorescent stains was the same in both the ''good'' and ''poor'' groups of oocytes, approximately 80%. Of the 132-apoptotic oocytes, 130 (98.5%) took up both stains, whereas two oocytes were stained with Yo Pro-1 only. The percentage of live oocytes was significantly greater in the ''good'' group (12.6 versus 1.3% in the ''poor group'', $P < 0.0001$), whereas the percentage of dead oocytes was greater in the ''poor'' group (18.2 versus 7.0% in the ''good'' group, $P < 0.0001$; two-tailed Fisher's Exact test).

	Good (%)	Poor (%)	Total
Live	11 (12.6)[a]	1 (1.3)[a]	12
Apoptotic	70 (80.4)[b]	62 (80.5)[b]	132
Dead	6 (7.0)[c]	14 (18.2)[c]	20
Total	87	77	164

[a–c]Within a column, values without a common superscript differed ($P < 0.05$).

The Yo Pro-1 stained the same population of cells stained by Annexin V; therefore, Yo Pro-1 has potential as a vital stain to identify early apoptosis in bovine oocytes. In addition, it appeared that a substantial number of cow oocytes shipped overnight may have been undergoing apoptosis, regardless of their initial morphologically classification as ''good'' or ''poor''. Further studies are ongoing to assess the viability and developmental potential of oocytes and embryos sorted into apoptotic and non-apoptotic populations using this vital stain.

Keywords: Oocytes; Apoptosis; Fluorescent stains; Bovine; Cattle

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Species Abstracts

Equine

Can vaginal electrical impedance be used to characterize estrus and ovulation in the mare?

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The Ovatec® (Key Business Services, Batavia, NY, USA) intravaginal probe measures vaginal electrical impedance (VEI) of cervical mucus. This study investigated whether this device could be used to characterize the mare's estrous cycle, and in particular, to predict ovulation. The hypothesis tested was whether VEI of mare's cervical mucus is cycle- and ovulation-