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

DOI: 10.1016/j.theriogenology.2008.05.018

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

S.L. Ayres
Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA

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

DOI: 10.1016/j.theriogenology.2008.05.019

Species Abstracts

Equine

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

J.E. Larsen, S.T. Norman
School of Animal and Veterinary Science, Charles Sturt University, Wagga Wagga, New South Wales, Australia

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-