

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. Propidium 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-

dependent. A preliminary study was conducted to determine whether VEI readings were repeatable and whether regular use of the probe was deleterious to the mare. Mare behaviour was compared to an ethogram and analysed with Chi-square. The objective of the second experiment was to correlate VEI with concurrent observations of reproductive status, as assessed by transrectal palpation and ultrasonography, steroid hormone concentrations, and cervical mucus characteristics. To standardise and focus VEI measurements on the endocrinological events of estrus and ovulation, 10 sexually mature and proven mares were synchronized with controlled intravaginal drug release (CIDR) devices, estradiol benzoate and prostaglandin $F_{2\alpha}$ [1]. Every 8 h during the peri- and post-ovulation periods, VEI was determined, plasma and cervical mucus samples were collected, and mares were observed for estrous behaviour, to determine the relationship between these variables and to ascertain whether VEI could predict ovulation. The Spearman correlation coefficient for ranked data was used. In this study, the ability to safely use the probe was quickly attained by a novice operator. Furthermore, the Ovatec[®] probe was less stressful to the mare than repeated transrectal palpation. When used once or thrice daily (in accordance with the manufacturer's instruction), the Ovatec[®] probe could be used to detect estrus in the mare. Over the preovulatory period, there was a positive correlation of VEI with time ($r = 0.76$, $P < 0.0001$) and follicle size ($r = 0.72$, $P < 0.0001$). There was a significant, negative correlation between VEI readings and plasma estradiol concentrations ($r = -0.52$, $P = 0.006$), but no significant correlations between VEI and either plasma progesterone concentrations or characteristics of cervical mucus. Although the Ovatec[®] probe appeared to be a repeatable and relatively reliable non-invasive method of detecting estrus, it was not suitable for determining time of ovulation, neither predicting it prospectively nor detecting it retrospectively.

Keywords: Estrus; Vaginal electrical impedance; Ovatec[®]; Ovulation; Mare

Reference

- [1] Norman ST, Larsen JE, Morton JM. Oestrous response and follicular development in mares after treatment with an intravaginal progesterone releasing device in association with single injections of oestradiol benzoate and PGF_{2α}. Aust Vet J 2006;84:47–9.

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Centrifugation has minimal effects on motility, viability, and acrosome integrity of equine sperm

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The objectives were to determine the effects of centrifugation on progressive motility, plasma membrane integrity (viability), and acrosome integrity of equine sperm. We hypothesized that high centrifugation forces will be detrimental to equine sperm, but will increase recovery rates. Ejaculates from six stallions were collected, extended (INRA96, IMV Technologies, Maple Grove, MN, USA) to a concentration of 25×10^6 cells/mL, and subjected (10 min) to: (1) no centrifugation (NC); (2) $400 \times g$ (400); (3) $900 \times g$ (900); and (4) $4500 \times g$ (4500). Before and after centrifugation (Day 0), and after 24 h of cooling (Day 1), sperm motility was assessed (Sperm Vision[®], Minitube, Verona, WI, USA), and samples were stained with SYBR-14/propidium iodide (PI) and PI/FITC-PNA (*Arachis Hypogaea*; Molecular Probes, Eugene, OR, USA), and assessed by flow cytometry. Data were analyzed using Shapiro–Wilk's statistics, and a mixed linear model was used to determine effects of treatment and day. The 4500 treatment group had reduced ($P < 0.05$) motility, viability, and intact acrosomes as compared with the other groups (Table 1). The 400 and 900 treatment groups had lower recovery rates than the

Table 1
Mean ($\pm$ S.D.) equine sperm progressive motility, viability, and acrosome integrity after centrifugation (Day 0) and 24 h after cooling (Day 1)

Treatment	Progressive motility (%)		Viability (%)		Acrosome (%)	
	Day 0	Day 1	Day 0	Day 1	Day 0	Day 1
NC	82.6 $\pm$ 6.7 ^a	76.4 $\pm$ 7.1 ^a	81.1 $\pm$ 6.2 ^a	76.4 $\pm$ 5.2 ^{ab}	81.9 $\pm$ 4.6 ^a	76.0 $\pm$ 5.7 ^{ab}
400	85.6 $\pm$ 8.7 ^a	74.9 $\pm$ 12.2 ^a	85.5 $\pm$ 5.6 ^a	81.1 $\pm$ 2.8 ^a	83.7 $\pm$ 2.4 ^a	80.3 $\pm$ 5.8 ^a
900	84.2 $\pm$ 7.4 ^a	73.4 $\pm$ 10.8 ^a	81.7 $\pm$ 5.2 ^a	79.0 $\pm$ 4.6 ^a	81.3 $\pm$ 5.0 ^a	79.6 $\pm$ 5.3 ^a
4500	74.2 $\pm$ 6.2 ^b	66.5 $\pm$ 11.5 ^b	72.8 $\pm$ 6.6 ^b	70.7 $\pm$ 7.9 ^{ab}	72.5 $\pm$ 6.1 ^b	69.5 $\pm$ 8.5 ^{ab}

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