

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 ($P < 0.05$).

4500 treatment group ($NC = 100.0 \pm 0.0\%$, $400 = 54.4 \pm 8.6\%$, $900 = 75.0 \pm 7.1\%$, and $4500 = 97.9 \pm 2.8\%$; $P < 0.05$). Centrifugation at 400 or $900 \times g$ did not damage equine sperm. Further studies of centrifugation forces between 900 and $4500 \times g$ are warranted to find optimal forces that maximize recovery rate, minimize sperm damage and do not affect fertility.

Keywords: Centrifugation; Spermatozoa; Plasma membrane; Acrosome; Recovery rate

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Serum concentrations of ergovaline/ergot alkaloids in late-term pregnant mares grazing endophyte-infected tall fescue pastures: A preliminary report

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Ergot alkaloid toxicities such as tall fescue toxicosis from *Neotyphodium coenophialum*-endophyte-infected [E+] tall fescue pastures are important veterinary and economic problems. Tall fescue toxicosis apparently results from ingestion of vasoconstrictive ergot alkaloids produced by symbiotic fungal endophytes; ergovaline is generally considered the critical toxin. To date, ELISA and HPLC with UV/fluorescence detection have been the predominant means of ergot alkaloid determination. These techniques, however, lacked sufficient sensitivity and/or specificity, to detect serum concentrations of specific ergot alkaloids. Thus, the objective of this study was to employ highly sensitive analytical techniques that included the application of liquid-liquid extraction, HPLC and Electrospray(+) ionization mass spectrometry (ESI-MS) with multiple reaction monitoring (MRM), to detect specific ergot alkaloids in equine serum with a limit of detection estimated at 1 pg/mL . To this end, weekly blood samples were collected by venipuncture from late-term pregnant mares ($>290 \text{ d}$) exposed to toxic endophyte-infected ($>90\%$ contaminated) tall

fescue pastures. Serum samples were analysed from four mares that showed clinical signs of ergot alkaloid reproductive toxicity (i.e., agalactia, prolonged gestation, placental thickening, mare and neonatal mortality). We now report that serum concentrations of ergovaline in mares grazing E+ tall fescue pastures ranged from 0.7 to 3.8 pg/mL , and its 8-positon epimer ergovalinine, from 1.5 to 5.5 pg/mL . Conversely, concentrations of ergocryptine and its epimer, ergocryptinine, ranged from 1.2 to 6.8 pg/mL and 2.2 to 7.5 pg/mL , respectively. These very low serum concentrations of ergovaline were consistent with a relatively small daily dose (approximately 1 mg/d on E+ pastures), and with ergovaline being an exceptionally potent xenobiotic/toxin. However, it is likely that these serum samples also contained an apparent mixture of ergot-related compounds, each of which may contribute to the overall clinical fescue toxicosis syndrome, and the specific identification of which will require broadening the scope of this highly sensitive and specific analytical method.

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Keywords: Equine; Fescue toxicosis; Endophyte-positive; Ergovaline; Ergovalinine

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Safety and efficacy against uterine infections of an equine in-tranasal *Salmonella* vector vaccine

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Attenuated *Salmonella enterica* serotype Typhimurium (*S. typhimurium*), expressing the SzP protective protein of the MB 9 serovar of *Streptococcus zooepidemicus* (SzP-MB9) was tested for its safety and efficacy as a vaccine against streptococcal uterine infections in mares. A laboratory-prepared $\Delta cya \Delta crp$ -*pabA* mutant, attenuated strain of *S. typhimurium* (MGN 707), originally isolated from a case of equine