4500 treatment group (NC = 100.0 ± 0.0%, 400 = 54.4 ± 8.6%, 900 = 75.0 ± 7.1%, and 4500 = 97.9 ± 2.8%; $P < 0.05$). Centrifugation at 400 or 900 × *g* did not damage equine sperm. Further studies of centrifugation forces between 900 and 4500 × *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

DOI: 10.1016/j.theriogenology.2008.05.027

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

A.F. Lehner [a], B.P. Fitzgerald [a], C.G. Hughes [a], T. Tobin [a], F.C. Camargo [b], J. May [c], L. Dirikolu [d], D.L. Christiansen [e], P.L. Ryan [e]

[a] *Department of Veterinary Sciences, University of Kentucky, Lexington, KY, USA*

[b] *Department of Animal Sciences, University of Kentucky, Lexington, KY, USA*

[c] *Environmental Research Training Laboratory, University of Kentucky, Lexington, KY, USA*

[d] *Department of Veterinary Biosciences, University of Illinois, Urbana, IL, USA*

[e] *Department of Pathobiology and Population Medicine, Mississippi State University, Mississippi State, MS, USA*

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 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.

Acknowledgments

This work was funded by KY and MS Agricultural Experiment Stations; USDA-ARS Forage-Animal Product, Research Unit.

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

DOI: 10.1016/j.theriogenology.2008.05.028

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

R.C. Causey [a], S.C. Artiushin [b], I.F. Crowley [a], A.D. Homola [a], A. Kelley [a], J.A. Weber [a], H.M. Opitz [a], L.A. Stephenson [a], S. Guilmain [a], J.F. Timoney [b]

[a] *Department of Animal and Veterinary Sciences and the Maine Agriculture and Forestry Experiment Station, University of Maine, Orono, ME, USA*

[b] *Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA*

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 Δ*cya* Δ*crp-pabA* mutant, attenuated strain of *S. typhimurium* (MGN 707), originally isolated from a case of equine

salmonellosis, was electroporated with plasmid pGP1-2 containing T7 RNA polymerase, and pET20-b containing the gene encoding SzP-MB9. It was previously demonstrated that mares vaccinated with this construct had serum, nasal and uterine antibody responses to *Salmonella* lipopolysaccharide (St-LPS) and to SzP-MB9. In the present study, responses to SzP-MB9 in reproductively healthy, vaccinated mares were compared to similar mares vaccinated with the *Salmonella* vector only. Serum, uterine and nasal washings were collected during estrus on two successive estrous cycles pre-vaccination, and at the first estrus $\geq$3 weeks post-vaccination. Antibody levels were assessed by an SzP-MB9-specific ELISA; cross-reacting antibodies were absorbed out using a heterologous streptococcal strain. Data were reported as optical density (OD), and analyzed by linear regression, with post-vaccination data as a reference (intercept) to which pre-vaccination values (regression coefficients) were compared. Vaccine vaccinated mares (expressor group, $n = 7$, 3–7 years old) had significant increases in SzP-MB9-specific uterine IgA (0.77 ± 0.54 vs. 2.13 ± 0.38), nasal IgA (0.84 ± 0.48 vs. 2.26 ± 0.32) and serum IgG (1.16 ± 0.23 vs. 2.06 ± 0.22); combined pre-vaccination data versus post-vaccination, mean OD $\pm$ S.E.M., $P < 0.05$). However, vector vaccinated mares (control group, $n = 7$, 3–16 years old) did not have significant changes in anti-SzP-MB9-specific antibody. Both groups had significant responses to St-LPS in serum, nasal and uterine secretions. When expressor ($n = 5$, age 4–17 years) and control ($n = 5$, age 3–8 years) groups were challenged intrauterinely with 6.3×10^9 cfu of *S. zooepidemicus*, significantly less *S. zooepidemicus* was cultured from uterine flushings of the expressor group (0.69 ± 0.41) compared to the control group (3.20 ± 0.65; $\log_{10}$ cfu, mean $\pm$ S.E.M., $P < 0.01$). There were no adverse reactions to vaccination. Most mares developed clinically apparent, transient endometritis after challenge. Since protective levels of SzP-MB9 antibodies have not been defined in the mare, it cannot be claimed that the reported antibody increases are protective *per se*. Based on the challenge study, vaccinated mares eliminated an inoculum typical of natural infection more quickly than controls; however, follow-up studies are necessary for confirmation. It was particularly noteworthy that uterine IgA mucosal responses were induced in the mare by intranasal vaccination.

Keywords: Uterus; Vaccination; Intranasal; *Salmonella*; Horse

DOI: 10.1016/j.theriogenology.2008.05.029

Food Animal

An estimate of the normal variation in the sperm DNA fragmentation index of Holstein bulls, and its association with serum testosterone and prolactin, over two spermatogenic cycles

R. Kasimanickam [a], W.H. Eyestone [a], J.M. DeJarnette [b], W.D. Whittier [a]

[a] *Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*

[b] *Select Sires Inc., Plain City, OH, USA*

Numerous factors affect sperm DNA stability in bulls including age, breed, frequency of semen collection, sperm concentration, freezing and laboratory procedural differences, toxins, and infections. The objective of this study was to determine the variation in the sperm DNA fragmentation in bi-weekly ejaculates collected via artificial vagina over two spermatogenic cycles (19 wk) from 10 Holstein bulls (14–18 mo of age) housed in a commercial AI center. On sampling day, two ejaculates were collected in succession from each sire and pooled (within sire) for processing and cryopreservation. The frozen semen samples were thawed at 35 °C and subjected to flow cytometry analysis of sperm chromatin structure to estimate the DNA fragmentation index (DFI). Blood samples (for testosterone and prolactin) were collected bi-weekly (on sampling day). Correlations between the DFI and hormone concentrations were determined using Pearson's correlation coefficient. The DFI were analyzed using repeated measures ANOVA. Differences in the sperm DFI were compared both within and across bulls and weeks of collection. There was no significant correlation between serum testosterone and sperm DFI ($r = 0.03$; $P > 0.1$) and between serum prolactin and DFI ($r = 0.04$; $P > 0.1$). For DFI, there were no differences for bulls between weeks ($P > 0.1$), but there were differences among bulls within weeks ($P < 0.001$). In conclusion, within bulls, the sperm DNA fragmentation index was similar during the study period (two spermatogenic cycles). However, observed variations in the DFI among bulls within bi-weekly samples may contribute to differences in sire fertility potential. In this population of reproductively sound AI sires, there were no correlations among bi-weekly testosterone, prolactin, and DFI levels.

Keywords: Sperm; Semen; Chromatin; DNA fragmentation index; Bull

DOI: 10.1016/j.theriogenology.2008.05.021