

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 ≥ 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

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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

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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

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