

THE EFFECT OF DIFFERENT LUBRICANTS ON LONGEVITY OF MOTILITY AND VELOCITY OF STALLION SPERMATOZOA

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Collection of spermatozoa for artificial insemination requires that the stallion be stimulated with an artificial vagina (AV) using proper pressure, temperature and lubrication. However, a detrimental effect of lubricants on sperm function has been widely reported, largely thought due to their often hyperosmotic formulation. Recently, several “non-spermicidal” lubricants have become available for equine reproductive work. This study was done to compare the effect of several of these “non-spermicidal” lubricants on stallion sperm motility over time. For this experiment, extragonadal reserves were stabilized and three ejaculates from five stallions of proven fertility were collected with a Missouri AV lubricated with 5 mL of petroleum vaseline. The number of sperm/mL was determined using a Spermacue and 1 part (v/v) of NFG semen extender was added for every 50×10^6 sperm present. Each ejaculate was then divided five ways and 10% (v/v) of each of four different lubricants was added. A sample with no lubricant was kept as a control. The lubricants evaluated were Priority CareTM (PC, First Priority Elgin, IL, USA); Pre-SeedTM Equine (PEq, INGfertility Valleyford, WA, USA); MiniLubeTM (ML, Minitube Verona, WI, USA) and EquiLubeTM (EL, Boehringer Ingelheim St. Joseph, MO, USA). Evaluation of progressive motility (PM) was performed at 0, 24, 48 and 72 h by two experienced individuals blinded to treatments. After the 0 h evaluation, semen treatments were placed into a cooling container (Equitainer) for storage. Aliquots were brought to body temperature for motility determination. Analysis (two-way ANOVA) revealed significant treatment effect over time and by treatment. There were no significant differences between any treatments and control sperm at 0 h, with PM ranging from 73% to 79%. PM data are presented as mean $\pm$ S.D. However, by 24 h of storage, sperm motility was decreased ($P < 0.001$) in the PC ($28 \pm 8\%$), ML ($54 \pm 16\%$) and EL ($39 \pm 19\%$) treatments, as compared to sperm motility seen in the control ($64 \pm 11\%$). Motility of sperm in PEq ($61 \pm 12\%$) did not differ from that in controls at 24 h. Likewise, at 48 h, sperm motility was decreased ($P < 0.001$) in the PC, ML and EL

treatments as compared to the control treatments, whereas motility in the PEq did not differ from that of the control. By 72 h, sperm motility had decreased in all treatments from that seen in the control ($39 \pm 12\%$; $P < 0.01$). However, sperm motility in the PC ($8 \pm 7\%$) and EL ($11 \pm 14\%$) treatments had decreased more ($P < 0.05$) than that seen with PEq ($20 \pm 15\%$) and ML ($18 \pm 12\%$). We inferred that stallion sperm motility was affected by even “non-spermicidal” lubricants. This effect could not be attributed solely to osmolality (mOsmo/kg) of the lubricants (i.e. EL 368; PC 2199; PEq 328; ML 336), as 10% EL, PC and ML caused declines in sperm motility at 24 and 48 h, even though only the PC was hyperosmotic. The detrimental effect of these lubricants was not evident immediately (0 h), although it did occur thereafter. PEq caused the least sperm damage, with motility consistent to the control throughout the first 48 h of storage.

Keywords: Stallion; Semen; Motility; Lubricants; Artificial insemination

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PERTURBATIONS IN MACROPHAGE INFLAMMATORY CYTOKINE mRNA EXPRESSION IN MARES EXPOSED TO ENDOPHYTE-INFECTED TALL FESCUE PASTURES

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Tall fescue is a cool season forage grown extensively in the southeast United States, but $>75\%$ of pastures are infected by the endophyte *Neotyphodium coenophialum*, resulting in the presence of ergot alkaloids (EA) known to be toxic to mares in late gestation. However, the mechanism of action of EA toxicity on the estrous cycle, early embryonic and fetal development and survival in horses is less well understood. This study investigated whether inappropriate expression of maternal inflammatory cytokines is associated with poor reproductive performance observed in mares

when exposed to E+ fescue during the spring months (March to June). To this end, 24 non-pregnant mares (Quarter Horse, Thoroughbreds) were matched by age and assigned to one of three pastures: (1) endophyte-free (E–, $n = 8$; 11.8 ± 1.9 years); (2) endophyte-infected (E+, $n = 8$; 11.4 ± 1.8 years); (3) endophyte-infected (E + D, $n = 8$; 11.8 ± 1.8 years) and treatment with domperidone (DP; 1.25 mg/kg BW, q 24, PO), a dopamine receptor antagonist. In pregnant mares, domperidone was given daily from 14 to 40 days after ovulation. Mares were placed on E+ (>94% infected) or E– (<6% infected) pastures March 21, 2005 and observed daily for estrus and examined by rectal palpation and ultrasonography (US) for ovarian activity. Mares were bred by AI or natural cover and pregnancy was confirmed by US on 14, 16, 18, 21 and 35 days post-ovulation, maintained on trial pastures for the first 100 days of gestation, then transferred to bermudagrass or rye grass pastures through to foaling (spring 2006). Blood samples were collected twice weekly (PaxGene™ tubes; Qiagen, Valencia, CA, USA) for isolation of macrophage mRNA and analysis of tumor necrosis factor alpha (TNF α) interferon gamma (INF γ), interleukin 1 β and 15 (IL-1 β , IL-15) by quantitative real time RT-PCR. Data was analyzed using ANOVA for repeated measures with group, time and time by group as the main effects, and expressed as mean $\pm$ S.E.M. Of the 24 mares, four E+ mares failed to conceive (March to June). The number of cycles and inseminations per cycle was higher in E– (2.0 ± 0.42 and 2.8 ± 0.5) versus E+ mares (1.7 ± 0.2 and 2.1 ± 0.3). From March to mid-April, there was an increase ($P < 0.05$) in INF γ mRNA expression over time in mares exposed to E+ versus E– pastures and a difference between groups (E+ versus E–, $P < 0.001$). There were no differences among groups for TNF α , IL-1 β or IL-15 mRNA expression. From mid-April to July, IL-1 β mRNA expression was higher ($P < 0.01$) in E+ versus E– mares, but treatment of E+ mares with DP (i.e., E+D) did not alter IL-1 β mRNA expression, which remained higher ($P < 0.0001$) compared to E– mares. All mares confirmed pregnant at 100 days (E–, $n = 8$; E+, $n = 6$; E + D, $n = 6$) delivered viable foals at or near term without complications with no differences in birth weights (43.9 ± 2.6 , 41.7 ± 1.1 and 43.9 ± 2.7 kg for E–, E+ and E + D, respectively). This is the first report to show an elevation in inflammatory cytokines following exposure of mares to E+ pastures, suggesting that inappropriate expression could be detrimental to embryonic development and survival.

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Keywords: Mares; Endophyte-infected; Tall fescue; Cytokines

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EXPRESSION OF ANTI-MÜLLERIAN HORMONE (AMH) IN THE EQUINE TESTIS

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Anti-Müllerian hormone (AMH) is a distinct member of the transforming growth factor β (TGF β) superfamily, and functions to induce regression of Müllerian ducts during male fetal development. In the human male, AMH is expressed in the Sertoli cells during fetal development and expression of AMH continues up through puberty. The purpose of this study was to characterize expression of AMH in the fetal, neonatal, prepubertal and adult equine testis as well as to examine expression of AMH in equine cryptorchid testes based upon immunohistochemistry (IHC) using an antibody directed against human AMH.

Testes were recovered from equine fetuses at 5.5 ($n = 1$), 10 ($n = 2$) and 11 ($n = 1$) months of gestation, as well as at 12 months of age ($n = 2$) and from adult stallions ($n = 3$). In addition, cryptorchid testes ($n = 10$), and testis tumors (teratomas ($n = 4$); seminoma ($n = 1$); Sertoli cell tumors ($n = 1$)) were examined by IHC for expression of AMH. For IHC, 5- μ M paraffin sections were deparaffinized through a graded alcohol series, rinsed and endogenous peroxidase activity was quenched with H₂O₂. Sections were then processed for antigen retrieval followed by routine IHC using a goat polyclonal primary antibody directed against a C-terminal peptide antigen from human AMH (Santa Cruz Biotechnology; Santa Cruz, CA, USA) followed by a biotinylated second antibody (donkey anti-goat IgG) and detection using the Vectastain ABC detection kit (Vectorlabs; Burlingame, CA, USA). Controls included omission of the primary antibody as well as incubation of the primary antibody with the corresponding blocking peptide prior to IHC.