

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.

Immunolabeling with α -AMH was localized to Sertoli cells within the developing seminiferous tubules of fetal, neonatal and prepubertal equine testes. Presumptive gonocytes within the developing seminiferous tubules did not express AMH. AMH expression was not detected in Sertoli cells from normal adult equine testes. Cryptorchid equine testes demonstrated expression of AMH in Sertoli cells in animals up to 3–4 years of age. Tubules which demonstrated severe vacuolization and degeneration in cryptorchid testes appeared to have less immunoreactive AMH than did tubules with more normal Sertoli cells. Omission of the primary antibody or incubation of the primary antibody with the corresponding blocking peptide eliminated immunolabeling

of Sertoli cells in fetal, prepubertal and cryptorchid testes, thus confirming the specificity of the immunolabel for AMH.

In summary, this study demonstrated that AMH was strongly expressed by Sertoli cells in fetal, neonatal and prepubertal equine testes and that the expression was not detected in normal adult equine testes. AMH was expressed in cryptorchid testes although the level of expression appeared more variable. AMH may therefore provide a useful biomarker for the detection of cryptorchid testes in the horse.

Keywords: Testis; Equine; Anti-Müllerian hormone

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