Preliminary evidence of fetal hypothalamic-pituitary-adrenal axis activation in an experimental model of infective preterm delivery in the mare


aDepartment of Veterinary Clinical Sciences, School of Veterinary Medicine
bDepartment of Animal Sciences, College of Agriculture
Louisiana State University, Baton Rouge, LA, USA
cGluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

Activation of the hypothalamic-pituitary-adrenal axis (HPAA) is a key event in the control of labor at term, and immaturity of the HPAA at birth is a major factor leading to poor neonatal outcome with preterm delivery. Cortisol production in the fetal horse rises only immediately prior to term gestation; manipulations to accelerate fetal maturation precociously are frequently complicated with a negative neonatal outcome. Previous research suggests that the HPAA in equine fetuses less than 295 d are immature, as evidenced by failure of intrafetal ACTH administration to elicit cortisol production by the fetus. Women with intrauterine infection and preterm delivery had significantly higher amniotic fluid concentrations of cortisol than patients with preterm delivery without intrauterine infection. Changes in equine fetal plasma cortisol concentration in response to in utero infection have not previously been reported, nor have concentrations of cortisol in equine fetal fluid. The objectives of this study were to measure changes in cytokine expression in the chorioallantois and cortisol concentrations in fetal fluid from mares in an experimental model of infective pre-term delivery. Thirteen adult pony mares of various ages were used in this study over a 2-year period. Allantoic catheters were placed in sedated standing animals under local anesthesia using laparoscopic visualization. Seven mares had in utero infection, six mares were uninfected. Fetal fluid and maternal plasma was collected at twenty-four hour intervals, centrifuged, and the supernatant stored at -70 ºC. The concentration of cortisol in fetal fluid was assessed using commercially available radioimmunoassay reagents (Cortisol RIA, Diagnostic Systems Laboratories, Inc., Webster, TX, USA), previously validated for equine samples. Samples were assayed in duplicate. Four areas of the chorioallantois were collected at delivery and stored in RNA stabilization reagent (RNAlater, Qiagen Inc., Valencia, CA, USA), until analyzed for expression of a panel of eleven equine-specific cytokines. Experimentally-induced infection increased the expression of IL-1β, IL-18, IL-15, IFN-γ, in a site dependant manner. Mares spontaneously aborting also had increased expression of IL-1β, IL-18, IFN-γ, and iNOS in a site dependant manner. Data from eleven mares were included for fetal fluid cortisol analysis; five mares had in utero infection, six mares were uninfected. Substantial increases in cortisol concentration in fetal fluids were observed prior to spontaneous abortion in three mares (two with fetal infection and placentitis, one with fetal aseptic fibrinous pneumonia and placental edema). Maternal plasma cortisol concentrations are pending. These results suggest that increased cortisol concentration in fetal fluid may be seen with infection or inflammation at 80% gestation. The signaling pathways responsible for release of cortisol from the equine fetal adrenal gland subsequent to intrauterine infection are unknown, but data from other species would suggest
that exposure to pro-inflammatory cytokines is one likely mechanism resulting in fetal HPAA activation. All of the mares with increased fetal fluid cortisol concentrations had a greater than 25-fold expression change in IL-1β at the cervical star, and in normal and abnormal areas of the chorioallantois. Further investigations, such as in vitro fetal adrenal cell responsiveness to IL-1β and ACTH at various stages of gestation may provide insights into the signaling pathways leading to cortisol secretion subsequent to IL-1β treatment or exposure.

**Keywords:** Equine, fetus, hypothalamic-pituitary-adrenal axis, pre-term delivery, inflammation