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Cortisol and DHEA as markers of placentitis in pregnant mares: a preliminary study

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Placentitis in mares is a subclinical cause of abortion, highlighting the importance of early detection for successful treatment. Sexual steroids could serve as biomarkers, but cross-reactivity and a limited detection range of immunoassays may hinder accurate measures. This study evaluated some steroids as potential markers of placentitis using liquid chromatography coupled to mass spectrometry (LC-MS), known to improve detection and quantification of steroids with good selectivity, specificity, and allowing multiplexing capabilities (Conley et al., Reproduction. 2019;158:197-208; Ledeck et al., Theriogenology. 2022;189:86-91). From the 7th month of pregnancy, mares were transrectally scanned to measure the combined thickness of the uterus and placenta (CTUP), and blood samples were collected at a fixed time of day. Ten healthy mares (HM) and nine mares diagnosed with non-experimentally-induced placentitis (PM) between 8 and 10 months were enrolled, based on ultrasonographic placentitis signs: heterogeneous echogenicity and/or thickened CTUP. *Post-partum* allantochorion examination confirmed the diagnosis. Subsequently, PM were further excluded from the study as they received treatments for placentitis. Serum concentrations of progesterone, 17α-hydroxyprogesterone (17αOHP), dehydroepiandrosterone (DHEA), and cortisol were assayed using LC-MS. Non-normally distributed data were presented as medians, and groups were compared at the same month of pregnancy using the Mann-Whitney test. There were no significant differences in 17αOHP and progesterone concentrations between groups at any month. DHEA concentrations were significantly higher (p=0.0297) at 8 months in PM (3.552μg/L) compared to HM (2.240μg/L). A similar trend was observed at 9 months, with DHEA concentrations of 2.126μg/L and 1.445μg/L in PM and HM, respectively (p=0.0604). In contrast, HM had significantly higher (p=0.022) cortisol concentrations at 7 months (71.75μg/L) compared to PM (38.62μg/L). This difference tended (p=0.0529) to be observed at 8 months with cortisol concentrations of 52.25μg/L (HM) and 37.28μg/L (PM). The DHEA/cortisol ratio was significantly higher in PM than in HM at 9 months (p=0.013) and tended to be increased at 7 and 8 months (respectively, p=0.0659 and p=0.0529). Placentitis has been shown to increase pregnenolone production (Ousey et al., Theriogenology. 2005;63:1844-1856). In this preliminary study, PM were observed to metabolize pregnenolone into DHEA rather than cortisol, without changes in 17αOHP concentrations. Reduced cortisol concentrations at 7 months could be an early but non-specific biomarker of placentitis. Conversely, elevated DHEA levels only appear together with ultrasonographic signs at 8 months but could become a more specific biomarker, which differs from previous findings in mares with experimentally-induced placentitis (Canisso et al., Equine Veterinary Journal. 2017;49:244-249). These changes result in a higher DHEA/cortisol ratio in PM at 9 months. However, further research should explore modifications of steroids' pathways in PM to confirm the value of DHEA and cortisol for early diagnosis of placentitis in mares.