Serum and placental oxytocinase in healthy late pregnant and postpartum mares
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Oxytocinase (OTase)/insulin regulated aminopeptidase (IRAP) or leucyl-cystinyl aminopeptidase (LNPEP) varies in pregnant women with higher levels in the third than the first trimester of pregnancy. Oxytocinase is present in the chorionic microvilli of human placenta, hydrolyzes several peptides including oxytocin and vasopressin, and is suggested to be responsible for maintaining pregnancy homeostasis. There is paucity of information on the activity and presence of OTase in placental tissues and serum of pregnant mares. The objectives of this study were: 1) characterize serum OTase of pregnant mares in the last month of gestation and post-partum; and 2) characterize the effect of location on OTase expression in placental tissues of mares. Jugular blood samples were taken from 18 Standardbred mares: (a) prepartum (320-336 days of gestation), (b) 24 hours before parturition, (c) 20 minutes and (d) 2 hours after foaling. Serum was stored frozen until analysis. Chorioallantoic membrane tissues were retrieved from the body (B), pregnant horn (PH), and non-pregnant horn (NPH) immediately after placental delivery in (n=8) mares, then divided and stored frozen at -80°C, or 10% formalin. Tissue samples (100 mg) were thawed, rinsed and homogenized in phosphate buffered saline using sonication and then centrifuged at 1500 x g for 15 minutes. The resulting supernatant was stored at -80°C until assayed. A commercial ELISA (LNPEP for horses, MYBioSource, San Diego, CA) with a detection range of 6.25-200 U/L and an intra and interassay coefficient of variation <15%, which was validated in our laboratory, was used. Immunohistochemical (IHC) staining for LNPEP with negative and positive controls (Rabbit anti-LNPEP, Thermo Fisher Scientific, Rockford, IL) using an automated slide strainer (Autostainer Plus, Dako Canada Inc., Mississauga, ON) was conducted. Proprietary software (STATA/SE version 13.1, College Station, TX) using p<0.05 was used including: Shapiro-Wilk for normality, ANOVA and Kruskal Wallis to evaluate the effect of Day and Placental Region on OTase. Post-hoc analysis was performed using Dunn’s Test. Mare’s mean gestational age was 342±7.1 days (range 332 to 361 days). There was no significant effect of Day on serum OTase levels. The OTase levels (U/L; mean±SD) at sampling times were: (a) (41.34±10.80), (b) (42.74±13.38), (c) (40.36±15.68), and (d) (38.78±13.50). There was a significant effect of Placental Region on OTase (p=0.0058), with body and pregnant horn (p=0.0098 and p=0.001, respectively) having significantly higher levels than non-pregnant horn. The placenta OTase activity U/gr [median (quartiles)] by placental region were: B (29.55 [15.49, 36.47]), NPH (14.65 [11.74, 17.40]), and PH (28.88 [21.14, 36.88]). Evaluation of IHC showed strong staining of OTase in the PH. This is the first description of the presence of OTase in equine placenta. Further investigation is required to determine if serum and placental OTase activity varies in healthy or abnormal equine pregnancies, or if levels are associated with retained fetal membranes.

Keywords: Oxytocinase, late term pregnancy, placenta, serum