

### Evaluation of angiogenesis in equine hydrops

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Hydrops conditions refer to excessive accumulation of fluid in the amniotic (hydramnios) or allantoic (hydrallantois) cavities during the last trimester of pregnancy. Although this condition is uncommon in mares, consequences of untreated hydrops allantois/amnion can be, very negative, including abdominal wall hernias, prepubic tendon rupture, cardiovascular shock associated with unattended abortion or foaling and dystocia. It has been postulated that hydrallantois is associated with structural and/or functional changes in the chorioallantoic membrane such as dysfunction of chorionic ion pumps. Macroscopic examination of affected placentas suggests areas with small, sparse villi or avillous areas on the chorionic surface. However, it remains undetermined whether this is due to villi atrophy or hypoplasia. In humans and mice, disruption in expression of the imprinted RTL1 gene (Retro Transposon Gag Like) is associated with hydrops. In previous studies, we evaluated expression of the RTL1 locus in the equine placenta throughout gestation and localized this protein in the endothelial cells within microvilli capillaries. In the present study, we hypothesized that the expression of angiogenic genes and the number of chorioallantoic (CA) capillaries are altered in hydrops placentae. Therefore, we evaluated angiogenesis in the hydrops placenta. Expression of 14 angiogenic genes was compared between equine hydrops (n = 10) and normal, 10 month pregnant CA samples (n = 4). Protein expression of RTL1 was evaluated by immunohistochemistry and vascular density was evaluated by immunostaining with Von Willebrand factor. ANOVA analyses revealed 4 differentially expressed transcripts between normal and hydrops samples. DVL1 (dishevelled segment polarity protein 1) had lower expression, whereas EDNRA (endothelin receptor type A), NRP1 (Neuropilin), and TYRO3 (TYRO3 Protein Tyrosine Kinase) had higher expression in hydrops samples ( $p < 0.05$ ). Protein expression of RTL1 and the number of vessels were lower in hydrops samples. In agreement with our hypothesis, there was an alteration in expression of select angiogenic genes and disruption in capillary vessel formation. Interestingly, in human placentae, altered expression of DVL1 significantly reduced placental estradiol production through modification of cytochrome P450 family 19 subfamily A member 1 (CYP19A1) promoters. Furthermore, there are lower plasma estradiol concentrations in ewes with hydrallantois. However, a direct link between reduced DVL1 expression and estradiol production in equine hydrops needs to be elucidated. In conclusion, equine hydrops was associated with an alteration in placental angiogenic features.

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