

## PhD Dissertation Defense

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**Title:** The Influence Of Pregnancy, Metabolic Status And Diet On Uterine Protein Secretions In The Domestic (*Equus Caballus*) And Endangered Przewalski's Horse (*Equus Ferus Przewalskii*)

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### ABSTRACT

Equines exhibit several unique reproductive characteristics. Specifically, mechanisms involved in maternal recognition of pregnancy, physiological basis for the high incidence of early embryonic loss, and insulin resistance (IR) remain poorly understood. An improved understanding of these conditions could enhance breeding management of both domestic and endangered equids in captivity. The use of shotgun proteomics via mass spectrometry enables complete protein identification in uterine secretions (histotroph) in real time. The overall objective of this research was to better understand the proteome of histotroph corresponding to various physiological states (stages of estrous cycle and IR vs. insulin sensitive (IS)) and compare the proteome between the domestic and Przewalski's mare. Study 1 analyzed uterine fluid collected from three domestic mares during estrus (-1 d), diestrus (12.5 d), pregnancy (12.5 d) and un-successful breeding (12.5 d). Mass Spectrometry identified proteins associated with metabolic and immune function in the equine endometrial lumen throughout the estrous cycle, which may help identify biomarkers for failed pregnancy. Study 2 examined the impact of IR as well as effects of omega-3 fatty acids (DHA) supplementation on the uterine proteome of pregnant (12.5 d) domestic mares. Serum amyloid A and afamin were identified for the first time in equine histotroph and were elevated in IR mares and supplementation with DHA reduced the abundance of these proteins in IR mares. Both proteins were detectable in serum and could potentially serve as a biomarker for IR. Study 3 analyzed uterine fluid from estrus (-1

d), diestrus (13.5 d) and artificially inseminated but non-pregnant (13.5 d) Przewalski's mares. Detection of uteroglobin in both domestic and Przewalski's horse histotroph during diestrus supports the notion that this protein is associated with progesterone, but not pregnancy, in equids; this is in opposition to uteroglobin's function in other species. The studies described in this dissertation identified and characterized for the first time, the uterine proteome during various stages of the estrous cycle, early pregnancy, and in IS vs. IR mares. A significant finding was that dietary supplementation of omega-3 fatty acids (DHA) improves the overall uterine environment and could support normal function in IR mares. Overall, findings have not only generated new information about domestic horse reproduction, but can assist with improved management of all equids. Furthermore, the ability to use domestic horse as a model is exemplified by the identification of proteins such as serum amyloid A that can serve as biomarkers of an insulin resistant state for both domestic and non-domestic equids managed in captivity.