Uterine flush fluid proteome of mares in estrus and diestrus: qualitative differences and functional classification

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Analysis of the uterine flush fluid proteome is a minimally invasive approach for evaluating changes in uterine luminal composition across stages of the estrous cycle in mares. Objectives were to evaluate qualitative differences in the uterine flush fluid proteome between mares in estrus and mares in diestrus and to perform a functional classification of proteins either unique to each stage or common between the 2 stages. Uterine flush fluid samples were collected from 8 light breed mares in either estrus (n = 5) or diestrus (n = 3). Mares were 3 - 8 years of age and reproductively healthy, based on transrectal examination, gross appearance of uterine flush fluid and endometrial cytology, culture and biopsy. Proteomic analysis of uterine flush fluid was conducted using label-free liquid chromatography tandem mass spectrometry. Proteins exclusively detected in estrus or diestrus and those common to both stages were identified using Scaffold software (version Scaffold_4.4.8, Proteome Software Inc., Portland, OR). Functional classification of identified proteins into gene ontology (GO) categories [cellular component (CC), molecular function (MF) and biological process (BP)] was performed using protein analysis through evolutionary relationships (www.pantherdb.org) classification system version 11.0.[1] Of 172 proteins identified, 51 and 28 were exclusively detected in mares in estrus and diestrus, respectively, with 93 proteins common to both stages. Most represented terms in various GO categories were similar among the 3 subsets of proteins. Most represented CC terms were cell (GO:0005623) and extracellular region (GO:0005623), most represented MF terms were binding (GO:0005488) and catalytic activity (GO:0003824) and most represented BP terms were cellular process (GO:0009987) and metabolic process (GO:0008152). However, in relatively less represented terms, there were some differences between protein subsets unique to estrus or diestrus. For instance, immune system process (GO:0002376) was represented by proteins unique to estrus, whereas developmental process (GO:0032502) was represented by proteins unique to diestrus. In conclusion, proteomic analysis of the uterine flush fluid enabled identification of subsets of proteins unique to estrus or diestrus, or common to both stages. Based on functional classifications, a majority of the proteins were involved in basic functions such as maintenance of cellular and metabolic processes. Results of this study can serve as a baseline for future research focused on finding stage-specific protein markers or evaluating differences in the uterine flush fluid proteome between normal mares and those with uterine disease.

Keywords: Equine, uterus, proteome, estrus, diestrus

Reference

1. Mi H, Huang X, Muruganujan A, Tang H, et al: PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res 2017;45:D183-189.