^{1}H-NMR metabolomic profiling of allantoic and amniotic fluid in pregnant mares

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Metabolomics and nuclear magnetic resonance (NMR) spectroscopy identifies, quantifies, and characterizes products of metabolism in cells, tissues, or biofluids in a multitude of normal and abnormal physiologic and disease states, i.e., "the metabolome". While no applications have been developed for horses yet, the field of metabolomics is becoming more widely used both in research and clinical settings for human obstetric medicine. The objective of this study was to characterize the metabolome of both allantoic and amniotic fluid in the pregnant mare.

Allantoic and amniotic fluid were collected as a component of a separate study via transabdominal ultrasound guided puncture from 10 pregnant pony mares between 270 and 295 days of gestation. Mares were five to 13 years of age and weighed between 400 and 850 pounds. At the time of collection, samples were labeled according to color as allantoic (brown), amniotic (clear) or admixed samples (yellow), which were later confirmed based on creatinine content.[1] One-milliliter samples were immediately flash frozen in liquid nitrogen, lyophilized, and then resuspended in 700-µL of deuterated water with 0.1 mM trimethylsilyl propionate (TSP). Samples were analyzed in 5-mm NMR tubes, using ^{1}H-NMR spectroscopy with a 500-MHz NMR spectrometer. Spectra were then processed with a one-dimensional NMR processor (ACD/Labs 12.0). Peak identification was performed using databases of known compounds (Chenomx NMR Suite 8.1 and HMDB) and assignment was validated based on two-dimensional HSQC and HMBC ([1]H-^{13}C) long-range correlation experiments. Individual peaks were integrated and normalized to TSP. Differences between fluid types were assessed using a Kruskal-Wallis test at $p < 0.05$ significance.

All samples produced spectra with identifiable peaks. Twenty-five metabolites were identified from allantoic fluid (n=10) while 21 metabolites were identified from amniotic fluid (n=5) and admixed (n=4) samples. Of distinctly identifiable peaks, integrals of the eight metabolites betaine (p=0.0030), citrate (p=0.0021), creatine (p=0.0013), creatinine (p=0.0001), histidine (p=0.0032), π-methylhistidine (p=0.0230), nitrophenol (p=0.0257), and an unknown metabolite at 0.5 ppm (p=0.0034) were significantly elevated in allantoic fluid compared to amniotic and admixed fluid samples. In comparison, the metabolite lactate (p=0.0101), was significantly elevated in amniotic fluid samples in comparison to allantoic and admixed fluid samples. In conclusion, this study established a metabolic fingerprint of equine allantoic and amniotic fluids and can serve as a foundation for future metabolomic studies in equine pregnancy.

Keywords: Equine, metabolomics, allantoic, amniotic

Reference

1. Williams MA, Wallace SS, Tyler JW, et al: Biochemical characteristics of amniotic and allantoic fluid in late gestational mares. Theriogenology 1993;40:1251-1257.