**Untargeted Metabolomic Analysis of Gestationally Matched Human and Bovine Milk samples at 2-weeks Postnatal**

Dominick J. Lemas1, PhD; Xinsong Du1; Bethany Dado-Senn2, Marina Magalhães6; Larissa Iapicca6; Alexander Kirpich, PhD3; Magda Francois1; Nicole Cacho, DO4; Lindsay Thompson, MD1; Leslie Parker, PhD, ARNP6; Josef Neu, MD4; Jimena Laporta, PhD2, Timothy Garrett, PhD5

1 Department of Health Outcomes and Biomedical Informatics, College of Medicine, University of Florida

2 Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida

3 Department of Population Health Sciences, School of Public Health, Georgia State University

4 Department of Pediatrics, University of Florida, College of Medicine, Gainesville, Florida

5 Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida

6 Department of Behavioral Nursing Science, College of Nursing, University of Florida

**ABSTRACT**

**Background**: Human breast milk is the ideal nutrition source for infant development during the first year of life. Epidemiological data demonstrates that bovine whole milk is often substituted for human milk during the first 12-months of life and may be associated with adverse infant outcomes.

**Objective**: The goal of this project is to interrogate the human and bovine milk metabolome at 2-weeks postnatal to identify unique and overlapping metabolites that may impact infant health outcomes.

**Methods**: Human milk (n=10) was collected at 2-weeks postpartum from normal weight mothers (pre-pregnant BMI<25kg/m2) that vaginally delivered term infants and planned to exclusively breastfeed for at least 2-months. Similarly, bovine milk (n=10) was collected 2-weeks postpartum from normal weight primiparous Holstein dairy cows. Dairy cattle were housed in sand-bedded, shaded barns with access to fans and water soakers and fed a common transition cow total mixed ration. Untargeted metabolomics was completed on all milk samples using high-resolution mass spectrometry. Metabolomic analysis was implemented using an open-source containerized metabolomics pipeline. Data processing was completed using MZmine, mummichog and Python were used for statistical analysis.

**Results**: We detected 716 metabolomic features in human and bovine milk samples after quality control. Our analysis also revealed that 43% (312) of metabolomics features were present in both human and bovine milk, 23% (167) of metabolomics features were unique to human milk and 33% (237) of metabolomics features existed only in bovine milk samples. Pathway analysis revealed that sialic acid and glycosphingolipid metabolism (p<0.0009) were common to human and bovine milk samples. We also found that amino acid (tryptophan, tyrosine, purine) metabolism (p<0.005) was unique to bovine samples and vitamin B3 pathways (p=0.03) was unique to human samples.

**Conclusions**: Our analysis revealed a core milk metabolome shared between human and bovine samples. Collectively, these results highlight untargeted metabolomics as a potential strategy to identify unique and overlapping metabolites in bovine and human milk that may impact infant health outcomes.

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