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

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**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. Despite these observations, comparative investigation of the human and bovine milk metabolome from a similar lactation stage has not been extensively studied.

**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 as part of the Breastfeeding and Early Child Health (BEACH) study. Normal weight mothers (pre-pregnant BMI<25kg/m2) enrolled in the BEACH study were recruited between 36-38 weeks of pregnancy, delivered vaginally and planned to exclusively breastfeed their infant for at least 2-months. Similarly, bovine milk (n=10) was collected 2-weeks postpartum from normal weight primiparous Holstein dairy cows. Briefly, starting at 36 weeks of gestation, 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 and Python were used for statistical analysis.

**Results**: We detected 716 metabolomic features in human and bovine milk samples after background subtraction using blank control samples. Principle component analysis demonstrated that human milk metabolites segregate from bovine milk. 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 .

**Conclusions**: 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. Collectively, our results demonstrate . .

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