

## Remodeling of the Uterine Luminal Metabolome prior to Embryo Elongation in Beef Cattle

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In cattle, pregnancy losses that occur in the initial three weeks of gestation remain a significant challenge for the industry. For proper embryonic growth during that window, the uterine luminal environment must contain adequate concentrations of molecules required by the growing embryo. We hypothesized that luminal concentrations of amino acids and lipids change over time in association with specific milestones of embryonic growth. The objective was to compare the concentrations of amino acids and lipids and evaluate their temporal dynamics of change in the uterine luminal fluid on days of the estrous cycle that coincide with the blastocyst stage (seven days after estrus, D7), embryo hatching (D10) and conceptus initial elongation (D14) in beef cows. The estrus of 22 *Bos indicus-influenced* cross-bred, primiparous, non-lactating, cyclic cows was synchronized by injecting prostaglandin F2alpha twice, 14 days apart. Five animals whose estrus were synchronized remained in the study. On D7, D10 and D14, luminal fluid was collected from the uterine body of each animal using a cytology brush. Fluid was subsequently diluted in PBS, centrifuged, filtered, and stored at  $-80^{\circ}\text{C}$  for analysis. Concentrations of 630 metabolites from 26 biochemical classes were measured on samples by targeted metabolomics. The effect of day was tested using univariate analysis (one-way ANOVA).

Overall, the concentration of 86 metabolites was affected by time ( $P < 0.1$ ). There were 11 amino acids, four amino acids-related, 56 lipids (diacylglycerols, glycerophospholipids, glycosylceramides, sphingolipids and triacylglycerols) and 15 metabolites from other classes. In general, luminal concentrations of amino acids and amino acids-related metabolites increased from D7 to D10, ranging from 210.44% increase for glutamine ( $P = 0.006$ ) to 62.3% increase for asparagine ( $P = 0.07$ ), then decreased from D10 to D14 ranging from 53.78% decrease for glycine ( $P = 0.09$ ) to 3.7% decrease for tryptophane ( $P = 0.1$ ). Amino acid metabolism was also affected over time. Glutaminase activity increased 62.92% between D10 and D14 ( $P = 0.06$ ). Glycine synthesis was greater on D7 ( $29.72 \pm 8.5$ ) than on D10 ( $9.50 \pm 1.94$ ) and D14 ( $8.05 \pm 1.63$ ;  $P = 0.004$ ). The concentration of essential amino acids increased 134.96% between D7 and D10 ( $P = 0.09$ ) and ratio of non-essential to essential amino acids decreased from  $26.79 \pm 4.48$  on D7 to  $11.60 \pm 0.80$  on D14 ( $P = 0.02$ ). Lipids, in general, exhibited a gradual increase in concentration from D7 to D14, ranging from 466.66% increase for PC aa C34:3 ( $P=0.02$ ) to 87.77% increase for PC aa C36:1 ( $P=0.08$ ).

In summary, the uterine luminal biochemical milieu changes dramatically in composition during the second week of the estrous cycle. Such changes are likely connected functionally with the transition from blastocyst to initial elongation of the conceptus. We speculate that alterations on such dynamics impact embryo development and determine the pregnancy outcome in cattle. This study is supported by USDA NIFA Award Number 2022-67015-36839.