Decoding the biochemical uterine environment essential for successful establishment of pregnancy in pigs: a metabolomics view

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Synchronous communication between the uterus and conceptus (embryo/fetus and its extraembryonic membranes) is imperative for establishment and maintenance of pregnancy in pigs, sheep and other ruminants. The biochemical milieu within the uterine lumen, orchestrated by a myriad of metabolic pathways, is pivotal in facilitating cytokine secretion, conceptus elongation, and placentation in pigs. However, little is known about the metabolic dynamics among the endometrium, conceptuses, and their crucial intermediary, histotroph, during the peri-implantation period of pregnancy in pigs. Thus, this study aimed to determine the metabolome in pig endometrium, conceptus, and uterine fluids between days 10 and 16 of pregnancy. We hypothesize that the metabolism status varies distinctly across different tissue types and gestational stages within the uterine environment. Gilts were bred via artificial insemination at 12 and 24 h after onset of estrus (day 0). On gestational day (GD) 10, 11, 12, 13, 14, 15, and 16, uteri (n=5-6 gilts per day) were flushed with 20 ml sterile PBS (pH 7.2) after gilts were subjected to a midventral laparotomy. Recoverable conceptuses and endometrium tissue were then subjected to metabolomic analyses by Liquid Chromatography-Mass Spectrometry (LC-MS), raw data was extracted and normalized to tissue weight (endometrium and conceptus) or recovered fluid volume (UF) prior to further analysis. A total of 231 metabolites were detected in endometrium and conceptus, and 192 in the uterine fluids (UF). Clear separations between the endometrium and conceptus were observed on days 10 to 16 based on principal component analysis (PCA), suggesting different metabolic pathways employed by endometrium and conceptuses. Through longitudinal analyses using KEGG pathways, we revealed significant alterations in metabolite profiles across different days of porcine gestation: specifically, 47, 24 and 18 metabolites were found to be significantly altered (t-test, $P<0.05$) when comparing days 10 vs. 12, 12 vs 14, and 14 vs. 16, respectively. Notably, these alterations were observed to enrich in key metabolic pathways, with arginine and proline metabolism, vitamin B6 metabolism, and arachidonic acid metabolism emerging as prominent pathways within the endometrium. Moreover, within the conceptus, thiamine metabolism, ether lipid metabolism, and synthesis/degradation of ketone bodies were predominant among the 42, 61, and 20 altered metabolites identified in the respective comparisons. This study also revealed significant changes in amino acid concentrations across different uterine environments during the peri-implantation period. Interestingly, the quantitative trends of amino acids were highly similar in both UF and conceptus, but not in the endometrium. For instance, histidine and glutamine exhibited a significant increase in concentration throughout the peri-implantation period (day 16 vs. day 10) in both UF and conceptus (10.1- and 8.9-fold increase, respectively, $P<0.05$), while remaining unchanged and

decreasing by 2.5-fold in the endometrium, respectively. Conversely, serine and tryptophan showed a significant decrease in UF and conceptus but remained unchanged and increased by 1.5-fold, respectively, in the endometrium (P<0.05). Unexpectedly, glycine decreased in total amount/concentration across all three types of milieus, warranting further functional investigation into metabolism differences within the uterine lumen during the peri-implantation period of pregnancy. Overall, these findings provide comprehensive insights into the biochemical environment within the endometrium, conceptuses, and UF, highlighting unique metabolic needs dictated by spatial and temporal changes and guiding future research directions to facilitate conceptus growth during the critical development stage. This research was supported by Agriculture and Food Research Initiative competitive Grant 2022-67015-36491 from the USDA-NIFA.