Evaluating Pregnancy Induced Changes to Peripheral Immune Cell Populations in Cattle Using Single Cell RNA Sequencing.

John J. Bromfield[1]; Haipeng Yu[1]; Mackenzie J. Dickson[1]; M. Belen Rabaglino[2]

[1]Department of Animal Sciences, University of Florida, Gainesville FL USA.

[2]Department Population Health Sciences, Utrecht University, Netherlands.

Describing the role of the maternal immune system during pregnancy is critical to our understanding of physiology and pregnancy complications. While these evaluations have been performed in humans and rodents using single cell RNA sequencing technology, information pertaining to reproductive immune system changes in cattle are lacking. We hypothesized that peripheral blood immune cell populations would be altered by pregnancy in cattle. Whole blood was collected from two pregnant Holstein heifers on day 33 of gestation, and two virgin heifers on the day of estrous synchronization immediately before removal of a controlled intravaginal drug release device containing progesterone. All heifers were later confirmed to have carried a pregnancy to term following a single insemination. CD45 positive cells from each heifer were isolated from whole blood using FACS and approximately 10,000 cells from each heifer were subjected to 10x Chromium library preparation and sequenced using an Illumina NovaSeq X Plus. After initial data preparation and cleaning, each heifer had an average of 6,653 cells, 49,693 reads per cell, and 1,091 genes per cell analyzed. When the data for all four heifers was integrated a total of 11 cell clusters were identified in peripheral blood of cattle. Each cluster was manually annotated using expression of cell receptors and transcription factors to identify the immune cell types corresponding to each cell cluster. Pregnancy at d 33 increased the absolute number and relative proportion of activated B-cells, naïve B-cells and gamma delta T-cells in circulation compared to virgin heifers. Pregnancy modulated the expression of several transcripts in specific cell types, including an upregulation of *JSP.1* in B-cells, CD4 T-cells, CD8 T-cells, gamma delta T-cells, granulocytes, NK cells, and monocytes. Similarly, pregnancy increased the expression of *CCL5*, *IDO* and *IL7R* in CD4 T-cells, while the expression of *IFIT1* and *MX2* were increased and *IL18* was decreased by pregnancy in granulocytes. Pathway analysis was performed for transcripts effected by pregnancy from each cell cluster. The eukaryotic initiation factor 2 (eIF2) signaling pathway, involved in translational stress responses, is downregulated by pregnancy in B-cells, CD4 T-cells, CD8 T-cells, gamma delta T-cells, NK cells and monocytes. Conversely, the estrogen receptor signaling pathway is upregulated by pregnancy in B-cells, CD4 T-cells, CD8 T-cells, gamma delta T-cells, NK cells and monocytes. As expected, interferon signaling was increased by pregnancy in granulocytes, likely due to the secretion of trophoblast derived interferon tau. Further analysis suggests the involvement of numerous cytokines, kinases, transcription factors, receptors, and enzymes in controlling pregnancy regulated genes in each cell cluster. Of interest is the potential involvement of IL-4 and ESR1 in regulating differentially expressed genes in B-cells, CD4 T-cells, gamma delta T-cells, CD8 T-cells and NK cells. While all the data obtained from single

cell RNAseq is too numerous to report here, these analyses reveal clear proportional and transcriptional changes to peripheral immune cell populations due to pregnancy at d 33 of gestation. By using single cell resolution, we have a greater appreciation for the immune cells present in peripheral blood of cattle and their potential involvement during pregnancy. Evaluating pregnancy induced changes to maternal immune cell populations will allow us to better understand the role of the immune system in pregnancy and pregnancy complications, while potentially developing new tools for early pregnancy diagnosis and interventional strategies in cattle.