#56: Changes in uterine immune regulatory factors and effects of pregnancy associated glycoproteins on uterine immune cells during early pregnancy in dairy heifers

Sreelakshmi Vasudevan, Manasi M Kamat, Joy L Pate, Troy L Ott

The bovine uterus has an abundance of lymphoid and myeloid lineage immune cells during early pregnancy. We hypothesized that these cells play roles in tissue remodeling and maintenance of tolerance. To assess tissue remodeling we analyzed expression of vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFB) by immunofluorescence (IF) labeling. For immune regulatory factors we analyzed the inhibitory proteins lymphocyte-activation gene 3 (LAG3), programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Endometrium was collected from Holstein dairy heifers (n=4-5/group) on Day 17 of the estrous cycle (D17C) and Day 17 (D17P) and 20 (D20P) of pregnancy, prepared for IF of immune regulatory proteins and RNA was extracted for qPCR for mRNA abundance. For IF, images from five different areas of the uterine wall (UW): luminal epithelium (LE), shallow stroma (SS), shallow glands (SG), deep glands (DG) and myometrium (Myo) were captured and percent area labeled was quantified using ImageJ. Abundant VEGF was observed in tissue collected on D17P with less VEGF expressed ($p<0.05$) on D20P across the entire UW. A similar pattern was detected for TGFB ($p<0.05$). The decline in VEGF and TGFB protein expression between D17P and D20P led us to determine if immune regulatory molecules changed during this same period. Abundance of mRNA of PDL1 was18-fold greater ($p<0.05$) in D17P compared to D17C endometrium, followed by a reduction ($p<0.05$) in D20P. There was a tendency ($p=0.067$) for LAG3 mRNA to be greater in pregnant than cyclic animals. Immunofluorescence analysis for CTLA4 revealed an increase ($p<0.05$) in percent area labeled across UW in pregnant compared to D17C endometrium. We next determined if pregnancy associated glycoproteins (PAG) could affect immune cell function during this time. Results indicated a 200-fold increase in PAG concentrations in uterine flushes between D17P (n=5) and D20P (n=4) from 17 ng/mL to 3.2 Âµg/mL. To determine effects of PAG on immune cells, CD45+ immune cells from D17C endometrium (n=4) and blood (n=5) were labeled with carboxyfluorescein succinimidyl ester (CFSE) and proliferation in response to concanavalin A (ConA; 40 Âµg/mL) was assessed. The cells were treated with PAG (2 µg/mL, 20 µg/mL), interferon tau (IFNT; 10000 U/mL) or their combination for 72 hours. After culture, cells were labeled with CD3 antibody to determine the proliferation response of the T cell subset. Proliferation of CD45+ uterine immune cells and the T cell subset was reduced ($p<0.05$) in response to 2 and 20 µg/mL PAG compared to untreated controls. There was no effect of PAG or IFNT on uterine cell proliferation in the absence of stimulation. The effect of PAG on immune cell proliferation was specific to uterine CD45+ cells and was not detected in peripheral blood CD45+ cells from these heifers. In summary, the endometrium undergoes changes in immune regulatory proteins during early pregnancy. Some of these changes may be mediated by conceptus-secreted PAG, which can modulate immune cell proliferation.