Maternal infection with Bovine Viral Diarrhea Virus (BVDV) has life-long negative effects on progeny. Despite current preventative measures, BVDV continues to be an issue, costing the industry $1.5 billion annually and producing infected calves that remain the primary reservoirs of the virus. If fetal infection occurs prior to day 120 of gestation, then the fetus becomes persistently infected (PI) and sheds the virus throughout its life. The mechanisms of persistent infection and impact on postnatal health is still not well known. Previous in vivo studies revealed a substantial activation of the PI fetal innate immune response 22 days after maternal infection. The innate immune activation was then followed by an attenuation of both the innate and adaptive immune branches 115 days after maternal infection. It was concluded that attenuation of the immune system was caused by a lack of T-cell response in the fetus, resulting in an inability for T-cells and B-cells to mature properly. In this study, it was hypothesized that T-cell activation and signaling genes were epigenetically altered after fetal infection, thus impairing the expression of key genes of the innate and adaptive immune responses Splenic tissue from PI and control fetuses were collected on day 245 of gestation, 170 days post-maternal infection. DNA was isolated and sent to Zymo Research for reduced representation bisulfite sequencing. Methylation sequencing files were aligned to the bovine ARS-UCD-1.2 genome using the Bismark package, then processed and analyzed using the methylKit R package. Differentially methylated regions (DMR) were selected based on a 25% difference in methylation compared to controls as well as a p-value cutoff of < 0.05. Within these parameters, 2,641 regions were differentially methylated: 1,951 hypermethylated and 691 hypomethylated regions. Results revealed hypermethylation of nuclear factor of activated T cells (NFAT) 1 and 4, while NFAT2 was hypomethylated. Calcium signaling components, calcium release activated calcium channel protein ORAI and calmodulin, were hypomethylated. Additionally, signal transducer guanine nucleotide exchange factor VAV1 was hypermethylated. Calcium regulated NFAT family members consist of NFAT 1,2, and 4. The NFAT family is critical in T-cell activation/anergy as well as cardiac development. NFAT 1 and 4 are associated with T helper (Th) 1 cell differentiation, while NFAT 2 is associated with Th2 cell differentiation. Hypermethylation of NFAT 1 and 4 is likely to shift the Th cell differentiation from Th1 to Th2 cells. An increase in NFAT2 and VAV1 expression due to hypomethylation would promote anergy of T-cells, further exacerbating the shift from Th1 to Th2 cells. This shift of Th cells is associated with T-cell receptor hyper-
reactivity and lymphoproliferative disorder. Additionally, the hypomethylation of ORAI and calmodulin may contribute to the Th2 hyper-reactivity by increasing the amount of calcium transported into a cell upon T-cell activation. The observed epigenetic modification of critical T-cell genes may help explain inability of postnatal PI calves to fight secondary infections efficiently, contributing to performance loss and continued BVDV viral shedding. This work is supported by: USDA AFRI NIFA Predoctoral Fellowship 2019-67011-29539/1019321, 2016-38420-25289 and W3112 Project.