

# **Bovine Endometrial Response to Different Concentrations of Progesterone Before Estrus and Different Intensities of Estrous Expression**

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This study aimed to evaluate if different concentrations of progesterone before estrus and different intensities of estrous expression impact endometrial gene expression in lactating Holstein cows. A total of 125 lactating Holstein cows were randomly assigned into two experimental groups: Low progesterone (n = 64) and High progesterone (n = 61). Cows received a pre-synchronization protocol followed by the progesterone treatment (Low progesterone = received extra injections of PGF2 $\alpha$ ). Ovarian ultrasonography and blood samples were performed to monitor ovarian structures and progesterone levels. Physical activity was monitored by an activity monitor, and the duration and intensity of estrus were measured. A subsample of 33 cows (High progesterone: n = 16; Low progesterone: n = 17) were submitted to uterine biopsy 7 and 14 d after identified estrous event. Total RNA was extracted from each sample and NanoString nCounter system was used to determine the abundance of specific mRNA molecules for 92 genes. The data was normalized to housekeeping genes and analyzed using mixed linear regression models including the progesterone levels post estrus as fixed effect and cow as random effect nested within day. Adjusted *P*-values were calculated using Bonferroni correction. Preliminary analysis suggested that greater progesterone concentrations before estrus as well as greater intensity and duration of estrus were associated with changes in endometrial gene expression of lactating Holstein cows. These modifications were linked to significant changes in the normalized number of reads for a total of 26 and 47 gene transcripts on days 7 and 14, respectively ( $P < 0.05$ ). On day 7, molecular functions represented often in the list of increased gene transcripts included: eicosanoid metabolic process (AKR1C4, HPGD, PTGIS, AKR1B1), growth factor signalling (IGF1, IGF2, MEP1B, EGF), steroid signalling (PGR) and Wnt signalling (WNT5A, WNT6, WIF1). On day 14, genes with an increased number of reads and their functions included: growth factor signalling (IGF1, IGF2, MEP1B), steroid signalling (PGR, PGRMC1, PGRMC2) and nutrients/solute transport (FOLR1, FABP, SLC27A6, SSLP1, SLC27A6, CYP26A1). A considerable number of gene transcripts associated with immune response were included in the list of genes with decreased expression on day 14 (IL1B, CCL2, NFKB2, CCL4, CXCL8, IL1A, CXCL12, PTX3, CCL17, CCL22). Interestingly, a significant interaction between estrous expression and the progesterone treatment was found for several gene transcripts ( $P < 0.05$ ). On day 7, greater intensity and duration of estrus were found to increase the number of reads for genes related to growth factor signalling (IGF2, MEP1B) and nutrients/solute transport (FOLR1, SLC1A4, CYP26A1) only in low progesterone treatment. Similarly on day 14, greater intensity and duration of estrus increased the number of reads for genes related to steroid signalling (PGR, PGRMC2) and nutrients/solute transport (FOLR1, FABP, SLC1A4, SSLP1, SLC6A20), whereas it decreased the number of reads for genes associated with the immune response (CCL2, CCL4, CXCL8, IL1A, CCL17) only in low progesterone treatment. Together, greater progesterone concentrations before estrus and greater intensity and duration of estrous expression seem to be promoting changes in endometrial gene expression that might be related to the uterine support for early embryonic development. Additionally, when the levels of progesterone before estrus is low, estrous expression seems to be able to promote changes in endometrial gene expression that may favour the early development of the embryo. This study was supported by the 'Resilient Dairy Genome Project' and NSERC.