Transcriptomic Analysis of Luteal Tissue Supports the Delayed Timing of Luteolysis in Heifers with Greater Ovarian Reserve

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Antral follicle count (AFC) is reflective of ovarian reserve and linked to reproductive performance in mammalian females. We previously demonstrated a delayed upregulation of endometrial oxytocin receptors in heifers with greater ovarian reserve, indicating delayed induction of luteolysis. Luteal abundance of selected genes of the luteolytic cascade were investigated to further support these findings.

We hypothesized that in response to luteolytic signals, mRNA transcript abundance of thrombospondin-1 (*THBS1*) will be increased and luteinizing hormone/choriogonadotropin receptor (*LH/CG-R*) will be decreased in the corpus luteum (CL) of open heifers, with a pronounced effect in heifers with diminished ovarian reserve. Furthermore, we hypothesized that the increase of luteal interferon-stimulated gene 15 (*ISG15*) and interferon-induced GTP-binding protein Mx1 (*MX1*) transcript abundance in pregnant heifers is greater in animals with increased ovarian reserve.

Based on rectal ultrasonography, beef heifers were chosen from the top 10% (high AFC, 33.5±8.4, n=40) and bottom 10% (low AFC, 14.4±2.9, n=40) of the population distribution. All heifers were inseminated (d0) following a 7d-CO-Synch protocol. On day 15 or 16 after insemination, reproductive tracts were collected and flushed. Pregnancy status was defined by presence or absence of a conceptus. Corpora lutea were isolated, weighed and snap-frozen. Transcript abundance of *ISG15*, *MX1*, *THBS1* and *LH/CG-R* was determined by RT-qPCR from heifers with a single CL. Circulating progesterone concentrations were determined via radioimmunoassay. Data were analyzed using the MIXED procedure of SAS with AFC group, pregnancy status and their interaction as fixed effects and day as a covariate, followed by Tukey's test.

Progesterone concentrations tended to be greater in high vs low AFC heifers (9.3±1.1 vs 6.5±1.2 ng/ml, $P<0.09$) and pregnant vs open heifers (9.5±1.1 vs 6.4±1.2 ng/ml, $P<0.06$), but were not influenced by their interaction. Luteal weights and transcript abundance of *ISG15* and *MX1* were not affected by pregnancy status, AFC group or their interaction. Transcript abundance of *THBS1* was upregulated in open vs pregnant animals (8.4±1.2 vs 3.7±0.5-fold, $P<0.0001$) and showed greater abundance in low vs high AFC heifers (7.3±1.1 vs 4.3±0.6-fold, $P<0.01$). THBS1 expression was not influenced by the interaction of AFC group and pregnancy status.

An interaction of AFC group and pregnancy status was identified ($P<0.05$) for *LH/CG-R* abundance, with greater transcript abundance in pregnant vs open (228.5±39.0 vs 88.5±16.2-fold) and in high vs low AFC heifers (199.5±33.5-fold vs 101.3±18.7-fold). Open low AFC heifers showed the lowest *LH/CG-R* transcript abundance (46.7±13.5-fold, $P<0.01$).

These results suggest that day 15 and 16 might be too early to detect differences in luteal response to conceptus derived interferon tau in heifers differing in pregnancy status and/or AFC. The transcript patterns of *THBS1* and *LH/GC-R* support the concept of a delayed induction of luteolysis in open heifers with greater ovarian reserve. This may contribute to an extended window for maternal recognition of pregnancy and result in superior reproductive

performance in heifers with greater ovarian reserve. USDA is an equal opportunity provider and employer.