

Cervical *IGS15* and *PGR* mRNA expression during early pregnancy in Holstein heifers

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We aimed to determine the effect of pregnancy on gene expression of Interferon-stimulated gene 15 (*ISG15*) and progesterone receptor (*PGR*) in the cervix of Holstein heifers. Sixteen heifers were synchronized with two injections of prostaglandin (Estrumate[®], MSD, Argentina) separated by eleven days. Animals were sham-inseminated with semen extender only (open, n=6), or inseminated with frozen semen from one bull and were diagnosed pregnant by ultrasound at day thirty post insemination (pregnant, n=10). Blood by coccygeal venipuncture and cervical cells samples using a cytological brush were obtained at 14, 16, and 18 after insemination. Serum progesterone (P4) was determined by a solid-phase radioimmunoassay using a commercial kit. Total RNA was obtained with Trizol reagent (Life Technologies) and the concentration and purity of the RNA was determined using a spectrophotometer (nanodrop ND 1000). Total RNA was treated with DNase using a DNA-free[™] kit (Ambion, Austin, TX, USA). For each sample, copy DNA (cDNA) was synthesized by reverse transcription using a SuperScript III transcriptase (Invitrogen) with random primers and total RNA as a template. *ISG15* and *PGR* gene expression was determined by real-time PCR (qPCR) using the Rotor-Gene[™] 6000 kit (Corbett Life Sciences, Sydney, Australia). Gene expression was calculated by relative quantification to the exogenous control (β -actin) and normalized to the geometric mean of the endogenous control, taking into account the respective amplification efficiencies. Serum progesterone concentrations and the transcripts determined by real-time PCR were analyzed by a Glimmix procedure (Proc Glimmix; SAS Studio[®]) including group, day and their interaction as a fixed effect. Significance was considered with $\alpha \leq 0.05$, and tendency between 0.05 and 0.10. Serum progesterone concentrations were higher in pregnant than open heifers (6.5 ± 0.48 vs 4.2 ± 0.57 ng/mL, $P=0.0077$). There was no effect of day or the interaction between group and day on progesterone concentrations. The cervical expression of *ISG15* mRNA was greater in pregnant than open heifers (1.76 ± 0.46 vs 0.13 ± 0.04 , respectively, $P<0.0001$), being different in all days (Fold-Change: 12.0, 21.5, and 9.4, on days 14, 16, and 18, respectively). There was an interaction between group and day ($P=0.033$), as pregnant heifers presented greater *ISG15* mRNA expression on day 16 than on day 14 and 18 ($P<0.05$), but no differences according to the day were found in open heifers. The *PGR* mRNA expression tended to be affected by the interaction between group and day ($P=0.0779$), while open heifers increased during the estrous cycle, pregnant heifers maintained *PGR* expression. We concluded that the cervical expression of *ISG15* mRNA could be used as a pregnancy biomarker as early as day 14 post insemination.