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Embryology, developmental biology, and physiology of reproduction

Expression of RNAm for galectin 3 binding protein in the circulating immune cells and vaginal mucosa cells during early pregnancy in beef heifers**Thais Sayuri Imura Oshiro¹, Cecilia Constantino Rocha², Gabriel Dalmaso de Melo², Leonardo Marin Ferreira Pinto², Amanda Guimarães Silva², Guilherme Pugliesi²**¹UNIMAR - Universidade de Marília (Av. Higino Muzi Filho, 1001 - Mirante, Marília - SP); ²USP - Universidade de São Paulo (R. Duque de Caxias, 225 - Jardim Elite, Pirassununga - SP).

Galectin 3 binding protein (*LGALS3BP*) is a molecule stimulated during early pregnancy and has a crucial role in the process of cell adhesion in the bovine endometrium. We aimed: 1) to evaluate the expression of *LGALS3BP* transcript using vaginal cytology as an alternative to indicate pregnancy status; and 2) to compare this method with the isolation of peripheral polymorphonuclear blood cells (PMN) to determine *LGALS3BP* expression on day 20 after timed-AI (TAI) in beef heifers. Nelore heifers (n=31) weighting 422 ± 47.8kg were submitted to an E2/P4 based protocol to synchronize ovulation for TAI (D0). On days 16, 18 and 20, samples of vaginal cells were collected using cytological brush (Cytobrush; Viamed Ltd, West Yorkshire, UK), which was placed in the fornix surrounding the external cervix os to recover the superficial cells from vagina, in which the samples were stored in liquid nitrogen until RNA extraction. On D20, blood samples collected from jugular vein (30ml) were used for PMN isolation (Zymo Research, CA, USA). The presence of an embryo with heartbeat on D30 indicated if the animals were Pregnant (Preg n=16) or Non-Pregnant (NPreg n=15). Extracted RNA was treated with DNase I for genomic DNA contamination before synthesis of complementary DNA (cDNA) using 1000ng (Cyto) and 500ng (PMN) of total RNA. The *LGALS3BP* expression was evaluated by RT-qPCR in the vaginal cell samples on D16, 18 and 20 (n=6 Preg and 7 NPreg), and on D20, the relative *LGALS3BP* expression between Preg and NPreg heifers was compared between the vaginal cells and PMN (n=14 Preg and 13 NPreg/cell type). Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) and Actin Beta (*ACTB*) were used for normalization of relative expression in both cell types. For the comparison between the methods, the relative *LGALS3BP* expression on D20 in each Preg heifer was divided by the averaged expression in the NPreg. The results were analyzed by ANOVA and PROC MIXED procedure (SAS), considering the main effects of group (G), time (T) and its interaction (TG). No significant effects (P>0.75) of G, T and TG were observed for the *LGALS3BP* expression in vaginal cells from D16 to 20. Also, when evaluated on D20 with a large number of animals, no significant difference was observed in *LGALS3BP* expression between Preg and NPreg for the vaginal cells (relative expression to reference genes: 0.018±0.007 vs 0.004±0.001 P=0.29) and PMN (0.013±0.002 vs 0.01±0.003 P=0.46). When compared the relative *ISG15* expression in the Preg to NPreg group in the two cell types, no difference (P=0.43) was found between the PMN (fold change 1.22) and vaginal cells (fold change 4.23). The coefficient of variation within each group in samples analyzed on D20 ranged from 77% to 131% for vaginal cells, and 53% to 73% for PMN. In conclusion, the high variation in *LGALS3BP* expression within Preg and NPreg heifers impairs its use as an effective pregnancy predictor in cattle. Acknowledgments FAPESP (Grant number: 2019/05926-5).