



Presence of bovine conceptus modulates interferon-tau stimulated genes expression in peripheral blood polymorphonuclear cells at the beginning of pregnancy

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We aimed to compare the expression of type I interferon stimulated genes (ISGs) in peripheral blood polymorphonuclear cells (PMNs) between pregnant and non-pregnant heifers on days 14 and 18 after FTAI. Twenty-six Nelore heifers were synchronized by pharmacological treatment based on P4 and E2, and the day of FTAI was designated as D0. Pregnancy diagnosis was made by ultrasonography on days 25 and 28 through the detection of the embryonic vesicle and heartbeats. On days 14 and 18, 25mL of blood was collected in heparinized tubes by puncture of the jugular vein for the isolation of PMNs. The isolation was made by Ficoll®Paque Plus gradient (GE Healthcare – São Paulo, Brazil), in an adapted methodology from that described by Jiemtaweeboon et al. (Reproductive Biology and Endocrinology, 9: 79-89; 2011). PMNs samples from 5 pregnant and 6 non-pregnant heifers were subjected to total RNA extraction using the Direct-Zol RNA Miniprep kit (Zymo Research- Irvine, USA) according to the manufacturer's instructions. Five reference genes (GAPDH, PPIA, 18S, RPL30 and ACTB) were quantified by real-time polymerase chain reaction (qPCR), and through the NormFinder software, the two genes with most stable expression (GAPDH and ACTB) were selected. The expression of the target genes (OAS-1, MX2 and ISG15) evaluated by qPCR was normalized in relation to the two reference genes by the comparative Ct method (Pfaffl, Nucleic Acids Research, 29:2001-2007, 2001). The abundance of transcripts was evaluated by analysis of variance (ANOVA) with repeated measures of time, considering the random effect of the heifer and the fixed effects of the group (pregnant or non-pregnant), day and group by day interaction using the PROC MIXED SAS software (Version 9.2; SAS Institute). For the OAS-1 gene, effects of time ($P=0.03$), group ($P=0.03$) and interaction between time and group ($P=0.0002$) were detected. The abundance of this transcript did not differ ($P=0.88$) between pregnant (1.43 ± 0.76) and non-pregnant (1.09 ± 0.32) females on D14, but it was higher in pregnant heifers on D18 ($P=0.0003$; 7.11 ± 3.24 vs. 0.30 ± 0.07). There was a 7-fold increase ($P=0.02$) in the expression of this transcript between D14 and D18 in the pregnant heifer group, while in non-pregnant, there was a 0.57-fold decrease ($P=0.05$). A group effect ($P=0.03$) was observed for the MX2 and ISG15 genes, indicating a 3 and 7-fold increase, respectively, in the abundance of these transcripts in pregnant females. It is possible to conclude that a viable bovine conceptus stimulates the greater abundance of transcripts for the evaluated ISGs in circulating PMNs, with a most evident effect on D18 for the OAS-1 gene. Further studies are needed to elucidate the expression profile of ISGs in PMNs aiming the use of these genes as possible markers of gestation and as a form of early pregnancy diagnosis.

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