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Profile of new early pregnancy markers identified by transcriptomic analysis in peripheral blood immune cells in beef heifers

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We aimed with this study in pregnant (P) and non-pregnant (NP) heifers: 1) to discover new pregnancy markers (PM) by RNA sequencing (RNAseq) in peripheral blood mononuclear cells (PBMC) on day 18 post-AI; and 2) to assess the mRNA profile of new PM in PBMC and peripheral blood polymorphonuclear cells (PMN) at early pregnancy. Nelore heifers (N=21) were AI in fixed-time (D0). On D10, 14, 16, 18 and 20, blood was collected for isolation of PBMC and PMN, and P4 concentration assay and color Doppler ultrasonography was performed to evaluate the corpus luteum (CL). Pregnancy diagnosis was done on D28 and heifers were *classified* in P (N=9) and NP (N=12). Heifers (N=6/group) with different (P<0.05) plasma P4 concentration, CL area and blood perfusion on D18 were selected and RNAseq was done on PBMC samples. RNAseq analysis indicated 220 differentially expressed genes (200 up regulated in P). Twenty genes found on RNAseq of PBMC with the highest fold-changes or no overlapping between P and NP, were assessed by qPCR from D10 to 20 (N=6/group). Reference genes were used for expression normalization (*GAPDH* and *PPIA*). Data were analyzed by ANOVA using the PROC MIXED procedure (SAS) and considering the main effects of group (G), time (T) and their interaction (GT). For PBMC, G and T effects (P<0.1) and GT interaction were observed for *IFI6*, *RSAD2*, *IFI44*, *IFITM2*, *TNFSF13B* and *LGALS3BP*, reflecting a greater (P<0.1) expression in the P group on D18 and D20 for *IFI6*, *RSAD2*, *IFI44* and *IFITM2*, and on D16 and D18 for *TNFSF13B*. For *CLEC3B*, *OAS2* and *LOC100139209*, a T effect (P<0.05) and GT interaction (P<0.1) were detected, reflecting a greater (P<0.05) expression in the P group on D20 for *OAS2* and *CLEC3B*. For *DMKN*, a GT interaction (P<0.05) reflects an increase on D16 in the P group. For *A2M*, *BPI*, *ANG*, *PLSCR2*, and *DRAM1*, only a T effect (P<0.05) was observed, reflecting a progressive increase from D10 to D20. For *LIG1*, a greater (P<0.1) expression was observed in the NP group from D10 to D20. For PMN, a T effect and GT interaction (P<0.1) were observed for *IFI44*, *RSAD2*, *OAS2* and *LGALS3BP*, reflecting a greater expression in the P group on D18 and 20 for *RSAD2* and *LGALS3BP*, and on D20 for *IFI44* and *OAS2*. An interaction (P<0.05) was also detected for *IFI6*, *C1R*, *RHOT1* and *LIG1*, indicating an increase in the P group on D16, D18 and D20, respectively, for *RHOT1*, *C1R* and *IFI6*, and a decrease in *LIG1* expression in NP group on D20. However, no effect (P>0.1) was observed for *SIGLEC1*, *SORD*, *C1R* and *RHOT1* in *PBMC* and for *IFITM2* in PMN. In conclusion, 9 genes presented increased expression on PBMC of P in at least one-time point from D16 to D20; but only 4 of these genes retained the expression increased on PMN (*IFI6*, *IFI44*, *RSAD2* and *LGALS3BP*). Thus, results indicate potential genes to be used as novel pregnancy predictors in immune cells in cattle at early gestation. Acknowledgments: FAPESP (2015/10606-9; 2017/13994-5).