Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Embryology, developmental biology, and physiology of reproduction

Use of different methods to assess abundance of genes stimulated by the conceptus 20 days after IATF in dairy cattle

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We aimed: 1) to evaluate the abundance of two genes stimulated by the conceptus using samples collected by four methods (peripheral blood mononuclear cells [PBMC], whole blood, cervical cytology or immune cells from milk); and 2) to compare these four methods as pregnancy predictors on day 20 after timed-AI (TAI) in dairy cattle. Eighteen Holstein females (12 cows and 6 heifers) with BCS of 3.1±0.3 (1 to 5 scale), were submitted to an E2/P4-based protocol to synchronize ovulation for TAI (D0). On D20 post-TAI, blood samples were collected from coccygeal vessels for isolation of PBMC and in Tempus Blood RNA® tubes. Isolation of PBMC was performed by Ficoll® Paque Plus gradient. Samples of cervical cytology were collected using a cytological brush. Milk samples were collected before routine milking in cows, and immune cells were isolated as proposed by Schanzenbach et al. (Plos One, 12: 2, 2017). The RNA from PBMC, cervical cytology, and milk were extracted using Trizol® Reagent according to manufacturer's instructions. Pregnancy diagnosis was performed on D30 using transrectal ultrasonography and females were classified as pregnant (P; n=8) or non-pregnant (NP; n=10). Expression of target genes (*ISG15* and *LGALS3BP*) was quantified by RT-qPCR and normalized in relation to the reference genes (*GAPDH* and *PPIA* for PBMCs; and *GAPDH* and *ACTB* for whole blood, cervical cytology and milk). Data were analyzed by ANOVA using the PROC MIXED procedure (SAS). Abundance of *ISG15* was greater in the P group than in NP group for PBMC (0.08 ± 0.01 vs. 0.03 ± 0.01; P=0.004), whole blood (0.024 ± 0.003 vs. 0.014 ± 0.004; P=0.04) and cervical cytology (0.41 ± 0.12 vs. 0.13 ± 0.08; P= 0.04). No difference (P>0.58) was detected for milk samples between the P and NP groups. For *LGALS3BP* abundance, no difference was detected between P and NP groups for PBMC (P=0.31), whole blood (P=0.43), and milk (P=0.65), but a tendency for greater abundance in P group was observed for cervical cytology (0.035 ± 0.006 vs. 0,021 ± 0.004, P=0.07). When the fold change between the *ISG15* abundance in P and the mean of NP animals was compared among the four methods, a greater (P<0.001) fold change was observed in cervical cytology (3.22 ± 1.54) than in the PBMC (2.75 ± 0.26), whole blood (1.71 ± 0.25) and milk (0.005 ± 0.002). ROC curve analysis indicated that *ISG15* abundance was a significant (P<0.001) predictor of pregnancy in PBMC (AUC= 0.92), but not in whole blood (AUC=0.68, P=0.16), cervical cytology (AUC = 0.71, P=0.42) or milk (AUC=0.42, P=0.64) methods. In conclusion: I) milk method is not a good indicator of genes stimulated by pregnancy; II) although an increased *ISG15* abundance is observed in P dairy females for whole blood and cervical cytology, the PBMC method is the best pregnancy predictor on D20 post-TAI; and III) the use of *LGALS3BP* abundance for determination of pregnancy status is not indicated for any method. Acknowledgments: FAPESP (2015/10606-9; 2019/16040-8).