Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Extracellular vesicles from blood serum and uterine fluid are modified by endometritis and negative energy balance in dairy cows

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Dairy cows usually present a negative energy balance (NEB) and uterine diseases during the post-calving period, which has a significant economic impact due to the reduction in animals available to the next reproductive season. Extracellular vesicles (EVs) are mediators of intercellular communication and can modulate several reproductive processes. The aims of this study were: 1) to investigate if EVs present in the serum of animals 7 days post-calving can predict animals with endometritis at 30 days post-calving and; 2) evaluate if NEB intensity modifies EVs miRNA contents present in the uterine fluid of dairy cows at 60 days post-calving. First, blood samples were collected from individual dairy cows at weeks 1 and 2 post-calving. Serum samples were subjected to metabolite analysis to determine the NEB intensity (Low NEB: NEFAs 0.3 to 0.8 mmol/L and BHB 0.55 to 1.1 mmol/L; High NEB: NEFAs ≥ 0.9 mmol/L and BHB ≥ 1.2 mmol/L). At 30 and 60 days post-calving animals were evaluated and a total of six animals presented uterine alteration out of 13 animals total. Healthy animals (n=7, being 3 Low and 4 High NEB) had the estrous cycle synchronized and on Day 5 after artificial insemination (AI) the collection of uterine fluid (UF) was performed. EVs isolated were analyzed for particle and concentration by nanoparticle tracking analysis. Total RNA from uterine fluid EVs from dairy cows with Low and High NEB was isolated and analyzed for the relative expression levels of 383 miRNAs. Differences between treatments were assessed by Student's t-test. The EVs mean particle concentration at week 1 post-calving decreased in the endometritis compared to the control group (Control: 343 x10^8 ± 107.6x10^8 particles/mL; Endometritis: 135.3 x10^8 ± 16.3x10^8 particles/mL) and the mean showed no difference (Control: 156.8 ± 15.8 nm; Endometritis: 160.9 ± 7.0 nm). No differences were identified in EVs mean particle concentration isolated from uterine fluid (Low NEB: 57.3x10^8 ± 18.4x10^8 particles/mL; High NEB: 25.7x10^8 ± 6.5x10^8 particles/mL) or mean (Low NEB: 189.52 ± 5.6 nm; High NEB: 192.7 ±4.2 nm) between the groups. EVs isolated from uterine fluid 60 days post-calving presented 316 miRNAs in common between the two groups and one (miR-369-3p) exclusive to the Low NEB group. A total of 28 miRNAs were differently expressed being 27 upregulated in Low NEB and 1 upregulated in High NEB. The miR-369-3p, which is exclusive in Low NEB, is related with decrease inflammatory response, which may suggest that animals with low NEB have a more favorable uterine environment to receive an embryo at 60 post-calving days. In conclusion, the EVs evaluation in serum can help in the early diagnosis of endometritis as well as the understanding of the NEB intensity modulation of the uterine environment at 60 post-calving days in dairy cows, which can help decide the best time for the uterus to receive an embryo.

Financial support: FAPESP grants 2020/13075-2 and 2021/06645-0.