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Embryology, developmental biology, and physiology of reproduction

High body energy reserve cows have different mirnas profile in isthmus oviductal cells during early embryonic development

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Body condition score (BCS) can influence reproductive performance by changes in the follicular and oviduct environment, impacting reproductive efficiency by modulation of important physiological pathways within the oviduct affecting early embryonic development. Thus we tested the hypothesis that cows with high or moderated body energy reserves have different miRNAs profile in isthmus oviductal cells. For this, a homogeneous group of dry Nelore cows ($495,67 \pm 39,66$ Kg and $BCS=5,5 \pm 0,48$, in a 1 to 9 scale) were fed in a calan gate system for ~100 days and randomly assigned to 1 of the 2 feeding treatments: 1) cows receiving an *Ad libitum* diet to increase body energy reserves (HBER) and 2) cows fed 70% of the HBER group ingestion to maintain body weight and BCS (MBER). Animals were submitted to fixed-time artificial insemination and ~120 hours after ovulation induction, cows were slaughtered. Next, the ipsilateral oviduct to the corpus luteum was collected and dissected. Isthmus luminal epithelial cells were obtained only from animals whose embryos were recovered at the 8-cell stage (HBER n=3; MBER n=3). At the end of the feedlot period, body weight was $642,33 \pm 50,41$; $482 \pm 38,76$ Kg and $BCS 6 \pm 0,57$, $4,66 \pm 0,33$ in HBER and MBER, respectively. Additionally, animals presented similar serum biochemical profile. Mature miRNAs were reverse transcribed using miScript HiSpec Buffer and the profile of 383 bovine mature miRNAs was analyzed by qPCR in isthmus cells obtained from both groups. The relative levels were evaluated and data normalized by the geometric mean of miR-99b and Hm/Ms/Rt U1 snRNA. To identify differential miRNA expression between groups, Student's t-test was performed ($p<0,05$). A total of 243 miRNAs were identified in both groups; out of these, six miRNAs (miR-148a, miR- 192, miR-28, miR-296-5p, miR-664b and miR-1271) were upregulated in HBER group, and two miRNAs (miR-190a and miR-378d) were increased in MBER group. Bioinformatics analysis using miRWALK 3.0 platform demonstrated that the upregulated miRNAs in HBER group are predicted to modulate signaling pathways such as MAPK (77 genes), RAS (68), Hippo (45), Insulin (42), and Insulin Resistance (30). Moreover, the increased miRNAs in MBER group are predicted to regulate RAS (37 genes), WNT (25), mTOR (22), FoxO (19), and Insulin (19) pathways. These results show that high BER conditions modify the isthmus cell miRNAs profile when compared with moderate BER cows. Furthermore, pathways related to cell proliferation, differentiation and metabolism, such as Insulin signaling, are predicted to be regulated by miRNAs in both groups. However, the Insulin Resistance pathway is predicted to be regulated only by the HBER group. Thus, we suggest that high BER can metabolically modulate the oviduct tissue and possibly modulate important signaling pathways affecting early embryonic development and reproductive efficiency in cows. Funding CAPES (001), FAPESP (2014/22887-0), CNPq (#420152/2018-0).