Animal Reproduction

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Folliculogenesis, oogenesis and superovulation

Effect of palmitic acid on the miRNA biogenesis from bovine cumulus cells

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Resumo

The postpartum negative energy balance (NEB) is an important risk factor in the establishment of reproductive failure in high-producing dairy cows. In this period an excessive mobilization of body reserves increase serum and follicular fluid concentrations of non-esterified fatty acids (NEFAs), such as palmitic acid (PA). Such increased plasma NEFAs concentrations induce changes in the microenvironment of the ovarian follicle, which may alter the pattern of epigenetic marks in cumulus cells, oocytes, and embryos. However, the complete mechanism has not been fully elucidated. MicroRNAs (miRNAs) are a class of non-coding RNAs that play important roles in regulating gene expression. MiRNAs biogenesis is regulated at multiple levels, including at the level of miRNA pre- and posttranscriptional processing and their dysregulation is associated with many diseases. The aim of the study was to investigate the effect of PA on miRNAs biogenesis in bovine cumulus cells. Bovine ovaries were obtained from a local abattoir and the cumulus-oocyte complexes (COCs) were aspirated and classified according to the morphology of oocyte and cumulus cells. Grade 1 and 2 COCs were matured in groups of 15 - 20 in a 500 µL serum-free maturation medium (containing TCM 199 supplemented with human recombinant FSH (hrFSH), pyruvate, gentamicin, BSA, AREG, IGF-1, progesterone and estradiol) in four-well plates for 24 h in humidified air with 5% CO2 at 38.5o C. COCs were exposed during IVM to the following conditions: Control (physiological PA concentration – 23 µM) and High PA (PA equivalent to that measured in follicular fluid during NEB – 150 µM). The PA was dissolved in ethanol according to the recommended solubility and the concentrations used in this study are based on bovine in vivo studies in follicular fluid during a period of NEB (Leroy et al., Reproduction, 4:485-95, 2005). Cumulus cells were submitted to total RNA extraction using QIAzol, followed by DNase treatment and cDNA synthesis using High Capacity cDNA Reverse Transcription Kit. The relative gene expression of DGCR8, DROSHA, XPO5, DICER1, TARBP2, PRKRA, and AGO2 were determined using three genes (RPL15, PPIA, and YWHAZ) as references. Expression levels were calculated using the 2-ΔCt method and differences in continuous data between treatments were assessed by Student's t-test. A level of 5% significance was used. Maturation rates were similar between groups. High PA concentrations during IVM significantly increased DICER1 relative expression compared to the control. DICER1 cleaves pre-miRNA into miRNA in the cytoplasm; transforming precursor in mature miRNA and dysregulation of this enzyme can affect miRNA functionality. In conclusion, exposure to high PA concentration during maturation can affect miRNAs due to altered DICER1 levels in bovine cumulus cells, suggesting that miRNAs are involved in NEB response.

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