A172 Physiology of Reproduction in Male and Semen Technology

# miR-216b abundance level is different between embryos produced from high and low fertility bulls

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The aim of this study was to investigate miRNAs profile in sperm cells of bulls that present high and low field fertility. For that, six semen commercial batches provided by Alta Genetics® (Uberaba, Brazil) were used. The semen batches were obtained from six Aberdeen Angus bulls (*Bos taurus*) classified as high fertility (HF, n=3, 54.3±1.0%) and low fertility bulls (LF, n=3, 41.5±2.3%) based on field pregnancy rates (P=0.007). Two straws from each batch were thawed (37°C/30s) and analyzed regarding morphological and functional sperm features: motility characteristics using Sperm Class Analyzer (SCA, Microptics, Barcelona, Spain) software; sperm abnormalities by differential interference contrast microscope (Nikon 80i, Tokyo, Japan); and sperm plasma and acrosome membranes integrity and high mitochondrial membrane potential by fluorescent microscopy (Nikon 80i, Tokyo, Japan). The sperm profile of 380 bovine mature miRNAs was analyzed using real time PCR with 100ng of total RNA from each sperm sample using miScript PCR® (Qiagen) according to manufacturer's instructions. Two miRNAs consistently detected among the samples (bta-miR-99b and -425-5p) were used to evaluate the relative abundance levels of the miRNAs. Only miRNAs with Ct value lower than 38 were considered for analysis. Afterwards, sperm miRNAs with P<0.1 in the relative abundance level between high and low fertility bulls were analyzed in mature oocytes (n=6) and in zygotes (n=18) fertilized *in vitro* using sperm from the same batches previously evaluated. Data were analyzed by ANOVA using Mixed procedure of SAS® and means compared with Tukey test when necessary. Except to sperm miRNAs analyses, statistical difference was considered when P<0.05. Sperm samples from high and low fertility bulls were similar (P>0.05) in all morphological and functional features evaluated. Sperm miRNAs analyses detected 14 miRNAs with different abundance levels (P<0.1) between high and low fertility bulls. Among the evaluated miRNAs, bta-miR-216b presented different (P=0.01) abundance level between zygotes derived from high fertility sperm samples, zygotes from low fertility sperm samples and oocytes. The relative abundance level of this miRNA was high in zygotes from LF and low in zygotes from HF and oocytes. In sperm cells, bta-miR-216b levels were higher (P=0.08) in LF than in HF. Bioinformatics analyses performed using DAVID Functional Annotation Tool (DAVID Bioinformatics Resources 6.7) predicted the regulation of important signaling pathways including Wnt, MAPK and focal adhesion. These findings suggest that miR-216b is a potential biomarker for bull fertility that is important for initial embryo development. Our group has been engaged to describe the role of miR-216b on embryo development.

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