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Recipient cows carrying IVF or SCNT conceptus have different miRNAs profile in the corpus luteum during maternal recognition of pregnancy

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In mammals, corpus luteum (CL) secretes P4 that is necessary to establish and maintain pregnancy. Normal luteal function requires an intricate molecular regulation, which involves transcription and translation of several genes. MicroRNAs are small non-coding RNA molecules involved in post-transcriptional regulation of target genes leading to mRNA degradation or translation repression and may play a role in regulating CL function during maternal recognition of pregnancy. Previous studies have demonstrated an increased rate of embryo loss in SCNT due to embryonic developmental problems leading to impaired pregnancy recognition. This study tested the hypothesis that IVF and SCNT-derived conceptuses elicit a different miRNA profile in the corpus luteum of recipient cows. Slaughterhouse ovaries were collected to make SCNT embryos. IVF embryos were performed by commercial laboratory. On day 7, Nelore cows previously synchronized received one IVF or SCNT embryo. The CL samples from six cows were collected on day 19 of pregnancy, in animals carrying a single embryo produced by IVF (n=3) or SCNT (n=3). P4 concentration in serum were measured on days 9, 14 and 19 following embryo transfer. Total miRNA reverse transcription for mature miRNAs was performed in CL samples from recipient cows carrying IVF or SCNT embryos using miScript HiSpec Buffer. We evaluate the relative levels of 384 bovine miRNAs. Geometric mean of miR-99b, RNU43 snoRNA and Hm/Ms/Rt U1 snRNA was used to normalize the data and differences in relative levels were determined by Student's t-test. Progesterone concentration in serum was similar between IVF (11.40 ± 0.5ng/mL; 12.90 ± 2.1ng/mL; 16.97 ± 2.28ng/mL) and SCNT (10.97 ± 4.32ng/mL; 17.07 ± 6.87ng/mL; 16.40 ± 2.40ng/mL) groups on days 9, 14 and 19, respectively. A total of 288 mature miRNAs were identified in IVF and SCNT CLs, with 10 miRNAs differently detected between the groups. In CL from SCNT pregnancies, 8 miRNAs were downregulated, while 2 miRNAs were upregulated when compared with CL from IVF group. Bioinformatics analysis with DIANA (TarBase v7.0) was used to determine enriched pathways regulated by these miRNAs. The increased miRNAs (miR-130b, miR-149-5p, miR-222, miR-218, miR-454, miR-485, miR-490 and miR-584) in CL from IVF group regulated signaling pathways such as FoxO, TNF, estrogen, TGF-beta, mTOR and steroid biosynthesis. Furthermore, bta-miR-101 and bta-miR-33a, that were upregulated in CL from SCNT modulates MAPK, FoxO and TGF-beta pathways. Some individual miRNAs were previously identified as involved in modulation of angiogenesis, steroidogenesis and CL regression. The results suggest that on day 19, molecular changes may occur in CL of IVF and SCNT embryo recipients cows possibly caused by conceptus of different origins and may contribute to impaired pregnancy recognition during this time.

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