Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Male reproductive physiology and sperm technology

Delivery of exogenous sperm microRNAs increases cleavage rates and change gene expression in embryos from low IVP fertility bulls.

Thais Rose dos Santos Hamilton [1], Camilla Mota Mendes [1], Marcelo Demarchi Goissis [1], Juliano Coelho da Silveira [2], Mayra Elena Ortiz D'Ávila Assumpção [1]

[1] FMVZ USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (Av. Prof. Dr. Orlando Marques de Paiva, 87 Cidade Universitária Armando de Salles Oliveira Butantã - São Paulo - SP), [2] FZEA USP - Faculdade de Zootecia e Engenharia de Alimentos (Av. Duque de Caxias Norte, 225 - Campus Fernando Costa, Pirassununga - SP.)

Resumo

Different bulls present high or low in vitro fertility. This lower efficiency on in vitro embryo production (IVP) may represent financial losses and delays in breeding programs. Sperm RNAs play roles in early embryo development by the action of non-coding RNAs, such as microRNAs, which could influence bull fertility. Recently, four microRNAs were identified exclusively in sperm from high IVP fertility bulls (Hamilton et al. Reproduction, Fertility and Development, 33:157, 2021). We hypothesized that these microRNAs could improve in vitro embryo development of low IVP fertility bulls. We performed two functional experiments called Rescue and Proof of Principle experiments with 6 Nellore bulls, 3 with high and 3 with low IVP fertility, retrospectively selected from commercial IVP manipulations (n=7000) from 2016 to 2018. In the rescue experiment, we performed IVP using the 3 bulls with low IVP fertility and the zygotes, 18 h after IVF, were microinjected with 5 to 7 pl mimics of the 4 miRNAs (100 nM, miRCuryLNA®, miScript®, Qiagen - mimic group). In the proof of principle experiment, we used the other 3 bulls with high IVP fertility, and then the zygotes were microinjected as above with inhibitors of the same 4 miRNAs (inhibitor group). Zygotes in control groups were microinjected with negative control mimic or inhibitor molecules (scramble groups) in both experiments. We performed 6 IVP manipulations per bull with 30 oocytes microinjected by experimental group in each replicate. In vitro embryo development rates and gene expression (q-RT-PCR) of miRNAs target transcripts in 2-4 cell embryos were evaluated. After microinjection, on the second day of in vitro culture, 5 embryos at 2-4 cell stage from each group were collected for gene expression of target transcripts: TGB1, CDKN1, HDAC1, PTEN, BCL6 and IRF1. SAS System for Windows 9.3 was used to evaluate IVP data by GLM procedure and qPCR data by mixed procedure (Steibel et al. Genomics, 94:146-153, 2009). In the rescue experiment, cleavage rate was increased in mimic group compared to the scramble group (cleaved structures/total oocytes; 68.30 ± 2.65 vs. 54.23 ± 3.69 respectively; $p < 0.0001$). A lower expression of HDAC1 was found in 2-4 cell embryos in the mimic compared to scramble group. No differences were observed in the proof of principle experiment. As it was hypothesized, the results showed a positive action of exogenous sperm miRNAs in embryo cleavage when using bulls with known low IVP fertility, probably as a result of the increase in global gene expression, as a consequence of lower HDAC1 expression, enzyme responsible for histone deacetylation.