A319 Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

In vitro embryos production in bovine after metaphase plate nuclear transfer

Felippe Manoel Costa Caixeta[1], Regivaldo Vieira de Sousa[2], José Felipe Warmling Sprícigo[3], Sarah de Andrade Dias Rodrigues[1], Andrei Antonioni Fidelis[1], Margot Alves Nunes Dode[2]

[1]Universidade de Brasília (UNB), Brasilia, DF, Brasil; [2]Embrapa Recursos Genéticos e Biotecnolgias, Brasilia, DF, Brasil; [3]FAP-DF, Brasilia, DF, Brasil.

Studies have shown that damages of the cytoplasmic organization is one of the main alterations caused by vitrification in bovine oocytes, being one of factors responsible for the inefficiency of this technique. Genomic nuclear transfer (GNT), in which the DNA of a damaged oocyte is transferred to a viable oocyte cytoplasm with the aid of a micromanipulator, is an alternative to rescue genetic material from oocytes with compromised cytoplasm, such those that have been submitted to vitrification. The objective of this study was to evaluate the viability of the GNT technique in bovine oocytes. Two experiments were performed. In the first experiment, the ability of reconstructed structures to develop to blastocyst stage after Parthenogenetic activation (PA) was evaluated. The second experiment aimed to evaluate the capacity of the reconstructed structures to be fertilized and to determine if the sperm concentration could affect fertilization rate. For the first experiment, cumulus-oocyte complexes (COCs) obtained from slaughterhouse ovaries were matured for 21 hours and distributed into three groups: 1) previously enucleated cytoplasm (n=275) reconstructed with a metaphase plate from another oocyte (GNT-MP) and submitted PA; 2) PA control (n=141)and 3) IVP control (n=204). In the second experiment, COCs were matured, micromanipulated and divided into 3 groups: 1) GNT-MP fertilized with 1x106sptz / ml (n=64); 2) GNT-MP fertilized with 0.5x106sptz / ml (n=63); 3) Control IVP (n=92; fertilized with 1x106sptz / ml). After 18 hours of fertilization, the structures were denuded, fixed in acetic acid: alcohol (1: 3) for 48 hours and stained with lacmoid. Oocytes were then classified as fertilized, unfertilized, polyspermic and abnormal. The chi-square test was used for the rates of fertilization and blastocyst production considering the value of $P \leq 0.05$. In the first experiment, no difference was found between the control PA and control IVP groups for both cleavage (83% and 80.4%) and blastocyst rates (46.1% and 38.7%). However, the GNT-MP group had lower cleavage (63.4%) and blastocyst rates (18.8%) compared to the two control groups. In the second experiment, the fertilization rate of the control group (76.1%) was higher than the fertilized GNT-MP with 1x106sptz/ml (46.9%) and GNT-MP fertilized with 0.5x106sptz / ml (46%), which did not differ from each other. The polyspermic rate was similar between the control group IVP (18.5%) and the GNT-MP groups either fertilized with 1x106sptz / ml (17.2%) or with 0.5x106sptz / ml (17.5%). It can be concluded that the structures reconstructed by the GNT-MP technique are capable of developing into embryo, and they can be fertilized without increasing the polyspermic rate. Therefore, it is a possible tool for the use of cryopreserved bovine oocytes.