Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Zinc oxide nanoparticles supplementation on *in vitro* maturation of bovine oocytes and its effects on the embryo quality, lipid accumulation, and preimplantation development

Carolina Capobiango Romano Quintão[1], Naiara Zoccal Saraiva[1], Clara Slade Oliveira[1], Luiz Sérgio de Almeida Camargo[1], Michele Munk[2]

[1]Embrapa Gado de Leite
[2]Universidade Federal de Juiz de Fora
e-mail: carolina.quintao@embrapa.br

One of the biggest challenges in bovine genetic improvement programs that use *in vitro* embryo production techniques is improving embryonic quality and survival after cryopreservation. Among the associated factors are lipid accumulation and excessive production of reactive oxygen species (ROS) associated with *in vitro* culture conditions, which do not provide all the necessary nutrients for perfect embryo development, as occurs *in vivo*. Supplementation of IVM medium with zinc oxide nanoparticles (ZnO-NPs) has been shown to have a beneficial effect in reducing free radicals (Isaac, et al., Biochemical and Biophysical Research Communications, 494: 656-662, 2017) and increasing the rate of development and re-expansion of vitrified bovine blastocysts (Abdel-Halim BR.; Moslhy, WA.; Helmy, N.A.; Asian Pac. J. Reprod. 7:161-6, 2018). This study evaluated the effects of adding three concentrations (0, 1.0, and 1.5 µg/mL) of ZnO-NPs in IVM medium on the development, lipid accumulation, and total cell number of bovine embryos. ZnO-NPs were characterized by morphology, and stability using dynamic light scattering (DLS), Scanning Electron Microscopy (SEM), and zeta potential analyses. Oocytes obtained from slaughterhouse ovaries were *in vitro* matured (TCM199 with 10% FCS, hormones, and sodium pyruvate) and exposed to ZnO-NPs in the following concentrations: 0µg/mL (control), 1,0 µg/mL (treat.1) and 1,5 µg/mL (treat.2). Then, were *in vitro* fertilized (Talp-FIV with 0.6% BSA) and the presumptive zygotes were partially denuded and cultured in SOF medium supplemented with 1.5% FCS. On D7, the blastocysts were fixed in paraformaldehyde and subsequently exposed to Hoechst 33342 and Bodipy 493/503 dyes for one hour for nuclear evaluation and lipid accumulation, respectively, in epifluorescence microscopy. Images of each embryo (approximately 30 embryos per group) were captured on an EVOS 5000 inverted microscope, and data on lipid accumulation were obtained by relative fluorescence values of images analyzed with the ImageJ program (version 1.53e). Five replicates were performed, totaling approximately 100 oocytes per group. Statistical analyses were performed in GraphPad Prism 9 software, and proportions were analyzed by Chi-Square Test ($\chi 2$). The addition of ZnO-NPs did not interfere ($P > 0.05$) in the cleavage rates (0µg/mL: 81.5%; 1.0 µg/mL: 73.5%, 1.5µg/mL: 79.5%), blastocyst developmental (D7) (0µg/mL: 36.9%; 1.0 µg/mL: 37.5%, 1.5µg/mL: 37%) and the accumulation lipid profile of blastocysts on D7. However, mean total cells were higher ($P < 0.05$) in the group treated with 1.0µg/mL ZnO-NPs (n=112.23) when compared to the control group (n=89.9). We conclude that, at the concentrations tested, ZnO-NPs do not affect embryo development and that adding 1.0 µg/mL ZnO-NPs to IVM can improve the quality of bovine embryos produced *in vitro*.

Acknowledgment: PPGCBIO/UFJF and Embrapa Dairy Cattle.

Financial support: Embrapa Dairy Cattle.