Animal Reproduction

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Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

Phenazine ethosulfate added to maturation medium does not reduce lipid concentration in blastocyst stage embryos

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Resumo

The objective was to determine the effect of different doses of the phenazine ethosulfate (PES) added to the in vitro maturation medium (IVM) on lipid concentrations (triglycerides, phospholipids, and cholesterol) in oocytes, and embryos from slaughterhouse cows. Oocytes (n =2,232) grades 1, and 2 were randomly submitted to IVM with different concentrations of PES: 0 µM (Control); 0.16 µM (PES0.16); 0.4 µM (PES0.4); 1.0 µM (PES1); and 2.5 µM (PES2.5). Matured oocytes were fertilized, and cultivated in vitro using commercial media (Botupharma, Botucatu, SP). Sub-samples of oocytes (n =171), and blastocysts (n =180) were randomly selected for fluorescence optical microscope analysis – fixed in 4% paraformaldehyde, and stained with Nile Red (NR; Sigma-Aldrich) – measured in arbitrary units (a.u.). Lipid content was obtained by fluorescence using ImajeJ. Data on mean fluorescence intensity of oocytes and embryos were submitted to distribution analysis and their distribution was verified as Poisson. For oocytes, the treatment effect was analyzed using the generalized linear models procedure and the means compared using the Student method. The PES 2.5 µM dose was toxic and did not yield any blastocyst. The lipid concentration in oocytes was lower (P <0.01) in the treated groups in comparison to the Control. In embryos, the triglycerides concentrations of PES1 (494,2 a.u.) and PES0.4 (552,6 a.u.) did not differ (P >0.05) from control during culture, and was higher (P =0.01) in PES0.16 (610,8 a.u.) compared to the Control (463,2 a.u.). The phospholipid and cholesterol concentrations (combined) of PES0.4 (746,2 a.u.), and PES0.16 (926,1 a.u.) were higher (P <0.01) than in the Control (674,0 a.u.), and PES1 (713,4 a.u.). Triglycerides from the treated groups were more abundant (>494,2 a.u) than in the Control (463,2 a.u.; P =0.03). In the PES0.16 and PES0.4 groups, triglycerides were 1.4 and 1.1 times, respectively, more abundant than for PES1 (P =0.05). The concentrations of phospholipids and cholesterol were higher in the treated groups (>713,4 a.u.; P =0.01) than in Control (674,0 a.u.), and PES0.4 embryos had lower concentrations (746,2 a.u.) of phospholipids and cholesterol compared to PES0.16 (926,1 a.u.; P <0,001). Surprisingly, the concentration of triglycerides in control embryos was lower when compared to the groups treated with PES (P =0.03). The treatments PES0,16, and PES0.4 resulted in embryos with higher (P =0.05) lipid concentration than PES1 (610,8 a.u., 552,6 a.u., and 494,2 a.u., respectively). The use of PES in this study reduced the lipid concentration in oocytes. This effect and the metabolic change induced by it were not sustained when evalueted in the blastocists stage. A compensatory effect of PES was observed, characterized by an increase in lipids after in vitro culture. The PES in IVM reduced lipids in oocytes but was not able to reduce it in the embryos under the current conditions.