Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Does Phenazine Ethosulfate improve early embryo development?

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Abstract

Early porcine embryos need glucose through a correct balance between the pentose-phosphate pathway (PPP) in early cleavages and in subsequent glycolysis (1). However, *in vitro* procedures causes a shift in energy production towards glycolysis. The Phenazine Ethosulfate (PES) is an electron acceptor related to glucose production through the PPP by NADPH/NAD+ oxidation. Since the PPP is temporarily required during early cleavages, we hypothesized that a time-dependent exposure to PES would enhance embryo development. Sow ovaries were collected in a local abattoir and transported to the laboratory in warm saline solution within one hour, washed in the same solution, and kept warm during aspiration. Follicles with 3-6 mm diameter were collected with an 18G needle coupled in a syringe and deposed in a 50 mL tube. After settling for 10 min, the supernatant was removed, and the pellet was resuspended in TCM-HEPES containing 50 IU/mL of gentamycin, 10% FCS, and 3 mg/mL of BSA. After another 10 min-settling, cumulus oophorous-oocyte complexes were resuspended and selected in the same medium. Oocytes with homogenous cytoplasm and at least three layers of compacted cumulus oophorous cells were selected and rewashed in maturation media. Groups of 20 to 25 oocytes were matured in 90 µL droplets of TCM-199 (Thermo Fischer Scientific® 11150059) supplemented with 3.05 mM of D-glucose, 0.57 mM of cysteine, 0.91 mM of $C_3H_3NaO_3$, 50 IU/mL of both EGF and gentamycin, and 10% of porcine follicular fluid under mineral oil during 44h at maximum humidity, with 10 IU/mL of both eCG and hCG during the first 22h. After maturation, cumulus oophorous cells were removed by gentle pipetting, with oocytes being parthenogenetically activated by 5 min exposure to 15 µM of ionomycin, 15 min exposure to 200µM of TPEN, and four-hour exposure to 7.5 µg/mL of cytochalasin B. After activation, presumptive parthenotes were cultured in PZM 5 (2) until day 7 (D7), with feeding at day 5 (D5), by supplementing the media with 3 mg/mL of BSA. Treatments with 0.05 µM of PES (3) were conducted in five replicates (20 to 25 embryos per group). Structures were randomly distributed in four groups: Control (PZM 5), PES (0–48 h), PES (24–48 h), and PES (0–24 h). Embryos were evaluated for cleavage at 24 h and 48 h and the percentage of blastocyst stages was recorded at 168 h after activation. Data were analysed by ANOVA, followed by Tukey test for multiple comparisons. We found no difference among any evaluated treatments and stages ($p > 0.05$) as cleavage rates at 24h, at 48h, and total blastocysts for Control were 61,0%, 73,1% and 58,0%; for PES (0–48 h) were 54,5%, 70,0% and 43,6%; for PES (24–48 h) were 53,1%, 70,4% and 39,8%; and for PES (0–24 h) were 61,0%, 73,0% and 53,3%, respectively. Interestingly, porcine embryos produced *in vivo* and cultured *in vitro* from 24 h up to 120 h in the same PES concentration had better blastocyst development, fewer TUNEL positive cells, and higher survival after freezing and thawing procedures (4). Nevertheless, more studies are necessary to asses if our PES dose and time of exposure may influence embryo quality.

References

1. Prates EG, Nunes JT, Pereira RM. A Role of Lipid Metabolism during Cumulus-Oocyte Complex Maturation: Impact of Lipid Modulators to Improve Embryo Production. Mediators Inflamm [Internet]. 2014;2014:1–11. Available from: http://www.hindawi.com/journals/mi/2014/692067/

2. Yoshioka K, Suzuki C, Onishi A. Defined system for in vitro production of porcine embryos using a single basic medium. J Reprod Dev. 2008;54(3):208–13.

3. Braga JVC, Bohn AP, Soares LP, Komninou ER, Lucia Júnior T, Vieira AD, et al. Docosahexaenoic acid and phenazine ethosulfate are not efficient lipid modulators for porcine in vitro maturation systems. Vet Res Commun [Internet]. 2022;47(0123456789). Available from: https://doi.org/10.1007/s11259-022-09889-5

4. Gajda B, Romek M, Grad I, Krzysztofowicz E, Bryła M, Smorg Z. Lipid content and cryotolerance of porcine embryos cultured with phenazine ethosulfate. Cryo-Letters. 2011;32(4):349–57.