

Influence of cryoprotectants on DNA fragmentation of in vitro produced porcine blastocysts

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Abstract

Introduction: The aim of the present study was to investigate the effects of different cryoprotectants (CPs) on DNA fragmentation of in vitro produced blastocysts to determine an appropriate cryoprotectant for embryo cryopreservation. Therefore, the precise aims of the study were to assess the toxic effects of different cryoprotectants in terms of survival rate and to evaluate the effects of different CPs on DNA fragmentation in in vitro produced porcine blastocysts. Ethylene glycol, 1,2 propanediol and glycerol are common cryoprotectants widely used for embryo cryopreservation in different animals as well as humans.

Materials & Methods: 197 porcine blastocysts were produced in vitro and 160 blastocysts were randomly selected and divided into 4 groups. 40 blastocysts were placed in phosphate buffer solution (PBS) without any cryoprotectants for 1 hour in room temperature (23-25 °C) as the control group. The rest of the blastocysts were exposed to 3 different cryoprotectants (10% solutions) ethylene glycol (EG), 1, 2 propanediol (PG) and glycerol (Gly) for 1 hour in a 3- step method in room temperature. The survival rate was assessed after culture in NCSU-37 medium for 24 hours as the proportion of recovered embryos with the reformation of blastocele observed by stereomicroscopy at 40X magnifications. The apoptotic indices were evaluated after staining by TUNEL technique to label apoptotic nuclei and later were counter-stained by propidium iodide (PI) to label all nuclei and were analyzed by fluorescent microscopy. Then, the survival rate was compared with the data obtained from the control group. Through ANOVA and Fisher's exact test the data were analyzed while employing StatView software and the level of significance was considered as 0.05%.

Results: Exposing porcine blastocysts to different cryoprotectants results in an increase in DNA fragmentation, although the apoptotic index in blastocysts with blastocele compared to those without them were lower in the study, disregard of the kind of cryoprotectant.

Conclusion: It is concluded that CPs can decrease the survival rate of porcine blastocysts by increasing the percentage of DNA fragmentation but EG has the least effect on DNA fragmentation.

Key Words: Cryoprotectants, Cytotoxicity, Apoptosis, Pig, DNA fragmentation, Blastocysts.

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