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Embryonic survival, development and cryoinjury of repeatedly vitrified mouse preimplantation embryos

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ABSTRACT

Objective: The aim of this study is to investigate the embryonic survival, development, and expressions of cryoinjury- or antioxidant-related genes in once, twice, or three-time vitrified mouse preimplantation embryos. Study desisgn: Six hundred 8-cell stage embryos were obtained from 60 female mice and randomly assigned to control and three experimental groups. Embryos were vitrified by indirect methods The developmental outcomes such as survival rate, blastocyst-forming rate, and the percentage of hatching/hatched blastocyst were assessed. The cell numbers of hatching/hatched blastocyst were counted after nuclear staining. From hatching/hatched blastocysts, the mRNA expressions for Cirbp, Casp3, Sod1, Gpx3, and Cat were quantified by real-time quantitative RT-PCR.
Results: In once, twice, or three-time vitrified mouse 8-cell stage embryos, survival rates, blastocyst-forming rates, the percentages of hatching/hatched blastocyst, and the cell counts were all similar when compared with non-vitrified control group. The mRNA expression levels of Cirbp, Casp3, Sod1, Gpx3 and Cat were not affected.
Conclusion: Repeatedly vitrified mouse 8-cell stage embryos well developed up to blastocyst stage without cryoinjury and without decrease of antioxidant-related genes.

Introduction

Embryo freezing is regarded as an important part of infertility treatment. Embryo freezing technology could lower the risk of ovarian hyperstimulation syndrome and multifetal pregnancies. Although guidelines about the number of embryos to be transferred differ among countries or individual IVF center, efforts to reduce the number of transferred embryo in a single cycle have been continuously made [1]. Elective single embryo transfer (eSET) is now widely used in many IVF centers [2,3]. Therefore, the need for embryo freezing is ever increasing.

Along with the wide use of embryo freezing, technology about re-freezing of embryos has been commonly encountered in the IVF laboratory. If three embryos were frozen in the past cycle and two thawed embryos are transferred in the present cycle, one embryo should be re-frozen. The clinical outcomes after use of re-frozen embryos have been reported in several studies. There are three case reports with regards successful pregnancy or delivery after transfer of twice frozen embryos in human [4–6]. In three retrospective comparative studies, pregnancy and live birth rate was similar between once frozen and twice frozen embryos [7–9]. However, studies evaluating the outcomes after transfer of three-time frozen embryos are currently not available.

In a mouse model, similar outcomes between once frozen and twice frozen embryos have been reported, but studies evaluating the outcomes of three-time frozen embryo are lacking [10–12].

In a global trend of less embryo transfer, researches on embryonic survival and development after repeatedly frozen embryos are mandatory to ensure its safety. The present study is aimed to investigate the embryonic survival, development, and expressions of cryoinjury- or antioxidant-related genes in once, twice, or three-time vitrified mouse preimplantation embryos.

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Materials and methods

Animals

Sixty five-to-six-week old BDF-1 female mice (Orient Co., Seoul, Korea) were cared under a 12 h light: 12 h dark cycle at 23 °C and fed ad libitum and used in this study in accordance with the institutional guidelines established by the Animal Care and Use Committee (IACUC) of the Seoul National University of Bundang Hospital.

Retrieval of mature oocytes and in vitro fertilization

The mice were treated with intraperitoneal (ip) injection of 5 IU pregnant mare's serum gonadotropins (PMSG; Daesung Microbiological Labs, Uiwang, Korea) followed by ip injection of 5 IU human chorionic gonadotrophin (hCG; Daesung Microbiological Labs) 48 h later. After 16–20 h following hCG administration, the mice were killed by cervical dislocation. Then the oviducts were dissected and placed in 1-mL of washing medium (modified mouse tubal fluid, mMTF) supplemented with 0.8% (w/v) bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO, USA). Cumulus oocyte complexes were released by tearing the ampulla of the oviducts. The cumulus cells were removed enzymatically using 85 IU/mL hyaluronidase (Cook, Brisbane, Australia) and by mechanical dissociation using a glass pipette. Only morphologically normal mature MII oocytes, as judged by the presence of a first polar body, were used in our study.

The epididymal sperms were retrieved from the cauda epididymis twelve 8–12-week-old BDF-1 male mice. The sperm suspensions were pre-incubated for 1.5 h in capacitation medium (mMTF supplemented with 0.8% BSA). Then the oocytes were inseminated with sperms at a final concentration of 2 million/mL at 37 °C in humidified 5% CO_2 in air. After 6 h, the inseminated oocytes were washed twice by pipetting and transferred to the embryo culture medium (Global medium, Life Global, Guilford, CT, USA) supplemented with 0.4% BSA. Formation of 2-cell embryos was identified at 24 h after insemination and considered as normal fertilization. Those cleaved embryos were transferred to new embryo culture medium, and their development up to 8-cell stage was evaluated at 48 h after insemination. At this time, 8-cell stage embryos were randomly allocated to non-vitrified control group and three vitrified groups.

Vitrification/warming and culture up to blastocyst

Six hundred 8-cell stage embryos were obtained from 60 female mice and randomly assigned to non-vitrified control group and three vitrified groups (150 embryos per each group). Embryos were vitrified and warmed once, twice, and three times, respectively. Non-vitrified group served as control. In 'once vitrified' group, warming was performed one week later from the vitrification. In twice and three-time vitrified group, interval between warming and re-vitrification was 1 h and the interval between vitrification and warming was one week, as illustrated in Fig. 1.

Vitrification was performed by indirect vitrification method (Rapid-I kit, Vitrolife, Goeteborg, Sweden). Embryos were suspended in equilibrium solution composed of basic medium (HEPES-buffered TCM-199 supplemented with 20% FBS), 7.5% ethylene glycol (EG) and 7.5% dimethyl sulfoxide (DMSO) for 5 min and moved to vitrification solution containing basic medium, 15% EG, 15% DMSO and 0.5 M/L sucrose (Sigma-Aldrich) for 45–60 s at room temperature (RT). Embryos were placed onto a cryo-container (five embryos per each) and then plunged immediately into liquid nitrogen for storage. For warming, the cryo-containers were immersed directly in a 37 °C warming solution (containing 1.0 M/L sucrose in basic medium) for 1 min. The warmed 8-cell embryos were transferred to 0.5 M/L and 0.25 M/L sucrose in basic medium for 3 min, respectively, and then washed twice with washing medium in basic medium.

Warmed embryos were transferred to culture medium at 37 °C and maintained with 5% CO_2 in humidified air. Survival rate of the embryos was assessed 1 h after incubation on the basis of morphologic appearance of membrane integrity and discoloration of the blastomere. The surviving embryos were further cultured for 2 days in Global medium (Life Global) supplemented with 0.4% BSA. The obtained blastocysts were classified as early, mid, hatching, or hatched. In non-vitrified control group, embryos were not vitrified and continuously cultured up to 4 days after insemination.

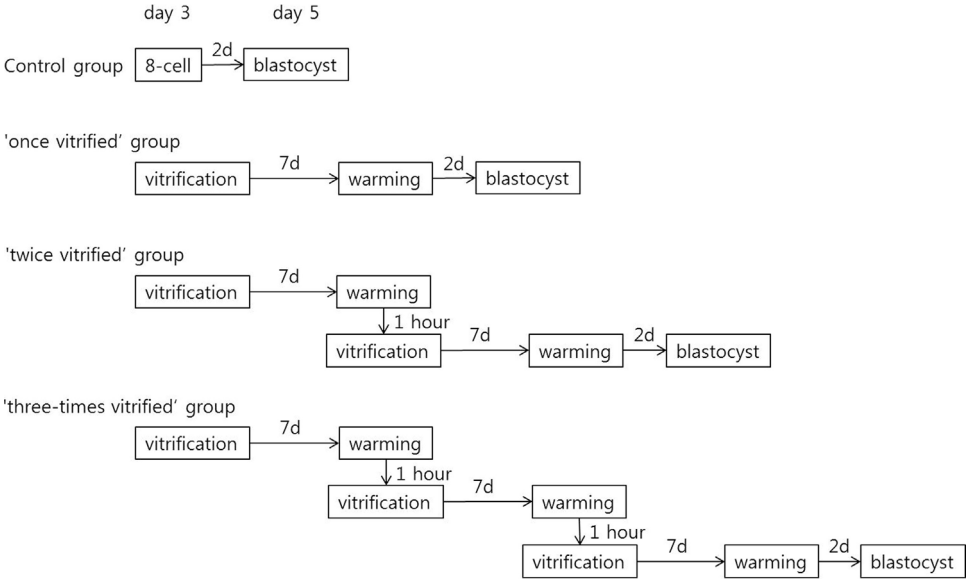

Fig. 1. Schematic illustration of duration of vitrification or re-vitrification in experimental groups.