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Freezing of Preimplanted Mouse Embryos by Vitrification

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A limited number of reports are available in Bulgaria on freezing of preimplanted embryos. The aim of this study was to determine the *in vitro* survival and the viability of vitrified preimplanted mouse embryos using super-cooled liquid nitrogen. Vitrification has been the method of choice for their cryopreservation by 6.85 M glycerol. The survival rate was assessed after thawing and dilution of the cryoprotectant by 0.35 M sucrose.

Thawed embryos were cultured to the blastocyst stage. The viability was assessed by cleavage and blastocyst rates on 24 h and 48 h of *in vitro* culture respectively.

These results indicated that vitrification of mouse preimplanted embryos using 6.85 M glycerol resulted in viable expanded blastocysts and hatched blastocysts at 56 and 44 % respectively following *in vitro* embryo biotechnology culture.

Key words: embryobiotechnology, freezing, vitrification.

Introduction

The biotechnology method for preimplanted embryo cryopreservation has become a useful tool for manipulating of reproductive cycle in mammals. Such interruption of embryogenesis for any period of time provides of powerful method of controlling animal reproduction. Mouse embryos are used for basic and applied research on cryobiological mechanisms. Basic and applied research has resulted in two approaches for embryo cryopreservation over the past years – convetional and vitrification respectively. During the vitrification procedures no ice forms in embryo blastomeres during freezing, storage and thawing therefore prevents lysis of the cytoplasm and membranes. On the other hand, highly concentrated cryoprotectant provokes characteristic osmotic changes in the entire volume of embryos during their dehydration and rehydration. A limited number of reports are available in Bulgaria on freezing of preimplanted embryos and most of them have described the conventional freezing procedure. Therefore systematic studies of the freezing and thawing mechanisms of the animal preimplanted embryos associated with cryoprotectants are consecutive needed.

The aim of this study was to determine the *in vitro* survival and the viability of vitrified preimplanted mouse embryos using 6.85 M glycerol and super-cooled liquid nitrogen.

Materials and Methods

The hormonal stimulation and estrus synchronization of 7 BALBc fertile donors was done by PMSG and HCG treatment described previously. The embryos were collected at 8-12 blastomers on 3 day after mating by forceps of the oviduct. The mice were in general narcosis and the ethical principles and legal requirements for the welfare of the animals were kept at embryo recovering. The manipulating medium (MM) was Krebs-Ringer solution with 20 % fetal calf serum supplement. The freezing medium (FM) was prepared by addition of 6.85 M glycerol to MM. The embryos in a group were placed and frozen in 0.5 ml straws (IMV, Cassue, France) by direct transfer in liquid nitrogen at -196°C. They were thawed by immersing of the straws in water bath at 38°C for 1 min. For cryoprotectant diluted medium (DM) 0.35 M sucrose was added to FM. The embryos were *in vitro* cultured in 5% CO_2 incubator at 38°C in Dulbecko medium.

Results and Discussion

The results of the experiments, in which collected preimplanted mouse embryos at early morula stage were vitrified then thawed and cultured *in vitro,* are summarized in Table 1.

A total of 176 mouse embryos were collected but 24 of them (Fig. 1-1) were retarded or degenerated and eliminated of the trials. The experimental 152 embryos in early morula stage of development (Fig. 1-2) with expanded blastomers and intact zona pelucida were assesed morphology normal and divided in 2 groups.

The first group of embryos was estimated as proper for the freezing program. After vitrification and following thawing procedure of 138 embryos the blastomers were contractive and zona pellucida was intact at 113 embryos (Fig. 1-3) and they were morphologically evaluated as saved. Dilution of the cryoprotectant indicates initial shrinkage and subsequent swelling to the normal isotonic volume (Fig. 1-4). A sign of zona pellucida hardening was also observed which reduced permeability of the cryoprotectant. Nevertheless the procedure decreased the survival rate and 96 embryos showed rehydration of the blastomers. The results of *in vitro* embryo culture demonstrates on 24 h and 48 h comparable saved viability and efficiency of about 56 and 44 % respectively (Fig. 1-5 and 1-6). It is also showed that gene expression and development of mouse embryos were preserved after vitrification procedure like mouse pronuclear zygotes [2].

As control group 14 of them were only cultured without applying on the freezing program. Their viability was 86% on 24 h of cultivation at blastocyst stage (Fig. 1-7) and 71% on 48 h at expanded or hatched blastocyst (Fig. 1-8) respectively.

In comparison similar results have been reported when the same protocol was applied to mouse and bovine embryos [4, 6]. On the other hand an alternative vitrification solutions and procedures have reported alternative success [1, 5, 8]. Using of ethylene glycol vitrification solution and propanediol may be especially appropriate for

Table 1. Survival and viability of vitrified preimplanted mouse embryos

Methods	Treated embryos	Survived embryos		Viable embryos	
		normal after thawing (%)	normal after dilution (%)	24 h culture *in vitro* (%)	48 h culture *in vitro* (%)
Vitrification	138	113 (82)	96 (70)	78 (56)	61 (44)
Nonvitrified	14	–	–	12 (86)	10 (71)

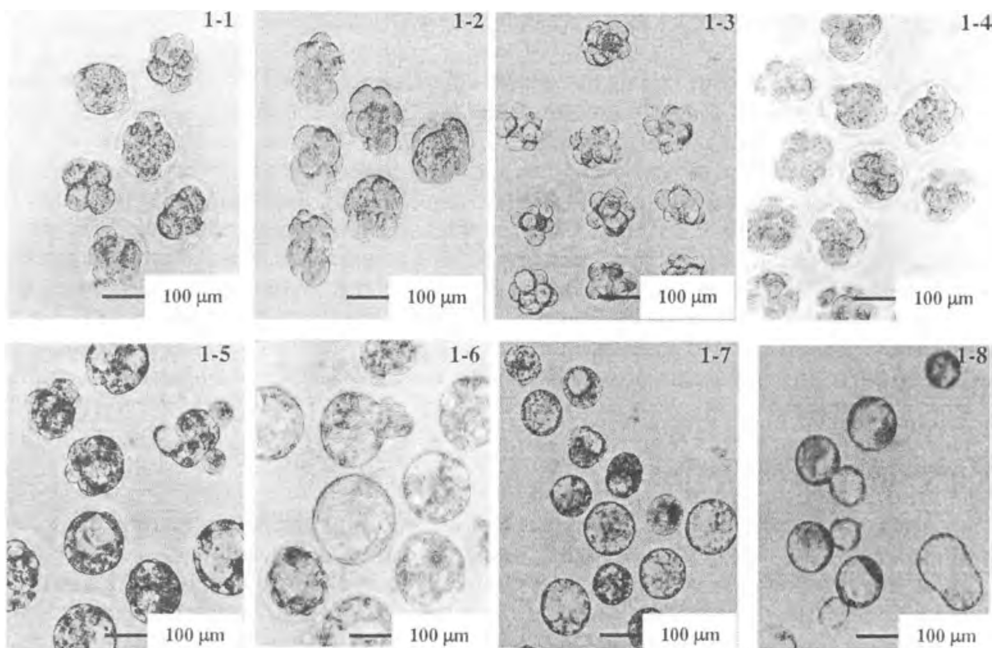

Fig. 1. Mouse preimplanted embryos exposed on vitrification and nontreated
1-1 – retarded embryos; 1-2 – normal early morula stage; 1-3 – embryos after vitrification; 1-4 – vitrified embryos after dilution; 1-5 – cultured vitrified embryos 24 h; 1-6 – cultured vitrified embryos 48 h; 1-7 – cultured nontreated embryos 24 h; 1-8 – cultured nontreated embryos 48 h

preimplanted embryos that exhibit limited permeability to glycerol [7]. Comprehensive vitrification protocol can outline its role among assisted reproductive technologies in human [3].

Conclusion

The current research demonstrates fundamental cryobiological approach aiming prevention of the deleterious effects of chemical toxicity and intracellular freezing of the living cells. The vitrification protocol saves the survival rate and viability of mouse preimplanted embryos in authentic value. However, more precise biology experiments as well as numerous embryo species are required for determination of the optimal mammal embryos vitrification procedure.

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