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Conference Paper

Comparison of Morula and Blastula Embryo Vitrification by Using Cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium

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Abstract

Vitrification is freezing method with low temperature (-196°C) using high concentrations of cryoprotectants with a view to preventing the formation of ice crystals that can damage cells and decrease the viability of the embryo blastomeres. Embryos post warming which has low viability when transferred to a recipient will decrease the pregnancy rate. Intracellular cryoprotectants used in vitrification is ethylene glycol, propanediol, or DMSO. The third type of cryoprotectants has different capacities to protect the morula and blastocyst stage embryos. This study aims to decide the exact type of cryoprotectants in protecting the morula and blastocyst stage embryos when vitrification process. Research methods were divided into three groups of cryoprotectants that group treatment 1 (P1): Ethylene Glycol 30% + Sucrose 1 M + Insulin Transferrin Selenium 15 mL, group treatment 2 (P2): Propanediol 30% + Sucrose 1 M + Insulin Transferrin Selenium 15 mL, treatment Group 3 (P3): DMSO 30% + Sucrose 1 M + Insulin Transferrin Selenium 15 mL. The data obtained were analyzed by one-way ANOVA. Results of research that use Propanediol at the morula stage embryo vitrification is not significantly different from the Ethylene glycol but significantly different from DMSO. Then use Ethylene Glycol at the blastocyst stage embryo vitrification significantly different with Propanediol and DMSO and DMSO Propanediol but usage is no different. The conclusion of this study is Propanediol used as cryoprotectants in the vitrification process morula stage embryos, while ethylene glycol used as cryoprotectants the blastocyst stage embryo vitrification process.

Keywords: vitrification; ethylene glycol; propanediol; DMSO; morula; blastocyst.

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1. Introduction

The method is widely used to store embryos is by cryopreserves. Cryopreservation or embryo freezing is a method of storing excess embryos produced by in vitro fertilization [1]. Cryopreservation consists of several kinds, among others slow freezing, rapid and ultra-rapid freezing. From ultra rapid growing freezing vitrification method. Vitrification has several advantages, among others, some of the advantages of fast, does not require equipment and not the formation of ice crystals that can cause cell damage. Vitrification method is by freezing embryos rapidly at low temperature (-196°C) using high concentrations of cryoprotectants with a view to prevent the formation of ice crystals that can damage cell blastomeres [2]. Ice crystals formed during freezing will damage the embryo, damaged organelles in the cristae mitochondria and lead to loss of cell plasma membrane integrity. Cell damage caused by the formation of ice crystals can cause the death of cells in the embryo [3].

The problem that occurred from this embryo storage method is the quality of embryos post warming is still very low. This can be seen when the embryo post warming then cultured and transferred to a recipient provides a very low pregnancy rate. The low implantation and pregnancy are influenced by many factors, among others, many cell blastomeres, trophoblast and the inner mast cell degeneration resulting in lower embryo viability and reduce the number of implantation.

In the process of vitrification used cryoprotectants with a high concentration is different from the process of slow and rapid freezing. High concentration of cryoprotectants is intended to protect the embryo during the process of cryopreservation. Cryoprotectants are indispensable to protect the embryo from external and internal to the drastic temperature changes very well when the freezing process or when warmed back (warming). During intracellular cryoprotectants used are ethylene glycol, propanediol, or DMSO. The third type of cryoprotectants is often used as intracellular cryoprotectants when performing vitrification of surplus embryos of fertilization embryos without seeing its infancy. The addition of Insulin Transferrin Selenium because it has compounds that can reduce the reaction oxygen species drastic temperature changes during the process of freezing and warming [4].

Ethylene glycol, propanediol, or DMSO has a different chemical content of course have different capabilities performance protect cell blastomere, trophoblast or inner mast cells by intracellular. Specificity of the chemical composition contained in cryoprotectant ethylene glycol, propanediol, or DMSO have different speeds in protecting the morula stage embryos which still consists of blastomere cell and blastocyst stage

embryo consisting of cells trophoblast, inner mast cell and blastocoel. Freeze blastocyst stage embryos provide a very high vitality after the frozen embryos in the melt back [5]. But it does not mean morula stage embryos can not be frozen. Selection of appropriate types of intracellular cryoprotectants will greatly affect the vitality or viability of embryos post warming. The viability of embryos post warming largely determines the occurrence of pregnancy after the embryos are transferred to the recipient. Therefore we need a study to optimize the selection of cryoprotectants toward types of embryos to be frozen so that the post is still warming embryos have high viability and worth to be transferred to a recipient.

2. Material and Methods

2.1. Materials and Devices Research

Materials used in this study were male mice were 5 months of age, female mice aged 3 months, Pregnant Mare Serum Gonadotropin (PMSG), Human Chorionic Gonadotropin (HCG), Phosphate Buffer Saline (PBS), Medium Engle Minimum (MEM), ethylene glycol, mineral oil, sulfates gentamycin, Co2. The equipment used in this study is a Co2 incubator, microscope inverted, microsurgical equipment, syringe, pipette pasture, Hemi straw, Petri dish disposable, Millipore, tube.

2.2. Observation Procedures

2.2.1. Superovulation and collection of eggs

Female mice were injected with hormone Gonadotropin Pregnant Mare Serum (PMSG or Folligon) at a dose of 5 IU. Forty-eight (48) hours later injected with the hormone Human Chorionic Gonadotropin (HCG or Chorulon) and directly mated with male mice that have been castrated by mono matting seventeen (17) hours after the examination of female mice mated plug vagina (vaginal plug). Female mice were positive stopper then performed vaginal egg collection. The egg is washed and prepared for in vitro fertilization.

2.3. In Vitro Fertilization

The egg cell that has been collected is then washed successively three times in PBS and MEM medium. Eggs that have been washed and then transferred to medium fertilization. To wait for the preparation of spermatozoa to be used for in vitro fertilization. Take spermatozoa from cauda epididymal of male mice were incubated for 1 hour, then immersed in the medium Fertilization existing egg cell. Egg is mixed with sperm and then incubated at 5% CO2 incubator with a temperature of 37°C for 7 hours, then threshed granulosa cells to observe the 2 pn.

2.4. Embryo Culture Until the Morula and Blastocyst

Once formed 2 pn, then zigot moved in culture medium and incubated in a 5% Co2 incubator at 37°C. Substitution culture medium is done 2 days until the embryos reached the morula and blastocyst stages.

2.5. Cryopreservation by the Vitrification Method

Overall this study consisted of three groups:

Treatment group 1 (P1): Ethylene Glycol 30% + Sucrose 1 M + Insulin Transferrin Selenium 15 ML. Treatment group 2 (P2): Propanediol 30% + Sucrose 1 M +Insulin Transferrin Selenium 15 mL.Treatment group 3 (P3): DMSO 30% + Sucrose 1 M +Insulin Transferrin Selenium 15 mLEmbryos that have been exposed by a combination of cryoprotectant medium ethylene glycol, Propanediol, DMSO and Insulin Transferrin Selenium, then placed at the end of the hemi straw. Furthermore hemi straw that has been revealed by liquid N2 dipped in liquid N2 and put in a big straw. When inputting a hemi straw into a large straw must be in liquid N2, so that the embryo is located at the end of the straw hemi not lost. Then the big straw fixed ends and put in a cassette straw. Further included in the cassette container goblet liquid N2 warming embryos. Before warming, warm medium consisting of V4 (medium PBS + 0.5 M sucrose), V5 (PBS medium + Sucrose 1 M) for 15 minutes. Embryos after warming included in the medium V4 for 2.5 minutes, then transferred in a medium V5 for 7.5 minutes. Further observed morula and blastocyst embryo viability post warming.

Table 1: Summary of Average life post morula warming frozen with different cryoprotectants.

Group	X ± SD	Signification
Ethylene Glikol+ITS	$20.00^{a} \pm 2.00$	0.002
Propanediol+ITS	$22.00^{a} \pm 2.65$	
DMSO+ITS	$10.33^{b} \pm 2.52$	

Table 2: Summary of Average life post warming blastocysts frozen with different cryoprotectant.

Group	X ± SD	Signification
Ethylene Glikol+ITS	$16.33^{a} \pm 3.22$	0.016
Propanediol+ITS	$11.00^{ab} \pm 1.00$	
DMSO+ITS	$9.33^{b} \pm 1.53$	

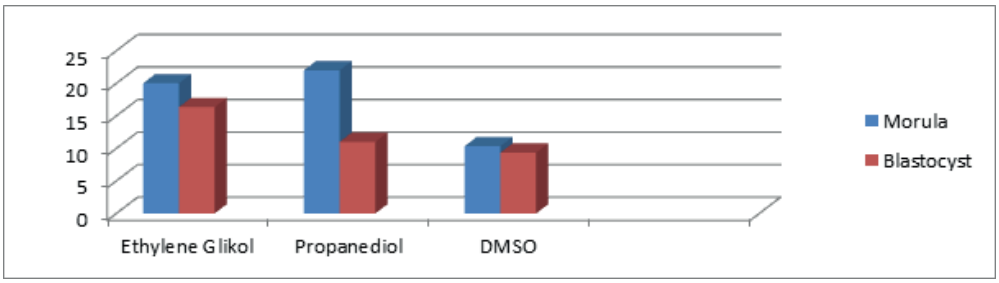

Figure 1: Bar chart of comparison of morula and blastula embryo vitrification by using cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium.

3. Results and Discussion

This study uses a vitrification method to freeze the morula and blastocyst embryos. Vitrification is relatively simple, does not require expensive equipment freezing, other than that vitrification as a solution to maintain optimal manner freezing [6]. The results showed that the type of cryoprotectants used when vitrification give different results on embryonic post viablitas warming. This is evident from the results of statistical analysis in tables 1 and 2 below.

Use of Propanediol cryoprotectant at the morula stage embryo vitrification gives results that are not significantly different from the Ethylene Glycol but significantly different with DMSO on the viability of the embryo. Use of Ethylene Glycol cryoprotectant at blastocyst stage embryo vitrification gives results that are not significantly different from the Propanediol but significantly different with DMSO on the viability of the embryo. The use Propanediol and DMSO at the morula stage embryo vitrification give different results on the viability of the embryo. The vitrified embryo blastocyst stage embryos provide a better vitality [5].

Selection of the type of cryoprotectants on stage embryos will be frozen determine the viability of embryos post warming. The viability of two cell stage embryos, 4 cells and 8 cells post thawing is no difference, while the second stage of embryo development post warming cells into blastocysts lower than 4 cells and 8 cell stage [7]. This indicates that the blastomere cell confetensi very influential when ICCV process.

4. Conclusion

The conclusion of this study is Propanodiol used as cryoprotectants in the vitrification process morula stage embryos, while ethylene glycol used as cryoprotectants the blastocyst stage embryo vitrification process.

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