



Establishment of an improved vitrification protocol by combinations of vitrification medium for isolated mouse ovarian follicles

Jaewang Lee ^{a, b}, Eun Jung Kim ^{b, c}, Hyun Sun Kong ^{b, c}, Hye Won Youm ^b, Seul Ki Kim ^{b, c}, Jung Ryeol Lee ^{b, c, *}, Chang Suk Suh ^{c, d}, Seok Hyun Kim ^{c, d}

^a Department of Biomedical Laboratory Sciences, Eulji University, Seongnam, South Korea

^b Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, South Korea

^c Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, South Korea

^d Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, South Korea

ARTICLE INFO

Article history:

Received 6 March 2018

Received in revised form

11 July 2018

Accepted 23 July 2018

Available online 3 August 2018

Keywords:

Ovarian follicle

Vitrification

Cryoprotective agent

Oocyte

Maturation

Meiotic spindle

ABSTRACT

In vitro follicle growth (IVFG) is an emerging alternative option for fertility preservation in women instead of ovarian tissue cryopreservation and transplantation. To widen the application of this technique, follicle cryopreservation should be established prior to clinical use. In the present study, we tried to determine the optimal vitrification protocol of mouse ovarian follicle for *in vitro* culture and oocyte maturation by comparing four different compositions of cryoprotective agents (CPA). Secondary follicles were mechanically isolated from 2-week-old BDF-1 mice and randomly assigned to fresh control and four different groups by the composition of CPAs (ES, EDS, EFS and EPS groups; E: ethylene glycol, D: dimethyl sulfoxide, S: sucrose, F: ficoll, P: 1,2-propanediol (PROH)). After vitrification and warming procedures, the follicles were cultured *in vitro* for 10 days and then treated with human chorionic gonadotropin and epidermal growth factor to induce oocyte maturation. Fourteen to 16 h later, oocyte maturation and quality were assessed. Follicle viability was evaluated by Calcein-AM/ethidium homodimer-1 staining immediately after warming, and their survival and diameters were measured during follicle culture periods. Antral cavity formation was observed at the end of the culture period (on the 10th day of culture). Following oocyte maturation, its maturational ability and meiotic spindle formation were assessed to evaluate their competence. There was no significant difference in viability after warming among the vitrification groups. From the 8th day of culture, the survival rate of ES and EDS were significantly higher than those of other vitrification groups (EPS and EFS). The follicle diameter was largest in the fresh-control group from the 6th day, while smallest in the EFS with statistical significance. On the 10th day of culture, the antral-cavity formation rate of EDS was comparable to that of the fresh control group. However, the oocyte maturation was significantly decreased in all four vitrification groups when compared with control group; especially, the EFS showed a more marked reduction in the oocyte maturation. There were no significant differences in meiotic spindle formation among all of those groups. Our results suggest that EDS combination for mouse follicle vitrification are the most effective vitrification protocols for mouse follicle and evaluated by an *in vitro* culture and oocyte maturation after warming.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Ovarian tissue cryopreservation and transplantation (OTCP) is a

promising option for fertility preservation (FP) in female cancer patients. Since the first live birth after OTCP, more than 60 babies have been born [1]. Despite this clinical success, cancer survival patients always have concerns about the risk of cancer re-implantation following OT transplantation exist [2,3]. To avoid the risk of cancer recurrence, cryopreservation of the isolated the follicle instead of OT, followed by an *in vitro* culture have emerged as a novel option for FP [4]. From a theoretical point of view, there

* Corresponding author. Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam, 463-707, South Korea.

E-mail address: leejrmd@snu.ac.kr (J.R. Lee).

are more advantages in cryopreservation of follicles than OT. Since the ovary is composed of various cell types while the follicles consist of the more simple kind of cells, the protocol of follicle cryopreservation much easier to be optimized, and the penetrability of the cryoprotective agent (CPA) is also higher in follicles than OT [5].

Vitrification has been widely applied in human assisted reproductive technology (ART) including oocyte and embryo banking, and recently, successful vitrification of human OT was also reported [6]. Even though vitrification has been rapidly advanced, there are still problems to improve. Exposure to high concentration of CPA, variations by expert's skill, and theoretical concerns of contamination during direct exposure and storage of cryopreserved samples in LN₂ have been raised [7]. There are diverse approaches to improve the efficiency of vitrification such as the type and concentration of CPA, temperature of exposure to vitrification solutions, stepwise addition of vitrification solution, sample size, carrier system, system quality and technical expertise [8–10]. To date, follicle vitrification has been attempted in various animals including rodents, domestic animals and even in humans [2,11–13]. However, no comparative study in CPA composition has yet been reported. In this study, four vitrification solutions composed of different permeable CPAs (EG, DMSO, and PROH) and/or non-permeable CPAs (ficoll and sucrose) were used for mouse follicle vitrification as previously attempted in OT [10].

As mentioned earlier, *in vitro* culture of ovarian follicle could be one of the alternatives for OCTP to prevent cancer cell re-implantation. To use such *in vitro* culture system clinically, ovarian follicles should be cryopreserved successfully for future uses, and so it is essential to set up the optimal cryopreservation method for isolated mouse follicle first. Therefore, this study aimed to improve the efficiency of vitrification method for mouse follicle by different composition of CPAs to provide insights into the human follicle.

2. Materials and methods

2.1. Animals and collection of ovarian tissue

A total of 150 2-week-old B6D2F1 female mice were purchased (Orient Bio, Seongnam, Korea) and were bred under controlled lighting and temperature conditions (13L:11D). The animal experiment protocol was approved by the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee (BA1506-178/028–01). Following sacrificed by cervical dislocation, the mouse ovaries were collected in collection medium (Leibovitz's, L-15, Gibco, Grand Island, NY) supplemented with 5% heat-inactivated fetal bovine serum (FBS).

2.2. Follicle isolation, vitrification and warming process

A total of 2752 follicles were used in this study. Follicles were mechanically isolated with a 25-G needle attached to a 1-mL syringe. Following isolation, secondary follicles with a diameter of 110–130 μ m and two to three layers of granulosa cells with intact basement membrane and theca cells were selectively pooled in collection medium [14]. Then, follicles were randomly distributed into four groups by the different composition of vitrification solution. The experimental groups were as follows: ES (ethylene glycol; EG and sucrose; S), EDS (EG, dimethylsulfoxide; DMSO and S), EFS (EG, ficoll; F and S) and EPS (EG, 1,2-propanediol; PROH and S) as shown in Table 1. The vitrification protocol and different composition of CPA in vitrification medium used in this study were slightly modified from previously described [2,9,15,16]. The time of equilibration and vitrification were selected according to the previous study [9]. All CPAs were purchased from Sigma (St. Louis,

MO), except propylene glycol (Amresco, Solon, OH).

In brief, five follicles were simultaneously incubated in equilibration solution for 5 min and vitrification solution for <1 min. Then, immersed follicles in a liquid microdrops of less than 2 μ L were loaded onto a Cryotop (Kitazato, Japan) and immediately plunged into liquid nitrogen.

After one week, vitrified follicles were promptly immersed into the 1.0 M sucrose solution for 5 min, then serially immersed with gradually decreasing concentrations of sucrose solution (0.5, 0.25 and 0 M) every 5 min for each step. L-15 supplemented with 20% FBS was used as a basic medium for each equilibration, vitrification and warming solution.

2.3. Evaluation of post-warming viability (calcein AM/ethidium homodimer-2 staining)

A total of 352 follicles were stained for the live/dead test to assess the survival of follicles immediately after the warming process according to the previous study [17]. In brief, the follicles were incubated with 2 μ mol/L calcein-AM and 5 μ mol/L ethidium homodimer-1 (CaAM/EthD-1, Live/dead® Viability/Cytotoxicity kit; Life Technologies, Waltham, MA) for 30 min at 37 °C in a humidified CO₂ incubator. Live and dead cells respectively stained with calcein-AM as green and ethidium homodimer-1 as red. Following vital staining, the follicles were washed and imaged on an LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany).

Then, the follicles were categorized into four groups according to the percentage of dead granulosa cells (GCs) as previously described [18].

Viability 1 (V1), live follicles: follicles with the oocyte and all the GCs viable

Viability 2 (V2), minimally damaged follicles: follicles with, 10% of dead GCs

Viability 3 (V3), moderately damaged follicles: follicles with 10–50% of dead GCs

Viability 4 (V4), dead follicles: follicles with both the oocyte and/or 50% GCs dead

2.4. In vitro follicle culture and assessment of viability, growth, and development during the culture period

In total, 2400 of follicles were used for *in vitro* culture. For fresh control, the follicles were isolated from mice 1 h before the warming process of vitrified follicles to induce oocyte maturation at the same time. Then, the follicles of fresh and four different vitrified-warmed groups were simultaneously plated in 96-well plates (SPL, Pochen, Korea) containing 75 μ L/well of culture medium [19]. The composition of culture medium was α -MEM Glutamax (Gibco) supplemented with 5% FBS, 10 mIU/mL of recombinant follicle-stimulating hormone (rhFSH, Merck-Serono, Darmstadt, Germany), ITS (5 μ g/mL insulin, 5 μ g/mL transferrin, and 5 ng/mL sodium selenite, Sigma), 50 IU/mL penicillin and 50 μ g/mL streptomycin (Gibco). The follicles were cultured for ten days, and 40% of culture medium was replaced with fresh medium every four days according to the previous study [19,20].

To evaluate the survival rate during the culture period, based on the morphology of the follicle, follicles with partially or entirely naked oocytes or dark atretic-looking follicle were regarded as dead. Survival rates of follicle were assessed every other day [20].

Additionally, follicle diameter was measured perpendicularly to assess the follicle growth every other day, and antral cavity formation was evaluated at the end of culture. It was considered as visible lucent space in the GCs complex around the oocyte [20].

Download English Version:

<https://daneshyari.com/en/article/8943830>

Download Persian Version:

<https://daneshyari.com/article/8943830>

[Daneshyari.com](https://daneshyari.com)