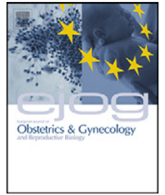
European Journal of Obstetrics & Gynecology and Reproductive Biology 167 (2013) 69–75

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb



The effect of vitrification on ultrastructure of human in vitro matured germinal vesicle oocytes

Abbas Shahedi [a], Ahmad Hosseini [b,**], Mohammad Ali Khalili [c,*], Mohsen Norouzian [a], Mohammad Salehi [a], Abbas Piriaei [a], Stefania Annarita Nottola [d]

[a] Department of Anatomy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Cell Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[d] Department of Anatomy, Histology, Forensic Medicine and Orthopaedics–Section of Anatomy La Sapienza University, Rome, Italy

ARTICLE INFO

Article history:
Received 30 June 2012
Received in revised form 15 September 2012
Accepted 22 November 2012

Keywords:
Cryopreservation
GV oocyte
Vitrification
Ultrastructure
In vitro maturation

ABSTRACT

Objective: To describe the possible effects of cryotop vitrification on maturation rate and ultrastructural morphology of human in vitro matured germinal vesicle (GV) oocytes.
Study design: A total of 301surplus immature GV oocytes obtained from infertile patients were allocated into two groups: (i) GV oocytes ($n = 150$) matured in vitro (fIVM), and (ii) GV oocytes ($n = 151$) that were first vitrified, then matured in vitro (vIVM). Supernumerary fresh in vivo matured oocytes ($n = 10$) were used as controls. The maturation media was Ham's F10 supplemented with FSH + LH and human follicular fluid. After 36 h of incubation, the oocytes were investigated for nuclear maturation and ultrastructural changes using transmission electron microscopy (TEM).
Results: Oocyte maturation rates were reduced ($P < 0.001$) in vIVM (45.92%) in comparison with fIVM oocytes (75.33%). The rate of degeneration was also significantly higher in vIVM than in the fIVM group (44.4% vs. 6.0%). Large and numerous mitochondria and minute vesicles of smooth endoplasmic reticulum (SER) complexes (MV complexes) were observed in both fIVM and vIVM groups. In addition, TEM revealed a drastic reduction in amount of cortical granules (CGs) at the cortex of vitrified-warmed GV oocytes, as well as appearance of vacuoles and small mitochondria-SER aggregates in the ooplasm.
Conclusion: The vitrification procedure is associated with ultrastructural alterations in specific oocyte microdomains, presumably related to the reduced competence of cryopreserved oocytes for maturation. This information emphasizes the need for further work on advancing the cryotechnology of human oocytes.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Oocyte cryopreservation has wider clinical implications than embryo freezing [1]. It is now possible for women who have no partner or are about to lose their ovarian function because of surgery or chemo/radiotherapy to store their oocytes for future use [2] It also provides an alternative to embryo preservation to avoid ethical issues and legal restrictions. For IVF patients, freezing the excess oocytes could avert repeated oocyte retrieval from the patients themselves or be a source for oocyte donation [3]. Recently, progress in improving human oocyte cryopreservation has been made, evident by numerous reports describing pregnancies and live births [4]. Recent progress involved application of vitrification that markedly improved the survival rate of oocytes, indicating that it is a promising cryopreservation technique [5].

One of the major problems associated with cryopreservation of mature oocytes is the sensitivity of the meiotic spindle to low temperature and cryoprotectants. This could be avoided by cryopreserving at the germinal vesicle (GV) stage when the chromosomes are within the nuclear membrane [6]. The disadvantage of cryopreservation of immature oocytes, however, is the fact that in vitro maturation (IVM) is required after thawing. It should be noted that IVM is not easily performed with human oocytes, and only a few successful pregnancies from cryopreserved immature oocytes have been reported so far [7]. Chian et al. reported pregnancies and live births as a result of natural IVF cycles with oocytes that were subjected to IVM [8]. This technology may therefore provide a benefit for patients, oocyte donation, and the study of cryopreservation of immature oocytes. Later, Cao et al. showed that there were no differences in survival rates between

* Corresponding author. Tel.: +98 0351 8247088; fax: +98 0351 8247087.
** Corresponding author. Tel: +98 021 22439957; fax: +98 021 22439958.
E-mail addresses: abas_shahedi2006@yahoo.com (A. Shahedi), Prof_hosseini@yahoo.com (A. Hosseini), Khalili59@hotmail.com (M.A. Khalili).

0301-2115/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejogrb.2012.11.006

oocytes vitrified at the GV or at the mature stage, but the potential of oocyte maturation is diminished by GV vitrification [9].

Evaluation of the oocytes by phase-contrast microscopy (PCM) is an important predictive marker of oocyte quality, currently utilized to evaluate the success of ART programs [10]. Low-resolution morphological assessment, however, is not always a sufficient measure of oocyte fertilization potential and developmental competence [11]. Therefore transmission electron microscopy (TEM) assessment, integrated with other investigational approaches, seems effective in estimating how cooling rates and cryoprotectants affect oocyte structural integrity during freeze-thawing. It seems that vitrification affects the ultrastructural morphology of the human mature oocyte [12]. This research aimed to investigate, by application of TEM, the possible effect of cryotop vitrification on maturation and ultrastructural features of human GV oocytes retrieved from ICSI cycles that were matured in vitro.

2. Materials and methods

2.1. Source of oocytes

A total of 301 GV and 10 MII oocytes were collected with informed consent from 137 women aged 20–40 years who underwent ICSI cycles. This study was approved by the ethics committee of our institute in Yazd, Iran. The patients underwent ovarian stimulation using a long stimulation protocol [13]. Human chorionic gonadotrophin (hCG; 10,000 IU) was administered 36 h before oocyte collection. The collected oocytes were cultured in IVF media (Vitrolife Co., Switzerland) and placed in an incubator (6% CO_2; 37 °C) for 1–2 h. Cumulus cells were removed enzymatically with 80 IU/ml hyaluronidase (Sigma Co, USA) and mechanically by glass pipettes. Denuded oocytes were observed under a stereo microscope (Olympus Co, Japan) to assess maturity. The oocytes that extruded the first polar body (1PB) were considered mature and used for ICSI. GV oocytes were determined by the absence of 1PB and presence of a GV nucleus. Only supernumerary MII oocytes from canceled cycles due to azoospermia were used as controls. Oocytes were divided into three groups of fresh IVM (fIVM; n = 150), vitrified IVM (vIVM; n = 151) and controls (n = 10).

2.2. Follicular fluid preparation

Human follicular fluid (HFF) was prepared as described previously [14]. HFF was obtained from patients who underwent follicular puncture. After centrifugation at 3500 RPM for 10 min, the blood and granulosa cells were settled, and pure HFF was inactivated in a water bath at 56 °C for 30 min. Finally, HFF was filtered with 0.22 μm filters.

2.3. In vitro maturation

Fresh (fIVM group) and vitrified (vIVM group) immature oocytes were transferred into maturation medium (2–3 oocytes per 50 μl droplet under mineral oil) for 36 h at 37 °C in a humidified atmosphere of 6% CO2 in air. The maturation medium was Ham's F10 (Biochrom Co, Germany) supplemented with 0.75 IU LH + 0.75 IU FSH (Ferring Co, Germany) with 40% HFF. To assess maturation, the oocytes were observed under an inverted microscope (Nikon Co, Japan) which was determined by the presence of the 1PB.

2.4. Vitrification

Immature oocytes were frozen utilizing a modified vitrification method [15]. Initially, the oocytes were equilibrated in a solution containing 7.5% ethylene glycol (EG, Merck Co, Germany), 7.5% dimethyl sulphoxide (DMSO, Merck Co, Germany) in Ham's F10 medium supplemented with 20% human serum albumin (HSA, Plasbumin Co, USA) for 10 min at room temperature (RT). They were then transferred to vitrification solution containing 15% EG, 15% DMSO and 0.5 M sucrose (Sigma Co, USA) in Ham's F10 medium supplemented with 20% HSA for 1 min at RT. Then, the oocytes were loaded on a cryotop in a volume of <1 μl, and immediately submerged into liquid nitrogen for storage.

For thawing, the cryotops were directly plunged into pre-incubated Ham's F10, 20% HSA and 1 M sucrose solution for 1 min. Subsequently, thawed oocytes were rehydrated in Ham's F10 and 20% HSA (v/v) containing 0.5 and 0.25 M sucrose at RT for 3–5 min in each, respectively. Finally, the oocytes were rinsed in Ham's F10 and 20% HSA for 3–5 times. After this stage, the oocytes were transferred into IVM medium for 36 h in incubator and checked after 1 h for survival [16]. Post-warming survival rate was assessed using morphological criteria, indicated by the absence of overt cell degeneration, elongated shape, thick or distorted zona, expanded perivitelline space (PVS) and dark cytoplasm.

2.5. Electron microscopy

Ten oocytes from each group were fixed and processed for TEM as described by Nottola et al. [12]. Oocytes were fixed in 1.5% glutaraldehyde (Sigma) for 2–5 days at 4 °C, embedded in 1% gelose (Sigma), and exposed to 1% osmium tetroxide (SIC, Rome, Italy). Then, the samples were dehydrated in increasing concentrations of ethanol, immersed in propylene oxide for solvent substitution and individually embedded in Epon 812 (SIC). The oocytes were then sectioned for both light and electron microscopy. For light microscopy, the oocytes were sectioned at a thickness of 0.5–1 μm and stained with toluidine blue. Ultrathin sections (60–80 nm) were cut and stained with uranyl acetate and lead citrate. These sections were observed and photographed with a TEM (Zeiss Co., Germany).

2.6. Statistical analysis

Differences in oocyte maturational stages between the groups were calculated and compared using Student's t-test and Chi-squared test. A P-value of <0.05 was considered statistically significant. Calculations were performed by SPSS software (version 16, USA).

3. Results

There were no significant differences in characteristics of age, etiology of infertility, and total number of retrieved oocytes between the fIVM and vIVM groups (Table 1). The majority of the patients had male factor infertility. The rates of oocyte survival and their maturation are shown in Table 2. The survival rate was as high as 89.4% post-thaw in the vIVM group. The maturation rate of the GV oocytes was 45.92% (62/135) when the oocytes were vitrified and then underwent IVM. This was lower than the fIVM

Table 1
Characteristics of patients in fresh IVM and vitrified IVM groups.

Variable	Fresh-IVM (n = 150)	Vitrified-IVM (n = 151)	P-value
Age (years) (mean ± SD)	29.3 ± 5.9	32.2 ± 5.5	NS
Female factor infertility	64 (50.6)	63 (49.4)	NS
Male factor infertility	69 (50.0)	69 (50.0)	NS
Both (male and female factors infertility)	17 (45.5)	19 (54.5)	NS

†NS, not significant; values inside parentheses represents (%)

Download English Version:

https://daneshyari.com/en/article/6174246

Download Persian Version:

https://daneshyari.com/article/6174246

Daneshyari.com