Supplementation of culture media with vitamin E improves mouse antral follicle maturation and embryo development from vitrified ovarian tissue

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Abstract

Aim: The aim of this study was to evaluate the effects of preventive vitamin E (α-tocopherol) on antral follicle development and embryogenesis of oocytes obtained after vitrification of mouse ovarian tissue.

Methods: Female Balb/c mice were killed by cervical dislocation after the injection of pregnant mare’s serum gonadotrophin (10 IU) and their ovaries were randomly divided into three groups: control or non-vitrified (n = 10), vitrification 1 (5, 10% ethylene glycol + 5, 10% dimethylsulfoxide) (n = 15), and vitrification 2 (10, 15% ethylene glycol + 10, 15% dimethylsulfoxide) (n = 15) with ascending concentration of cryoprotectants. After toxicity tests and vitrification–warming, mechanically isolated antral follicles were cultured in α-minimum essential medium, which was supplemented with or without α-tocopherol (100 μM). The follicular maturation rates and embryo development were collected and assessed. Also, the viability, morphology and ultrastructure of derived antral follicles from vitrified ovaries were analyzed.

Results: The morphology and ultrastructure of follicles were well preserved in the vitrified groups and α-tocopherol supplementation of culture media significantly increased the proportion of oocytes that reached metaphase II blastocyst rates compared to non-α-tocopherol supplemented media (P < 0.01).

Conclusion: Vitamin E improves in vitro maturation rates and blastocyst rates of oocytes that are isolated from vitrified ovarian tissue.

Key words: antral follicles, in vitro culture, vitrification, α-tocopherol.

Introduction

Although the vitrification method is still widely applied for cryopreservation of ovarian tissue, it has been shown that vitrification is often accompanied by ultrastructural damage to the granulosa cells and oocyte, such as accumulation of vesicles and loss of mitochondrial cristae.¹⁻⁴ Moreover, the developmental rates of ovarian tissue and embryo quality are still low. Vitrification protocols have depended on several factors, such as suitable cryopreservation techniques, number of equilibrations, type and concentration of cryoprotectant, warming steps and cryopreservation devices.⁵⁻⁷

Several studies have reported that low molecular weight cryoprotectant agents, such as ethylene glycol (EG) and dimethylsulfoxide (DMSO), could be useful solutions for preservation of ovarian tissue.⁸⁻¹² Vitrification causes changes in the structure of the membrane and follicles’ mitochondria.¹³,¹⁴ Thus, in this study, the ultrastructure of vitrified ovaries was analyzed for assessment of cryoprotectant solutions. We concluded in our previous investigation that the morphological

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integrity and ultrastructure of follicles were preserved after ovarian tissue vitrification with ascending concentration of EG and DMSO.\textsuperscript{14} In this regard, step-by-step vitrification in ascending concentration of EG and DMSO was used for ovarian tissue cryopreservation. Two methods, including graft of ovarian tissue and \textit{in vitro} maturation of follicles, were applied for obtaining a mature oocyte from frozen-thawed ovaries\textsuperscript{15,16} wherever possible as a useful method for vitrification solutions assessment.

The previous study showed that reactive oxygen species (ROS) levels were increased in the absence of antioxidants during follicle culture and maximum levels of ROS occurred in 48 h of culture in vitrified samples.\textsuperscript{17} Overproduction of ROS in cultured follicles can have a deleterious effect on oocyte quality, fertilization rate and embryogenesis. Therefore, ROS must be decreased during culture of ovarian follicles and where the follicular fluid is rich in antioxidants. Moreover, to protect cultured follicles from the deleterious effect of ROS, the addition of an antioxidant is essential.

Alpha-tocopherol (vitamin E) is a predominant lipid-soluble antioxidant that has been considered as a primary free radical scavenger in biological membranes.\textsuperscript{17-19} Alpha-tocopherol scavenges peroxyl radicals from polyunsaturated fatty acid in membrane phospholipids or lipoproteins that do not spread the radical chain, thereby protecting against lipid peroxidation.\textsuperscript{19} Some researches have reported that \textit{α}-tocopherol improves folliculogenesis, oocyte quality, fertilization rates and embryo development.\textsuperscript{20-22} Lisboa \textit{et al.} reported that supplementation of \textit{α}-tocopherol maintains the survival of cattle preantral follicles and promotes activation of primordial follicles after 6 days of \textit{in vitro} culture.\textsuperscript{23}

The antioxidant activity of \textit{α}-tocopherol is dose-dependent and higher concentrations may have a toxic effect on follicular development. In order to determine the optimal concentration, Jeong \textit{et al.} tested 0, 50, 100 and 200 μM of \textit{α}-tocopherol for embryo culture.\textsuperscript{24} A higher frequency of blastocyst formation was obtained in the 100-μM concentration.\textsuperscript{24} Considering the above, 100 μM \textit{α}-tocopherol is a suitable level for follicular development and might be chosen for the \textit{in vitro} culture system. We aimed to investigate the preventive effects of 100 μM \textit{α}-tocopherol on the development of antral follicle, rate of fertilization and embryogenesis of oocytes obtained from vitrified ovarian tissue in mice. In this way, we aimed to achieve the optimal culture conditions for follicular development and to improve oocyte maturation and development in \textit{in vitro} fertilization clinics.

**Methods**

**Chemicals**

All media and chemicals were bought from Sigma-Aldrich unless otherwise indicated.

**Animals and ovarian tissue preparation**

Female Balb/c mice (\( n = 40 \)), aged 5–6 weeks old, were lodged and used in accordance with the International Animal Care and Use Committee of Tabriz University of Medical Sciences. The mice were housed in an environmentally controlled room on a 12-h light/12-h dark photoperiod at 22–24 °C. Food and water were supplied without limitation. The mice were injected intraperitoneally with 10 IU of pregnant mare’s serum gonadotrophin (PMSG) to synchronize the estrus cycle. Forty-eight hours after the injection of PMSG by cervical dislocation, the mice were killed and their ovaries (~2 mm\(^3\)) were dissected free of fat and mesentery and straight away transferred to dissection medium in 100-μL drops, consisting of α-minimum essential medium (α-MEM) added with 20% fetal bovine serum (FBS).

**Preparation of the equilibration and vitrification solution**

After ovarian tissue collection, the mice were randomly divided into three groups: control or non-vitrified, vitrification 1 (5, 10% EG + 5, 10% DMSO) and vitrification 2 (10, 15% EG + 10, 15% DMSO). The cryoprotectant and warming solutions were prepared in Dulbecco’s phosphate-buffered saline (DPBS).

Three concentrations of cryoprotectants in the equilibration and vitrification solutions (VS) in this research consist of: 5% EG and 5% DMSO in DPBS with 20% FBS and 0.5 M sucrose (VS1), 10% EG and 10% DMSO in DPBS with 20% FBS and 0.5 M sucrose (VS2), and 15% EG and 15% DMSO in DPBS with 20% FBS and 0.5 M sucrose (VS3).

**Vitrification and warming**

First, ovarian tissue in the vitrification 1 group was equilibrated in the VS1 for 8 min, and then placed in VS2 at room temperature for 2 min. In the vitrification 2 group, ovarian tissue was also equilibrated in the VS2 for 8 min, then placed in VS3 at room temperature for 2 min; then the ovarian tissue was put in 1.8-μL plastic cryotubes with a minimum volume of the VS, placed on nitrogen vapor for 20 s and finally plunged into liquid nitrogen and kept for 1 week.

For warming, vitrified ovaries were placed on nitrogen vapor for 20 s, warmed at room temperature for 20 s and
then placed in a 25 °C water bath for 20 s. Then, the contents of each cryotube were expelled into 100 μL of descending concentrations of sucrose (1, 0.5 and 0.25 M) and DPBS at room temperature for 5 min.

Before follicle isolation, warmed ovaries were equilibrated in α-MEM medium with 20% FBS for 30 min in an incubator.

**Follicle morphology**

For histological assessment, vitrified and fresh ovaries were fixed in Bouin’s solution, dehydrated in ethanol, clarified with xylene, embedded in paraffin wax, serially sectioned at 5 μm, stained with hematoxylin–eosin and analyzed under a light microscope (magnification ×400).

For this study, antral follicles were those with two or more layers of cuboidal granulosa cells and those that had an antrum. Follicular quality was evaluated and follicles were classified as normal or degenerate. The follicles were classified as normal by intact oocyte and complete layer of granulosa cells or degenerated by vacuolation, pyknotic and shrinkage of oocyte and granulosa cells.

**Ultrastructure of follicle**

All chemicals were obtained from TAAB Laboratories Ltd. Vitrified and non-vitrified ovaries were randomly collected (n = 3 from each group) after equilibration in medium for 30 min, fixed in 2.5% glutaraldehyde in phosphate-buffered saline (pH 7.4) for 2 h, and post fixed with 1% osmium tetroxide in the same buffer for 2 h. After dehydration in an ascending series of ethanol, they were placed in propylene oxide and embedded in Epon 812. The ovarian tissue was cut into 0.5-μm sections (semithin sections), stained with toluidine blue and observed in light microscopy.

Ultrathin sections (60–80 nm) were contrasted with uranyl acetate and lead citrate and examined by electron microscopy (Zeiss). The granulosa cells and oocyte were analyzed by cytoplasmic organelles, vacuolation, mitochondrial formation and basement membrane of cells.

**Follicle isolation**

Antral follicles from ovaries were isolated by mechanical dissection under a stereomicroscope and were selected according to the following criteria: (i) intact follicle with several layers of granulosa cells and some adhering theca cells; (ii) antrum present; (iii) visible, round and central oocyte; and (iv) follicle diameter between 220 and 370 μm. Then, isolated follicles were transferred to main culture medium.

**Survival of antral follicles**

The survival rates of antral follicles from non-vitrified and vitrified ovaries were assessed using trypan blue staining. The isolated follicles from the ovaries were transferred to new microdroplets of medium (20 μL) and were stained by 0.4% trypan blue and examined under an inverted microscope. For each group, only follicles containing layers of membrane-enclosed granulosa cells with a centrally located oocyte were examined. The follicles were scored as viable or degenerate: viable ones were not stained and the oocyte and the surrounding granulose cells were clear and degenerated follicles stained blue.

**In vitro maturation of antral follicles**

Isolated antral follicles were cultured individually in a culture dish (35-mm Petri dishes) containing 50-μL droplets of culture medium under decontaminated mineral oil in a humidified atmosphere of 5% CO2, 5% O2 in air at 37 °C for 4 days.

The culture medium consisted of α-MEM (pH = 7.2) supplemented with 20% FBS, 19/0 mM sodium pyruvate, 75 μg/mL recombinant follicle stimulating hormone (rFSH or Gonal-f), luteinizing hormone (0/5 μg/mL), 50 μg/mL penicillin G, 50 μg/mL streptomycin, and 100 μM vitamin E (α-tocopherol). The α-MEM culture media supplemented with FBS and without vitamin E (α-tocopherol) was considered as the control group. Every 48 h of culturing, culture medium from each drop was replaced by renewed medium. The survival rate of the follicles was checked by assessment of follicle morphology under inverted microscope.

**In vitro ovulation induction**

On the 4th day of culture, final oocyte maturation and ovulation were induced by addition of 1.5 IU/mL recombinant human chorionic gonadotrophin (rhCG; Organon) to the media. Released oocytes were recorded as: germinal vesicle (GV); when the GV was absent, as germinal vesicle breakdown (GVBD); when absence of both a germinal vesicle and a first polar body, as metaphase I (MI); and when the first polar body was extruded, as metaphase II (MII). The proportions of GV, MI and MII were evaluated in all groups of study 48 h after hCG addition.

**Fertilization rates and embryo culture**

Spermatozoa were extracted from the cauda epididymis of 7–8-week-old male Balb/c mice and capacitated for 1.5 h into a 500-μL drop of Tyrods medium (T6)
supplemented with 5 mg/mL of bovine serum albumin in the incubator. The collected MII oocytes from all experimental and control groups were transferred to T6 medium containing capacitated spermatozoa, and after fertilization, they were cultured for 120 h.

**Statistical analysis**

The survival, degeneration and developmental rates of follicles and oocytes were evaluated by Tukey, analysis of variance (ANOVA) and follicular diameter analyzed by Means, ANOVA. *P* < 0.05 was considered to be statistically significant.

**Results**

**Survival rates of isolated antral follicles after vitrification–warming**

The survival rates of antral follicles derived from the fresh and the vitrified1 and2 groups were 84.9%, 66.2% and 67.2%, respectively (Table 1). There were significant differences in the survival of antral follicles between the fresh and vitrified groups (*P* < 0.05).

**Table 1** Number and percentage of intact and degenerated follicles of various stages isolated from vitrified and non-vitrified ovarian tissue after Trypan blue staining

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of follicles</th>
<th>Number of antral follicles (%)</th>
<th>Int (%)</th>
<th>Deg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>186</td>
<td>158 (84.9)</td>
<td>28 (15.1)</td>
<td></td>
</tr>
<tr>
<td>Vit1</td>
<td>275</td>
<td>182 (66.2)*</td>
<td>93 (33.8)*</td>
<td></td>
</tr>
<tr>
<td>Vit2</td>
<td>290</td>
<td>195 (67.2)*</td>
<td>95 (32.8)*</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage significantly different (*P* < 0.05) from the control and supplemented groups. Deg, degenerated; Int, intact; Vit, vitrification.

**Morphology of antral follicles after vitrification–warming**

The morphology of follicles was well preserved in the vitrified groups and was similar with the non-vitrified or fresh group (Fig. 1a). The vitrification 1 group showed more signs of degeneration and cryoinjury, such as shrinkage and picnosis of the oocyte, cytoplasmic retraction, numerous cytoplasmic vacuoles and detachment of innermost granulosa layer and oocyte (Fig. 1b). However, in the vitrification 2 group, slight cryodamage was observed, including the disruption of intercellular contacts among granulosa cells and the oocyte of antral follicles (Fig. 1c).

**Antral follicles ultrastructure after vitrification–warming**

The ultrastructural analysis showed that the integrity of cell organelles was well preserved in the vitrification 2 concentration and this was very similar to the non-vitrified groups. The granulosa cells exhibited a well-developed Golgi complex and both smooth and rough endoplasmic reticulum and highly variable numbers of vesicles spread throughout the ooplasm. Most mitochondria had continuous membranes and normal cristae but irregularly shaped or swollen mitochondria were rarely observed in the cytoplasm of granulosa cells. The oocytes were surrounded by several layers of cuboidal granulosa cells with a continuous basement membrane in antral follicles (Fig. 2a–c).

Although the ultrastructure of antral follicles that vitrified in the vitrification 1 cryoprotectant was similar to fresh control follicles, in some cases, granulosa cells, oocytes and techa cells had numerous vacuoles and elongated mitochondria with a few cristae (Fig. 2b).
Although vacuolization and deformity of mitochondria were observed in the ooplasm and the cytoplasm of granulosa cells, other organelles did not change after vitrification of ovarian tissue, compared with fresh ovaries.

**In vitro follicular viability**

Altogether, approximately 800 good-quality antral follicles were cultured with or without α-tocopherol in the present study.

By day 4 of culture, the survival rates of isolated follicles from the control, vitrification 1 and 2 groups that were cultured in the absence of α-tocopherol in medium were 74%, 59% and 59%, respectively. That of follicles cultured in vitamin-E-supplemented media were 89%, 77% and 80%, respectively (Table 2).

The control group had a significantly higher proportion of antral follicles that survived to the end of the culture as compared to the vitrified group ($P < 0.001$). The addition of α-tocopherol to the media significantly increased the viability rate of antral follicles ($P < 0.001$). Therefore, follicular viability was affected by α-tocopherol supplementation.

**In vitro follicular growth**

Follicular diameters and changes during culture on days 0, 2 and 4 are presented in Table 3. Follicular diameter did not differ among the treatment groups at the beginning of culture. However, follicles cultured in α-tocopherol had a larger diameter than those in the unsupplemented groups, but there was no difference in follicular diameter on different days of culture ($P > 0.05$).

**In vitro follicular ovulation rate**

After 4 days’ culture of antral follicles, hCG (1.5 IU/mL) was supplemented to induce ovulation. The numbers of cumulus-oocyte complexes (COC) that were derived (after 24 h) from the antral follicles were counted, in order to evaluate the ovulation rate. The addition of 100 μM α-tocopherol in culture medium increased ovulation rates of follicles (60% to 80%, $P < 0.05$) in the vitrified groups but the incidence of ovulation was not significantly different among the treatment and control groups (Table 2).

**In vitro follicular development**

The rates of maturation to the MII oocyte in the control, vitrification 1 and 2 groups that cultured in non-α-tocopherol-supplemented media were 41%, 19% and 24%, respectively. Those of follicles cultured in
The proportion of oocytes in the MII stage at day 4 of maturation was significantly higher in the control group compared with the vitrified group ($P < 0.001$). Addition of $\alpha$-tocopherol to the maturation medium revealed that a significantly higher ($P < 0.001$) proportion of oocytes reached MII compared to those in non-$\alpha$-tocopherol-supplemented media. There was no significant difference between the control and vitrified groups in the presence of $\alpha$-tocopherol. Therefore, follicular maturation was affected by $\alpha$-tocopherol supplementation (Fig. 3a,b).

**Fertilization rate and embryo culture**

After 24 h of sperm oocyte co-incubation, the proportions of oocytes that were fertilized were 69% (control), 30% (vitrification 1), and 38% (vitrification 2) in the absence of $\alpha$-tocopherol and 81% (control); and 79% (vitrification 1) and 82% (vitrification 2) in the presence of $\alpha$-tocopherol (Fig. 3c,d; Table 4). The number of matured oocytes that fertilized and reached blastocyst stage was higher in the control group compared to both the vitrified groups ($P < 0.001$) and 5 days after insemination, a significantly higher proportion of oocytes reached the blastocyst stages in the $\alpha$-tocopherol-supplemented medium compared with the unsupplemented groups ($P < 0.001$).

The blastocyst formation was significantly lower in follicles from the vitrification 1 group that cultured in $\alpha$-tocopherol media compared with treatment control (29% vs 41%). Moreover, the embryonic development was increased significantly in the control + vitamin E supplementation group compared to all other groups ($P < 0.05$).

**Discussion**

These studies were designed to determine whether the antioxidant vitamin E would protect in vitro maturation and development of isolated follicles from the deleterious effects of cryoprotectants. More mature oocytes developed into embryos during the blastocyst stages when the culture medium was supplemented with 100 $\mu$M vitamin E when compared with the non-treatment groups. Because the vitrification solution is normally supercooled at a low temperature in liquid nitrogen, it can be crystallized during warming, which leads to the cryodamage of ovarian tissue. In order to prevent cryodamage during freezing–warming, the presence of...
high concentrations of permeable cryoprotective agents is essential. The first successful method for vitrifying mouse ovarian tissue is addition of EG as permeable agents. Our previous study confirmed that EG in combination with DMSO had the highest protective effects and least ultrastructural damage on the ovarian follicle.

Thus, in the present study we used mixed cryoprotectants (EG and DMSO) for ovarian tissue vitrification. The cryopreservation of ovarian tissue leading to disruption of the meiotic spindles thereby decreases fertilization rate and embryo quality. Therefore the use of DMSO might have a protective effect by spindle polymerization.

The second successful method is the addition of a macromolecule, such as sucrose, in equilibration and vitrification solutions. The addition of sucrose confirmed previous reports that this is more efficient for follicular viability and ultrastructure. Sucrose has high solubility and low viscosity, which could facilitate the exit of water from the cell and decrease the formation of ice crystals; thus, it could protect the cells during the freezing–warming process.

In agreement with previous findings, this study indicated that optimum survival can be attained with 10-min equilibration in 10% EG + DMSO with 20% sucrose (VS2) and vitrification in 15% EG + DMSO with 20% sucrose (VS3) for ovarian tissue. This allows sufficient time for the cryoprotectant to permeate into the granulosa cells and results in a high proportion of oocytes of normal morphology, fertilization and development to two-cell embryos and blastocysts after thawing and dilution. However, in our study, no

### Table 4. Fertilization and developmental rates of oocytes derived from cultured follicles in vitrified and non-vitrified ovaries in the presence of vitamin E

<table>
<thead>
<tr>
<th>Group</th>
<th>No. oocytes that reached MII</th>
<th>No. fertilized oocytes (%)</th>
<th>No. two cells (%)</th>
<th>No. four cells (%)</th>
<th>No. blastocysts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45</td>
<td>31 (68.9)</td>
<td>18 (40.0)</td>
<td>12 (26.7)</td>
<td>9 (20.0)</td>
</tr>
<tr>
<td>Vit1</td>
<td>9</td>
<td>3 (30.3)*</td>
<td>2 (22.2)*</td>
<td>0 (0.0)*</td>
<td>0 (0.0)*</td>
</tr>
<tr>
<td>Vit2</td>
<td>13</td>
<td>5 (38.5)*</td>
<td>3 (23.1)*</td>
<td>1 (7.7)*</td>
<td>0 (0.0)*</td>
</tr>
<tr>
<td>Control + VE</td>
<td>54</td>
<td>44 (81.5)</td>
<td>33 (61.1)</td>
<td>26 (48.1)</td>
<td>22 (40.7)**</td>
</tr>
<tr>
<td>Vit1 + VE</td>
<td>34</td>
<td>27 (79.4)</td>
<td>17 (50.0)</td>
<td>12 (35.3)</td>
<td>10 (29.4)</td>
</tr>
<tr>
<td>Vit2 + VE</td>
<td>39</td>
<td>32 (82.1)</td>
<td>22 (56.4)</td>
<td>17 (43.6)</td>
<td>15 (38.5)</td>
</tr>
</tbody>
</table>

*Percentage significantly different (P < 0.001) from the control and supplemented groups. **Percentage significantly different with all groups without Vit2 + VE (P < 0.05). MII, metaphase II; VE, vitamin E; Vit, vitrification.
It is well known that the overproduction of ROS levels and lipid peroxidation of granulosa cells and oocyte membrane can affect follicular function after cryopreservation. Various antioxidants have been used to supplement in vitro culture media, especially α-tocopherol. Alpha-tocopherol (vitamin E) is well known as an ROS scavenger in in vivo and in vitro conditions and is the most important antioxidant present in ovarian tissue and follicular fluid. The antioxidant activity of α-tocopherol in preventing free-radical-induced tissue damage is accepted by most investigators and is believed to be the primary free radical scavenger and to inhibit lipid peroxidation in the mammalian cell membrane.

There was an increase in the number of viable and intact ovarian follicles after supplementation of α-tocopherol in culture media. Lisboa et al. reported that α-tocopherol maintains the survival of cattle preantral follicles and promotes activation of primordial follicles after 6 days of in vitro culture. Successful maturation of oocyte, fertilization and blastocyst rates depends on the coordination between growth and development of the ovarian follicles. Perhaps the supplementation of vitamin E may be enhanced by the effectiveness of hormones and growth factors in culture medium. In the present study, the follicle diameters, which were measured in order to evaluate growth, showed an increase of follicular diameter during culturing of isolated follicles from all fresh and vitrified ovaries. The main biological function of vitamin E is likely to protect polyunsaturated fatty acids in membranes and maintain iron and other metals in a reduced state. Peroxidation of cell membrane lipids can lead to structural damages, affecting function and permeability of membranes, eventually resulting in irreversible cell death. As a fat-soluble vitamin, α-tocopherol may be more efficient on the cell membrane than other antioxidants to protect against lipid peroxidation.

The results of the present study have shown that vitamin E supplementation of the culture media significantly increased the percentage of oocytes that reached MII stage and increased blastocyst formation. After α-tocopherol supplementation in the vitrified groups, the follicle viability, morphology and structure were significantly higher than that of the unsupplemented groups. Indeed, the increase of ROS was administrated in the absence of antioxidants during 48 h of follicular culture in vitrified samples. Therefore, in our sample, the frozen addition of α-tocopherol confirms the necessity of antioxidant supplementation during in vitro maturation of follicles.
These studies suggest that supplementation of culture media with α-tocopherol at a concentration of 100 μM allowed the maintenance of the equilibrium between antioxidants and pro-oxidants in the follicular culture, reducing the O2 concentration, and minimizing ROS production. Furthermore, this procedure inhibits establishment of oxidative stress conditions and thereby improves follicular maturation and embryo development. Kitagawa et al. showed that increased ROS levels and decreased antioxidant concentration in the culture media of oocyte maturation were responsible for embryonic fragmentation.

In conclusion, our results showed that culture of antral follicles from vitrified ovaries with 100 μM α-tocopherol improves the developmental rate of mouse follicles in vitro and the blastocyst formation. These results introduce a potential improvement of vitamin E to preserve the fertility potential by minimizing the level of free radicals that might occur during vitrification of ovarian tissue and this could be useful during assisted reproductive techniques.

Acknowledgments

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Disclosure

There is no conflict of interest.

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