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ORIGINAL ARTICLE

Effect of docetaxel on mitochondrial genes expression level in mouse MII oocytes following vitrification with cryotop

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ABSTRACT

Background: This study aimed to investigate the effect of docetaxel on the expression level of mitochondria- related genes including mitochondrial transcription A (TFAM) and mitochondrial-encoded cytochrome c oxidase subunit 1 (MT-COX 1) in mouse MII oocytes following vitrification by cryotop.

Methods: Oocytes were selected by simple random sampling and distributed amongst five experimental groups (control (n=126), docetaxel (n=132), docetaxel + cryoprotectant agent (CPA) (n=134), docetaxel+ Vitrification (n=132), and vitrification (n=123)). In this experimental study, the survival and fertilization of methaphase II (MII) mouse oocytes were assessed after vitrification by cryotop. In the second experiment, the effects of docetaxel on the expression of TFAM and COX1 genes were determined in vitrified-warmed oocytes by real-time RT-PCR. Each experimental group was compared with the control group.

Results: The results showed a significant reduction in the survival rate of each group in comparison with the controls ($P<0.05$). The survival rate was significantly lower in both vitrification groups (Docetaxel+ vitrification, vitrification) than in non-vitrification groups (fresh control and Docetaxel) ($P<0.05$). The survival rate of vitrified/warmed oocytes was significantly higher in the Docetaxel+ vitrification group compared with the vitrification group ($P=0.005$). There were significant differences in the fertilization rate between the docetaxel group with the other groups ($P<0.05$). There were significant differences between the expression level of Cox1 gene in the control group with docetaxel and vitrification groups ($P<0.05$). the results showed that the expression level of COX1, TFAM genes was significantly high in CPA, vitrification group ($P<0.05$) and pretreatment of oocytes with docetaxel can decrease the expression level of cox1, TFAM genes in the vitrified group (vitrification) and non-vitrified group (CPA) ($P<0.05$).

Conclusions: This study indicates that vitrification of mouse MII oocytes can lead to an increase in mitochondrial gene expressions (specifically TFAM and COX1). However, the pretreatment of oocytes with docetaxel is observed to result in a decrease in mitochondrial gene expression.

KEYWORDS

Docetaxel, Vitrification, oocyte, TFAM, COX1.

1. Introduction

Vitrification of oocytes is a powerful method for preservation of genetic diversity in endangered animal species and farm animals through establishment of oocyte banks (Sripunya *et al.,* 2010).

The method of vitrification is now the main effective approach for cryopreservation of human oocytes and embryos. Vitrification is a physical process through which highly concentrated cryoprotectant (CPA) prevents ice crystal formation (Khodabandeh *et al.,* 2010; Yoon *et al.,* 2003). A variety of factors during vitrification may affect the oocyte, including the toxicity of highly concentrated cryoprotectant, cold shock, and osmotic stress. These factors lead to disruption of the organelles, zona hardening, and genetic material array (Roozbehi, 2013). however damage to the cytoskeleton fibers (CSF) of oocytes during vitrification is the main cause of abnormal spindle configuration and reduced viability of frozen-thawed oocytes (Morató *et al.,* 2008). Recently, it was reported that the stabilization of CSF before vitrification could be beneficial for reducing CSF damage in oocytes (Jiménez-Trigos *et al.,* 2013). Docetaxel as a spindle fiber stabilizer plays a critical role in the successful vitrification of oocytes. It stabilizes binding with the b-subunit of tubulin in the microtubules and prevents microtubule depolymerization during vitrification, and therefore reduces cytoskeleton fibers (CSF) damages. Finally, it leads to improvement of oocyte viability after vitrification-warming (Jiménez-Trigos *et al.,* 2012; Ciotti *et al.,* 2009).

The level of oocyte viability after vitrification is dependent on CPA concentrations, time of exposure to CPA, temperature of equilibration solution, the speed of vitrification (all steps), and cell size. The mammalian oocytes which have a large size are more prone to damage by changing the temperature and concentration of CPAs (Almasi *et al.,* 2009; Morató *et al.,* 2008; Ciotti *et al.,* 2009). Moreover the high cooling and warming rate during vitrification lead to spindle damage and microfilament destructions. Therefore, the vitrification consequence can be improved by changing the vitrification conditions (Kuwayama, 2007). There are many different types of cryoprotectant used for vitrification including ethylene glycol(EG), 1,2-propanediol(PROH) and dimethylsulphoxide (DMSO) as permeable and sucrose as non-permeable CPAs. A previous study reported that the mixture of DMSO and EG in identical quantities was the most efficient method (Vincent *et al.,* 1990). EG has a low molecular weight, high permeation ability and low toxicity, which lead to its use for vitrification of human oocytes and embryos. Furthermore, EG permeability can be facilitated by DMSO (Dhali *et al.,* 2009). The high concentration of CPAs may induce osmotic stress, thus, establishing a stability between high concentration of CPAs, and low toxicity which can reduce this negative effect (Wang *et al.,* 2009).

Mitochondria has its genetic material which is known as mtDNA. In other words, mitochondria's function is interconnected with mtDNA (El Shourbagy *et al.,* 2006). The oocyte cytoplasmic maturation and developmental competence are related to mitochondrial distribution. There is little information available on the oocyte cytoplasmic maturation and regulation of mitochondrial transcription in response to cell metabolism. A previous study has shown the direct effect of the gene-specific transcription factors on the gene transcription in mitochondria (Falkenberg *et al.,* 2007). mtDNA transcription and replication were regulated by

mitochondrial transcription factor A (TFAM) in several tissues. TFAM, a nuclear-encoded high-mobility group box protein, promoter, acts as a control region to regulate the mtDNA transcription and replication (Scarpulla, 2002). Cytochrome c oxidase subunit 1 (COX1) is one of the three genomic components of the mitochondrial respiratory chain (Fernández-Silva *et al.*, 2003). Fernandez *et al*. have revealed that COX1 can be considered as an indirect indicator of activity and quantity of mtDNA (Chasombat *et al.*, 2015). There is little information and data about the effect of freezing and thawing on the expression level of TFAM and COX1.

To the best of our knowledge, gene expressions have not been completely investigated in mammalian vitrified oocytes. The objective of the present study was to determine the expression of some mitochondrial genes related to stress including TFAM and COX1. On the other hand, the effect of oocyte freezing by crypto method with the mixture of CPAs and also the effect of docetaxel on survival, morphology, and gene expression of the mouse oocyte was examined.

2. Material and methods

The present experimental study was conducted using mouse oocytes and sperm. The study protocol was approved by the Research Ethics Committee of Shiraz University of Medical Sciences (ethical code: IR.MIAU.REC.1397.801).

2. 1. Chemicals

All chemicals and media were purchased from Sigma-Aldrich Co (St.Louis, Mo, USA) unless otherwise stated.

2. 2. Experimental design

A total of 300 oocytes were selected and randomly divided into 5 groups including group I: untreated oocytes (Fresh control); group II: oocytes were pre-incubated with docetaxel for 20 min (Docetaxel); group III: oocytes were pre-incubated with docetaxel for 20 min and then exposed to CPAs, but not vitrified (Docetaxel+ CPA); group IV: oocytes were pre-incubated with docetaxel for 20 min and vitrified by cryotop method (Docetaxel+ Vitrification), and group V: oocytes were vitrified by cryotop method (Vitrification).

All the oocytes (vitrified oocytes after warming) were evaluated for morphology and survival. The oocytes were analyzed by real-time RT-PCR to evaluate the expression level of mitochondrial genes (TFAM, COX1) in all groups.

2. 3. Oocytes pre-incubation with docetaxel

Oocytes were pre-treated with docetaxel (Sigma-Aldrich, UK) at a concentration of 0.05 µM for 20 minutes, except in the control and vitrification groups (Amidi *et al.*, 2018).

2. 4. Vitrification solution

CPAs was supplemented with 7.5% ethylene glycol (EG; Sigma-Aldrich, Steinheim, Germany)+ 7.5% glycerol (Sigma-Aldrich)+ 0.5 M sucrose (Sigma-Aldrich, UK) in the base medium (G-MOPS™ (Vitrolife, Sweden AB, Göteborg, Sweden)). The equilibration solutions (ES) were half of CPA with no sucrose.

2. 5. Oocyte collection

Female (8-10 weeks old) and male (10-12 weeks old) NMRI mice were obtained from Razi Institute (Karaj, Iran) and maintained under the 12-hour light: 12-hour dark cycle for two weeks to adapt to laboratory conditions.

Superovulation of the female was done by intraperitoneal injections of 10 IU PMSG and followed 48 later by 10 IU HCG. All female mice were sacrificed by cervical dislocation 13-15 hours post-HCG administration. The

oocyte cumulus complexes (OCC) were isolated from the antral follicle and put in a base medium (G-mops) containing 300 microgram/ml hyaluronidase for 30 seconds to 1 minute. Typical M2 stage oocytes were selected based on the presence of a first polar body under a light microscope.

2. 6. Vitrification and Warming

Denuded MII oocytes were collected in groups of 15 and vitrified in cryotop device according to the protocol described by Amidi *et al*. (2018).

Oocytes were treated with docetaxel at concentration of 0.05 µM for 20 minute in each group except in the control and vitrification groups (Amidi *et al.*, 2018).

For vitrification, the oocytes were first placed in equilibration drop at room temperature for 3 minutes. Following, they were transferred to the vitrification medium for 1 minute. Finally, the oocytes were loaded on top of cryotop (Kitzato Ltd. Tokyo, Japan) and all the media were collected around the oocytes. Then, the cryotop was promptly immersed in liquid nitrogen (LN2) (Ciotti *et al.*, 2009) and stored for 1weak.

For warming, the oocytes were thawed into 1M sucrose solution (G-Mops) for 1 minute at 37˚C. Next, the oocytes were placed into reduced sucrose concentrations (0.5 M for 3 minutes and 0.25M for 5 minutes), and holding the medium for 3-5 minutes.

The vitrification process was done at room temperature (25˚C) according to the protocol described in our previous studies (Khodabandeh *et al.*, 2010; Huang *et al.*, 2008).

2. 7. Sperm preparation

Male mice (8-12 weeks) were sacrificed by cervical dislocation and cauda epididymis was excised and chopped. The sperms were capacitated in the medium (HAM's F10+ 4 mg/ml BSA) at 37°C in 5% CO_2 for 45-60 minutes.

2. 8. RNA extraction and Real-time PCR

Total RNA was isolated from the vitrified and non-vitrified oocytes using an RNA extraction kit (Cinnagen Inc., Iran). The quantity and quality of RNA were checked by evaluating the optical density (OD) of 260/280 nm (Nanodrop™ spectrophotometer) and agarose gel (1%) electrophoresis. 1000 ng of total RNA was used to synthesize the first-strand complementary DNA by RevertAid™ First Strand cDNA Synthesis kit (Fermentas Inc.). Quantitative real-time PCR was accomplished using the ABI Biosystems step one and the Real Plus 2x Master Mix Green (Amplicon Inc.,). The primers were designed by a macro gene company based on the mouse DNA sequences found in the gene bank Primer-BLAST online program as shown in Table 1.

Table 1. The sequences of primers used for Real-time PCR

Genes	Primer Sequence (5'-3')	Size (bp)
M-TFAM	F:CACCCAGATGCAAAACTTTCAG R:CTGCTCTTTATACTTGCTCACAG	147
M-COX1	F:GATTGTACTCGCACGGGCTAC R:GGATAAGGTTGGACCGCACT	202
M-B-actin	F:AGTGTGACGTTGACATCCGT R:TGCTAGGAGCCAGAGCAGTA	120

The β-actin gene was also used as the internal control of qPCR reactions. The qPCR conditions were set for 10 minutes at 94°C followed by 40 cycles of 15 seconds at 94°C, 60 seconds at 58°C and a final extension of 7 minutes at 72°C. The amplification signals of different samples were normalized to PGK Ct (cycle threshold), and then the delta-delta CT ($2^{-\Delta\Delta CT}$) method was applied to compare mRNA levels of activated versus the controls (normal LX-2), which represented as fold change in data analysis.

2. 9. Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS software version 21. Tuckey's test was used to determine the differences between the mean values of the gene expression in oocytes. P<0.05 was considered as significant.

3. Results

3. 1. Assessment of oocyte viability

After warming, the oocytes were observed under a stereomicroscope to select the healthy and degenerated oocytes. The oocytes with intact and homogenous ooplasm were selected for the experimental groups. Those with dark ooplasm were considered as deteriorated.

The survival rate of each group was separately compared to the controls. As shown in Table 2, there were significant differences between the control group with the other groups (docetaxel, docetaxel+ CPA, docetaxel+ vitrification, vitrification) (P<0.05).

The survival rate of vitrified/warmed oocytes was significantly higher in the Docetaxel+ vitrification group compared with the vitrification group (P=0.005).

Similarly, there was no significant difference in oocyte survival between the docetaxel and the docetaxel+ CPA group (P=0.533).

The results showed a significant reduction in the fertilization rate of each group in comparison with the controls (P<0.05).

There were significant differences in the fertilization rate between the docetaxel group with the other groups (P<0.05) while this difference is more significant with vitrification and Docetaxel+ vitrification groups (P<0.001).

The rate of two cell formation after IVF was significantly lower in both vitrification groups (Docetaxel+ vitrification, vitrification) than in the non-vitrification groups (fresh control and Docetaxel) (P<0.05).

3. 2. Quantitative Real-Time RT-PCR

The expressions of TFAM and Cox1 mRNA were evaluated in the vitrified/warmed and non-vitrified groups and compared to the fresh control groups. According to the results shown in Figure 1, there were no significant differences between the expression level of Cox1 gene in different groups of non-vitrified MII oocytes (P>0.05). The results showed that the expression level of COX1 gene was significantly high in the vitrification group (P<0.05) and pretreatment of oocytes with docetaxel can decrease the expression level of cox1 gene in different groups such as the vitrification group (P<0.05).

The results showed that the expression level of TFAM gene was significantly high in the vitrification group (P<0.01) (Figure 2). There were significant differences in TFAM expression level in vitrified different groups (P= 0.0014).

The expression level of TFAM gene was significantly high in vitrified group (vitrification) and non-vitrified group (CPA) (p<0.01) and pretreatment of oocytes with docetaxel can decrease the expression level of TFAM gene in different groups such as CPA and the vitrification group (P<0.05).

Tabel 2. Effects of docetaxel on the survival and the fertilization rate of the MII oocytes

Treatment group	No. (%) of surviving oocytes	No. (%) of oocytes fertilization (two cell)
Fresh Control	133/130 (97.79 ± 0.73)	94/84 (89.47 ± 2.003)
Docetaxel	136/124 (91.15±1.23)	99/83 (83.86±1.55)
Docetaxel+ CPA	146/130 (88.99±1/27)	121/97 (80.22± 0.83)
Docetaxel+ Vitrification	138/121 (87.68± 0.43)	98/65 (66.26±1.45)
Vitrification	145/120 (82.75±1.33)	98/61 (62.26± 2.15)
P valu	0.000	0.000

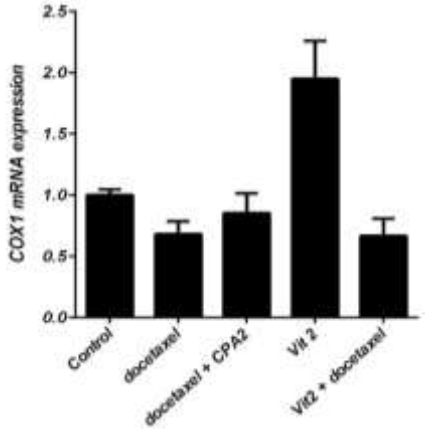

Figure 1. The expression level of Cox1 shown in different groups of vitrified and non-vitrified MII oocytes. (*significant difference between the control group and the vit2 group P= 0.0387). (P value: Control vs docetaxel= 0.0592; Control vs docetaxel+CPA2= 0.4336; Control vs Vit2+ docetaxel = 0.0910.)

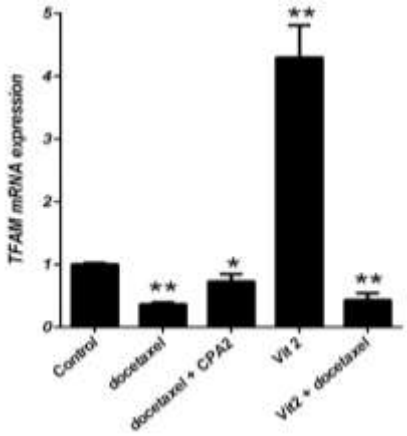

Figure 2. The expression level of TFAM shown in different groups of vitrified and non-vitrified MII oocytes (*significant difference between the control group and the docetaxel+CPA2) and (** significant difference between the docetaxel and vit2 ana vit2+docetaxel and the control group) ((P value: Control vs docetaxel= < 0.0001; Control vs docetaxel+ CPA2= 0.0217; Control vs Vit 2= 0.0001; Control vs Vit2+ docetaxel= 0.0007).

4. Discussion

In this study, we observed that the docetaxel changed the transcription levels of the mitochondrial genes including TFAM and COX1 in the vitrified-warmed MII-Oocytes.

The present study showed that the pretreatment of oocyte with docetaxel before vitrification can change the transcript level of TFAM and COX1, although some of the conditions did not. In our recent study, the vitrified-warmed oocytes that were pre-incubated with docetaxel showed higher survival and fertilization rates than those of oocytes without pre-incubation. It was also shown that the pretreatment of oocytes with docetaxel before vitrification as well as vitrification method can decrease mitochondrial gene activity (De Munck *et al.,* 2015). Mitochondria are considered a critical organelle in oocytes. The mitochondria are hereditary by the zygote completely from the oocyte, therefore the fertilizability of an oocyte. Mitochondrial genome is very important (Novin *et al.,* 2015). It was shown that a decrease in mitochondrial activity leads to damage to the oocyte maturation and produces a defect in embryo development (Bartolac *et al.,* 2018). Thus, there is a relationship between the oocyte maturation and

overexpression of mitochondrial-related (NRF1, TFAM, and MT-CO1) genes in the human oocytes (Bartolac *et al*., 2018).

Louise *et al*. reported that after warming, the survival rates of all groups were similar; therefore the differences between gene expression levels of various groups were not related to the post-warming survival (Xiao *et al.*, 2006). Also, Chasombat *et al*. showed that pre-incubation of IVM bovine oocytes with docetaxel (0.05µm) had no toxic effect on the survival and fertilization rate of vitrified-warmed oocytes (Amidi *et al.*, 2018).

The vitrification and warming mainly damage to oocyte CSF system and the normal fertilization rate of MII oocytes is dependent on normal cortical distribution (Amidi *et al*., 2018). Docetaxel, as a microtubule inhibitor, stabilizes it by preventing the disassembly of tubulin during vitrification (Dehghani *et al.*, 2019). The meiotic spindles in oocytes and the development of embryos are improved after warming. Hence, the vitrified-warmed oocyte is comparable in different steps including cleavage timings, cell number, and DNA methylation patterns with fresh oocytes (Zhou *et al.*, 2016; Diaz & Andreu, 1993).

In this study, the survival and fertilization rates of vitrified-warmed oocytes pre-incubated with docetaxel were better than those of oocytes without pre-incubation. It has been suggested that the normality of cytoskeleton fiber (CSF), cortical granules, and mitochondria after vitrification-warming might be associated with the level of cell metabolism, proliferation, and differentiation. One of the main cryoinjuries in the vitrified-warmed oocytes is damage to the oocyte CSF system. Normal fertilization is closely related to the normal cortical distribution in MII oocytes (Novin *et al*., 2015).

The rate and extent of tubulin assembly stimulate into stable microtubule by docetaxel, during vitrification, the cold may prevent the depolymerization of microtubule (Bartolac *et al.*, 2018). After vitrification and warming, the meiotic spindles recovered and the embryo development improved. Thus, the vitrified-warmed oocyte is comparable in cleavage timings, cell number, and DNA methylation patterns with the fresh oocytes (Xiao *et al*., 2006; Zhou *et al*., 2016).

Mitochondria were more sensitive during vitrification, thus to compensate for the loss of their function and oxidative damage, the mitochondrial gene expressions were increased. The expression of mitochondrial genes was increased in vitrified-warmed groups and non-vitrified compared to the controls. It should be noted that unexpectedly; the increase in the mRNA expression level of TFAM, COX1 genes may be the result of high mitochondrial biogenesis and also stimulation of mitochondrial respiratory activity. Our finding showed that the vitrification and warming solution as well as vitrification method can increase the mitochondrial activity. TFAM gene has an important role in the regulation of mtDNA copy number; therefore, the oocyte developmental stages are associated with the transcript levels of this gene. The data from the previous study showed a decrease in the expression of TFAM in the vitrified MII oocytes compared to the non-vitrified group. This result may be due to the critical role of TFAM in the stability of mitochondrial genome (mtDNA) (De Munck *et al*., 2015). There are 1000 TFAM molecules for one mtDNA in each nucleoid (Zhou *et al.*, 2016). Consequently, low expression of TFAM gene may be due to the decrease in the mtDNA copy number (De Munck *et al.*, 2015). Recent studies in various species showed that osmotic stress during vitrification and warming may lead

to damage to the mitochondrial structure, function, or distribution (Diaz & Andreu 1993; Zhou *et al.*, 2016), but the data in this study showed that vitrified-warmed oocytes with cryotop can increase the expression of mitochondrial genes that is due to increasing mtDNA copy number, quality and developmental competency of mouse MII oocytes and pretreatment of oocytes with docetaxel can decrease the expression level of the mitochondrial gene.

5. Conclusion

The present study demonstrated that pre-treating mouse MII oocytes with docetaxel before vitrification leads to a decrease in mitochondrial gene expression (TFAM and COX1). This result may be significant in enhancing the developmental competency of the oocytes after vitrification in ART.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Almasi, T.S., Rouzbehi, A., Aliabadi, E., Haeri, A., Sadeghi, Y., & Hosseini, A. (2009). Developmental consequences of mouse cryotop-vitrified oocyte and embryo using low concentrated cryoprotectants.

Amidi, F., Khodabandeh, Z., & Mogahi, M. H. N. (2018). Comparison of the effects of vitrification on gene expression of mature mouse oocytes using cryotop and open pulled straw. *International Journal of Fertility & Sterility*, *12*(1), 61.

Bartolac, L.K., Lowe, J.L., Koustas, G., Grupen, C.G., & Sjöblom, C. (2018). Vitrification, not cryoprotectant exposure, alters the expression of developmentally important genes in in vitro produced porcine blastocysts. *Cryobiology*, *80*, 70-76. https://doi.org/10.1016/j.cryobiol.2017.12.001

Chasombat, J., Nagai, T., Parnpai, R., & Vongpralub, T. (2015). Pretreatment of in vitro matured bovine oocytes with docetaxel before vitrification: Effects on cytoskeleton integrity and developmental ability after warming. *Cryobiology*, *71*(2), 216-223. https://doi.org/10.1016/j.cryobiol.2015.07.002

Ciotti, P.M., Porcu, E., Notarangelo, L., Magrini, O., Bazzocchi, A., & Venturoli, S. (2009). Meiotic spindle recovery is faster in vitrification of human oocytes compared to slow freezing. *Fertility and Sterility*, *91*(6), 2399-2407. https://doi.org/10.1016/j.fertnstert.2008.03.013

De Munck, N., Petrussa, L., Verheyen, G., Staessen, C., Vandeskelde, Y., Sterckx, J., ... & Van de Velde, H. (2015). Chromosomal meiotic segregation, embryonic developmental kinetics and DNA (hydroxy) methylation analysis consolidate the safety of human oocyte vitrification. *MHR: Basic science of reproductive medicine*, *21*(6), 535-544.

De Munck, N., Petrussa, L., Verheyen, G., Staessen, C., Vandeskelde, Y., Sterckx, J., ... & Van de Velde, H. (2015). Chromosomal meiotic segregation, embryonic developmental kinetics and DNA (hydroxy) methylation analysis consolidate the safety of human oocyte vitrification. *MHR: Basic science of reproductive medicine*, *21*(6), 535-544.

Dehghani, N., Dianatpour, M., Hosseini, S. E., Khodabandeh, Z., & Daneshpazhouh, H. (2019). Overexpression of mitochondrial genes (mitochondrial transcription factor A and cytochrome c oxidase subunit 1) in mouse metaphase II oocytes following vitrification via cryotop. *Iranian Journal of Medical Sciences*, *44*(5), 406.

Dhali, A., Anchamparuthy, V. M., Butler, S.P., Pearson, R.E., Mullarky, I.K., & Gwazdauskas, F.C. (2009). Effect of droplet vitrification on development competence, actin cytoskeletal integrity and gene expression in in vitro cultured mouse embryos. *Theriogenology*, *71*(9), 1408-1416. https://doi.org/10.1016/j.theriogenology.2009.01.011

Diaz, J.F., & Andreu, J.M. (1993). Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry*, *32*(11), 2747-2755.

El Shourbagy, S.H., Spikings, E.C., Freitas, M., & St John, J.C. (2006). Mitochondria directly influence fertilisation outcome in the pig. *Reproduction*, *131*(2), 233-245.

Falkenberg, M., Larsson, N. G., & Gustafsson, C.M. (2007). DNA replication and transcription in mammalian mitochondria. *Annu. Rev. Biochem.*, *76*, 679-699.

Fernández-Silva, P., Enriquez, J.A., & Montoya, J. (2003). Replication and transcription of mammalian mitochondrial DNA. *Experimental physiology*, *88*(1), 41-56.

Huang, J. Y., Chen, H. Y., Park, J. Y. S., Tan, S. L., & Chian, R. C. (2008). Comparison of spindle and chromosome configuration in in vitro-and in vivo-matured mouse oocytes after vitrification. *Fertility and Sterility*, *90*(4), 1424-1432.

Jiménez-Trigos, E., Naturil-Alfonso, C., Vicente, J.S., & Marco-Jiménez, F. (2013). Post-Warming Competence of In Vivo Matured Rabbit Oocytes Treated with Cytoskeletal Stabilization (Taxol) and Cytoskeletal Relaxant (Cytochalasin B) Before Vitrification. *Reproduction in Domestic Animals*, *48*(1), 15-19. https://doi.org/10.1111/j.1439-0531.2012.02018.x

Jiménez-Trigos, E., Naturil-Alfonso, C., Vicente, J.S., & Marco-Jiménez, F. (2012). Effects of cryopreservation on the meiotic spindle, cortical granule distribution and development of rabbit oocytes. *Reproduction in domestic animals*, *47*(3), 472-478. https://doi.org/10.1111/j.1439-0531.2011.01906.x

Khodabandeh, J.Z., Amidi, F., Nouri, M.S., Sobhani, A., Mehranian, K.M., ABBASI, M., ... & Ebrahimi, M. (2010). Expression of heat shock protein (HSP A1A) and MnSOD genes following vitrification of mouse MII oocytes with cryotop method.

Khodabandeh, J.Z., Amidi, F., NOURI, M. S., Sobhani, A., Mehranian, K.M., Abbasi, M., ... & Ebrahimi, M. (2010). Expression of heat shock protein (HSP A1A) and MnSOD genes following vitrification of mouse MII oocytes with cryotop method.

Kuwayama, M. (2007). Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology*, *67*(1), 73-80. https://doi.org/10.1016/j.theriogenology.2006.09.014

Kuwayama, M. (2007). Oocyte cryopreservation. *Journal of Mammalian Ova Research*, *24*(1), 2-7.

Montoya, J., López-Pérez, M.J., & Ruiz-Pesini, E. (2006). Mitochondrial DNA transcription and diseases: past, present and future. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1757*(9-10), 1179-1189. https://doi.org/10.1016/j.bbabio.2006.03.023

Morató, R., Izquierdo, D., Albarracín, J.L., Anguita, B., Palomo, M.J., Jiménez-Macedo, A.R., ... & Mogas, T. (2008). Effects of pre-treating in vitro-matured bovine oocytes with the cytoskeleton stabilizing agent taxol prior to vitrification. *Molecular Reproduction and Development: Incorporating Gamete Research*, *75*(1), 191-201. https://doi.org/10.1002/mrd.20725

Morató, R., Izquierdo, D., Paramio, M.T., & Mogas, T. (2008). Cryotops versus open-pulled straws (OPS) as carriers for the cryopreservation of bovine oocytes: effects on spindle and chromosome configuration and embryo development. *Cryobiology*, *57*(2), 137-141. https://doi.org/10.1016/j.cryobiol.2008.07.003

Novin, M. G., Noruzinia, M., Allahveisi, A., Saremi, A., Fathabadi, F.F., Farahani, R. M., ... & Yousefian, E. (2015). Comparison of mitochondrial-related transcriptional levels of TFAM, NRF1 and MT-CO1 genes in single human oocytes at various stages of the oocyte maturation. *Iranian biomedical journal*, *19*(1), 23.

Roozbehi, A. (2013). Mouse oocytes and embryos cryotop-vitrification using low concentrated solutions: Effects on meiotic spindle, genetic material array and developmental ability. *Iranian journal of basic medical sciences*, *16*(4), 590.

Scarpulla, R.C. (2002). Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene*, *286*(1), 81-89. https://doi.org/10.1016/S0378-1119(01)00809-5

Sripunya, N., Somfai, T., Inaba, Y., Nagai, T., Imai, K., & Parnpai, R. (2010). A comparison of cryotop and solid surface vitrification methods for the cryopreservation of in vitro matured bovine oocytes. *Journal of Reproduction and Development*, *56*(1), 176-181.

Vincent, C., Pickering, S.J., Johnson, M.H., & Quick, S.J. (1990). Dimethylsulphoxide affects the organisation of microfilaments in the mouse oocyte. *Molecular Reproduction and Development*, *26*(3), 227-235. https://doi.org/10.1002/mrd.1080260306

Wang, L.Y., Wang, D.H., Zou, X.Y., & Xu, C.M. (2009). Mitochondrial functions on oocytes and preimplantation embryos. *Journal of Zhejiang University Science B*, *10*(7), 483-492. https://doi.org/10.1631/jzus.B0820379

Xiao, H., Verdier-Pinard, P., Fernandez-Fuentes, N., Burd, B., Angeletti, R., Fiser, A., ... & Orr, G. A. (2006). Insights into the mechanism of microtubule stabilization by Taxol. *Proceedings of the National Academy of Sciences*, *103*(27), 10166-10173. https://doi.org/10.1073/pnas.0603704103

Yoon, T.K., Kim, T.J., Park, S.E., Hong, S. W., Ko, J.J., Chung, H.M., & Cha, K.Y. (2003). Live births after vitrification of oocytes in a stimulated in vitro fertilization–embryo transfer program. *Fertility and sterility*, *79*(6), 1323-1326. https://doi.org/10.1016/S0015-0282(03)00258-9

Zhou, C.J., Wang, D.H., Niu, X.X., Kong, X.W., Li, Y.J., Ren, J., ... & Liang, C.G. (2016). High survival of mouse oocytes using an optimized vitrification protocol. *Scientific reports*, *6*(1), 19465.