Original Article

Expression of Apoptosis-Related Genes in Cultured Ovarian Tissue of Mouse in Presence of Coenzyme Q$_{10}$

Shayesteh Behnam; M.Sc.[1], Saeed Zavareh; Ph.D.[2], Mojdeh Salehnia; Ph.D.[1]

1 Anatomy Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2 School of Biology, Damghan University, Damghan, Iran

Abstract

Objective: Oxidative stress affects the development of ovarian follicles during *in vitro* culture thus applying an antioxidant is necessary. The aim of this study was to investigate the effect of coenzyme Q$_{10}$ (CoQ$_{10}$) on the expression of apoptosis-related genes of mouse ovaries during *in vitro* culture.

Materials and methods: The immature mouse ovaries were cultured in the presence of 50 μM CoQ$_{10}$ for 7 days. Histological examinations were performed and the 17 beta-estradiol concentration was measured on the seventh day of culture. The relative expression of *Caspase* 3, *Bax, Bad,* and *Bcl* 2 genes were investigated by real-time RT-PCR.

Results: The rates of normal follicles in the presence of CoQ$_{10}$ was significantly increased compared to the control group. Also, in CoQ$_{10}$-trated group a significant increase in the level of 17 beta-estradiol was seen compared to the control group. The mRNA expression of anti-apoptotic gene *Bcl*2 was significantly increased while the expression of pro-apoptotic genes (*Caspase*3, *Bax* and *Bad*) significantly declined in CoQ$_{10}$ treated group compared to those of control group.

Conclusion: The supplementation of the ovarian culture media with CoQ$_{10}$ improved the follicular development through alteration in expression of apoptosis-related genes and stimulated the production of estradiol.

Keywords: Apoptosis; Coenzyme Q$_{10}$; In Vitro Culture; Ovarian Tissue

Introduction

In vitro culture of ovarian tissue is an alternative strategy for follicular growth and development to improve fertility potential (1-4). During *in vitro* culture of follicles and ovarian tissue the high level of reactive oxygen species (ROS) are produced by oxidative stress that affects the survival of ovarian follicles and their development (5, 6). Therefore, many attempts have been focused on the application of an antioxidant to reduce the harmful effects of ROS (7).

There are two main categories of antioxidants: enzymatic such as superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic ones including vitamin E and C, selenium, and CoQ$_{10}$ that are used to attenuate the effects of oxidative stress (8, 9).

CoQ$_{10}$ as a non-enzymatic antioxidant is distributed throughout several tissues of the body, especially in the heart, kidney, liver, and muscle tissues (10). CoQ$_{10}$ is a critical component of the electron transfer chain in mitochondria (11).

Correspondence:
Dr. Mojdeh Salehnia
Email: salehnim@modares.ac.ir

According to previous studies, CoQ10 improves the survival and the developmental competence of follicles, oocytes, and embryos (12-14). Also, another series of studies showed that the supplementation of culture media with CoQ10 increases follicular total antioxidant capacity, superoxide dismutase, and glutathione peroxidase levels (13, 15). Heydarnejad et al. demonstrated that CoQ10 could alter the expression of apoptotic-related genes such as *Caspase-3* and *Bax* in sheep oocytes and cumulus cells during in vitro culture of cumulus oocytes complex (16). In addition, it was revealed that by several investigators, CoQ10 could increase the mitochondrial mass and improve the distribution and polarization of mitochondrial membrane in bovine and mouse oocytes, also it could reduce the mitochondrial malfunction in the blastocysts (17-20).

During in vitro culture of ovarian tissue, apoptosis as a programmed cell death may be initiated by extrinsic or intrinsic factors that triggered the related pathways (7, 21). Both of these pathways could affect the viability and development of oocytes and follicles (22). Several investigators showed that the usage of CoQ10 in vitro or in vivo could decrease the incidence of oocyte apoptosis by altering the expression of genes related to apoptosis (16). While based on our knowledge there was no report to evaluate the improvement of ovarian tissue culture by using CoQ10 and evaluating the changes in the follicular expression of apoptosis genes. Therefore, the aim of the present study was to investigate the effect of CoQ10 on the follicular development and expression of genes involved in apoptosis during mouse ovarian tissue culture.

Materials and methods

Animal: In this experimental study, two weeks old Naval Medical Research Institute (NMRI) mice (n=9) were kept under a controlled condition (20-24°C, 40-50% humidity and 12/12h light: dark cycles) in the animal house of Tarbiat Modares University. All stages of the research were carried out with the approval of the Ethics committee for Animal Research of Tarbiat Modares University (Ref NO: 1399.008). The mice were sacrificed by cervical dislocation, and their ovaries were removed and dissected using two insulin syringe needles under stereomicroscope (Olympus; Japan). The collected ovaries were washed in 30 μl droplets of alpha-minimal essential medium (α-MEM; Gibco, UK) supplemented with 10% fetal bovine serum (FBS;

Gibco, UK), 100 IU/mL-1 penicillin (Pan biote, Germany), 100 μg/mL-1 streptomycin (Pan biote, Germany), 0.23 mMol sodium pyruvate (Sigma-Aldrich, Germany) and 2.2 g/L sodium bicarbonate (Carl Roth, Germany).

Experimental design: The ovaries were randomly divided into control and experimental groups (n = 9 in each group). In the experimental group the ovaries were cultured in media supplemented with 50 μM CoQ10 based on the previous research (13). After one week of *in vitro* culture, the morphology of ovaries, the level of 17 beta-estradiol in collected cultured media, and the expression level of genes related to apoptosis were studied.

In vitro culture of ovaries: In both groups the ovaries were cultured on the insert (0.4-μm pore size; Greiner Bio-One, Germany) in the 24-well plates (SPL, Korea) containing 300 μl of α-MEM supplemented with 10% FBS, 1% insulin, transferrin and selenium (ITS; Gibco, USA), 100 mIU/mL recombinant FSH (rFSH; Homa pharmed; Iran) in the CO2 incubator (5% CO_2, 100% humidity, 37°C) for one weethe k. In experimental group 50 μM CoQ10 (Nono kimia; Kore) was added to the culture media (12, 14). Every other day, the development of ovarian follicles was evaluated under an inverted microscope (Labomed, USA) and, half of the culture media (150 μl) was renewed. The collected culture medium was stored at -20°C until measurement of the 17-β-estradiol level.

Histological study: To evaluate the morphology of cultured ovaries in both groups (n=5 in each group) of the study, they were subjected to tissue processing including a fixation with Bouin's solution, dehydration with increasing concentration of alcohol (70%, 80%, 90%, 100%), clearing with Xylene and impregnation and embedding with paraffin wax. After serial sectioning at 5 μm thickness, the sections with 5 intervals were collected, stained using hematoxylin and eosin (H&E), and studied under a light microscope. The normal follicles had an intact oocyte surrounded by regular layers of granulosa cells with no condensed nucleus and degenerated follicles showed shrinkage oocyte which was surrounded by disorganized and detached granulosa cells. To avoid duplicate counting of the follicles, only follicles with a visible nucleus in the oocyte were evaluated (7) and classification of follicles was performed based on the previous studies (23). The preantral follicles according to their sizes were divided into small (80-130 μm), medium

(131-210 μm) and large follicles with prominent antrum cavities (211-330 μm).

Hormonal assay: The level of 17-β-estradiol (E2) in the collected culture media (n = 3 in each group and in three repeats) at the end of the culture period was measured using the electrochemiluminescence immunoassay kit (ECLIA; Abbott architect i2000SR, UK).

Real time RT-PCR: At the end of the culture period, the RNA of cultured ovarian tissues in studied groups (3 ovaries in each group in 3 replications) were extracted by TRIzol ® Kit (Thermo Fisher Scientific, Bremen, Germany) following the manufacturer's protocol. RNA quantification was evaluated by spectrophotometric read at 260 nm. cDNA preparation was obtained using cDNA synthesis kit (Thermo Fisher Scientific, Bremen, Germany) according to the manufacturer's instructions. Relative mRNA expression levels were investigated by a StepOnePlus real-time PCR system (Applied BioSystems, USA) using QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany). Relative quantification analysis was performed using the $2^{-\Delta\Delta CT}$ method. The primers of the *Bcl-2*, *Bax*, *Bad* and *Caspase-3* genes were designed by AlleleID software (Primier Biosoft, USA) and the elongation factor 1 (*Ef1*) gene was used as a housekeeping gene (24) (Table 1).

Statistical analysis: All data were analyzed by SPSS for the windows statistical package program (version 24, Chicago, IL, USA). The normal distribution of data and equality of variances were investigated with the Shapiro test and Levent test respectively. Then, an independent sample t-student test (parametric conditions) was used to compare the follicular count, hormone level, and gene expression level, between the two studied groups. A *P*-value less than 0.05 was considered statistically significant. The

data are presented as mean ± SD. Each experiment was repeated at least three times.

Results

Morphology of ovaries: The morphological observations of cultured ovaries under an inverted microscope during cultivation time in both groups of study are shown in Figure 1. As these figures showed the growing follicles in the peripheral area of the ovarian tissue are prominent. The central region of the cultured ovarian tissue in both groups had a dark and dens appearance.

Histological examination of studied ovaries using hematoxylin and eosin staining is demonstrated in Figure 2. Normal follicles at different developmental stages were seen in both groups and the morphology of ovarian sections is almost similar in both groups. In the central regions of ovarian tissue, some degenerated follicles were seen especially in the control group and in the periphery of the ovaries in both groups. The large preantral follicles were observed in treated ovaries with CoQ_{10}.

The rates of survived and degenerated follicles at different developmental stages in both groups are summarized in Table 2. As results showed the ovaries that were cultured in the presence of CoQ_{10} had a significantly higher mean percentage of normal follicles ($85.40 \pm 4.22\%$) and lower degenerated follicles ($14.60 \pm 4.29\%$) in comparison with the control group ($P < 0.05$). These rates regarding to primordial, medium preantral, and large preantral follicles were not significantly different between these two groups ($P > 0.05$) while these rates had significantly increased in relation to primary ($83.74 \pm 2.99\%$ vs $76.73 \pm 1.98\%$) and small preantral follicles ($67.52 \pm .13\%$ vs $42.32 \pm 4.36\%$) in CoQ_{10} treated and control groups respectively ($P < 0.05$).

Table 1: The sequences of designed primers

Target gene	Accession number	Primer sequences	PCR product size (bp)
Bad	NM-0011285423.1	F: 5′-GGAGCAACATTCATCAGCAG-3′	91
		R: 5′-TACGAACTGTGGCGACTC-3′	
Bcl-2	NM-177410	F: 5′-TGCTGCTATCCTGCCAAG-3′	108
		R: 5′-GTCTGTGTTCTTCATCGTTACTTC-3	
Caspase-3	NM-00981003	F: 5′-GCTGACTTCCTGTATGCTTA-3′	164
		R: 5′-GTTGCCACCTTCCTGTTA-3′	
Bax	NM-0075227.3	F: 5′-GCGAATTGGAGATGAACT-3′	129
		R: 5′-CAGTTGAAGTTGCCATCA-3′	
Ef1	NM-010106.2	F: 5′-AGTCGCCTTGGACGTTCTT-3′	124
		R: 5′-CCGATTACGACGATGTTGATGTG-3′	
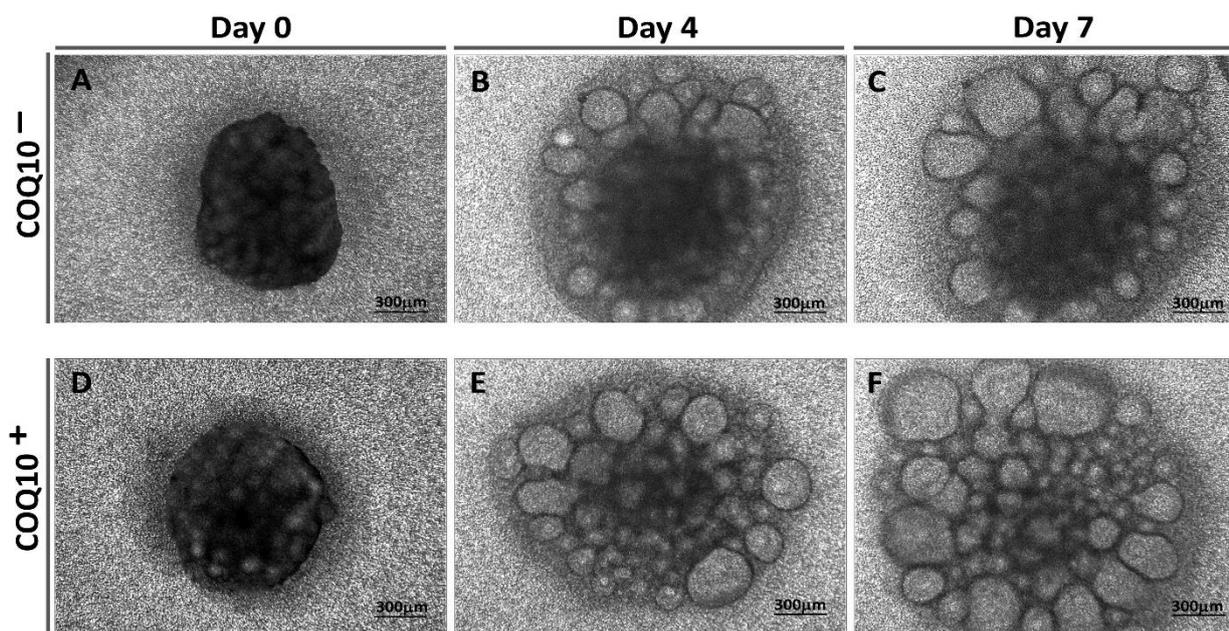
Figure 1: Photomicrographs of mouse ovaries under an inverted microscope on days 0, 5, and 7. A-C: Control, D-F: COQ10 treated group.

Hormonal assay: The average E2 levels in collected culture media after 7 days of in vitro culture of ovaries in both groups is shown and compared in Table 3. The level of E2 in the group treated with CoQ_{10} was significantly higher (8994.66 ± 1341.41 pg /ml) compared to that of the control group (5260.33 ± 1911.83 pg /ml; $P < 0.05$).

Real time RT-PCR result: The relative expression of target genes to the housekeeping gene (*Ef1*) in both studied groups is presented in Figure 3. As this figure shows the relative expression of *Bcl-2*, *Bax*, *Bad* and *Caspase-3* genes in control group were 0.97 ± 0.11, 1 ± 0.5, 0.99 ± 0.7 and 1.02 ± 0.99 and these ratios in CoQ_{10} treated group were 6.84 ± 1.03, 0.64 ± 0.01, 0.48 ± 0.07 and 0.46 ± 0.09 respectively ($P < 0.05$). In addition, the ratio of *Bcl-2/Bax* expression in the control and CoQ_{10} treated group were 0.98± 0.16 and 10.64 ± 1.6 respectively ($P < 0.05$).
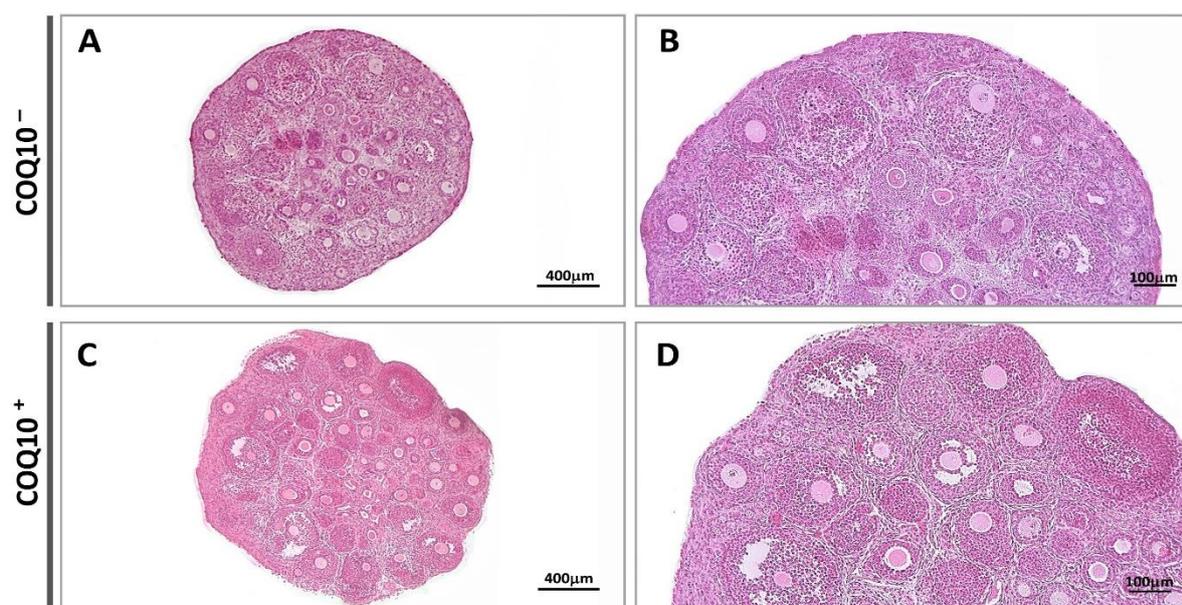
Figure 2: Photomicrographs of mouse ovarian tissue sections stained using hematoxylin and eosin after seven days of in vitro culture. A and B: Control; C-D: treated group with 50 µM COQ10.

Table 2: The mean percentages of normal follicles at different developmental stages in the studied groups

Groups		Total No. (Mean% ± SD)	No. Primordial (Mean% ± SD)	No. Primary (Mean% ± SD)	No. Small preantral (Mean% ± SD)	No. Medium preantral (Mean% ± SD)	No. Large preantral (Mean% ± SD)
Control	Normal	929	587	155	124	34	25
		(77.74 ± 5.76)	(95.44 ± 1.56)	(76.73±1.98)	(42.32 ± 4.36)	(73.37 ± 3.81)	(80.64 ± 4.62)
	Degenerated	266	28	47	169	16	6
		(22.26 ± 5.76)	(4.56 ± 1.56)	(23.27 ± 1.98)	(57.68 ± 4.36)	(29.63 ± 3.81)	(19.36 ± 4.62)
COQ10	Normal	1033	591	170	131	77	64
		(85.40 ± 4.22) *	(95.01 ± 1.00)	(83.74 ± 2.99) *	(67.52 ± 4.13) *	(66.37 ± 2.87)	(85.33 ± 2.18)
	Degenerated	177	31	33	63	39	11
		(14.60 ± 4.29) *	(4.99 ± 1.00)	(16.25 ± 2.99) *	(32.48 ± 4.130 *	(33.63 ± 2.87)	(14.67 ± 2.18)

COQ10: Coenzyme Q10;
*: Indicate significant differences compared with the control group in the same column ($P<0.05$).
Small preantral follicles (80-130 μm), medium preantral follicles (131-210 μm), and large preantral follicles (211-330 μm).
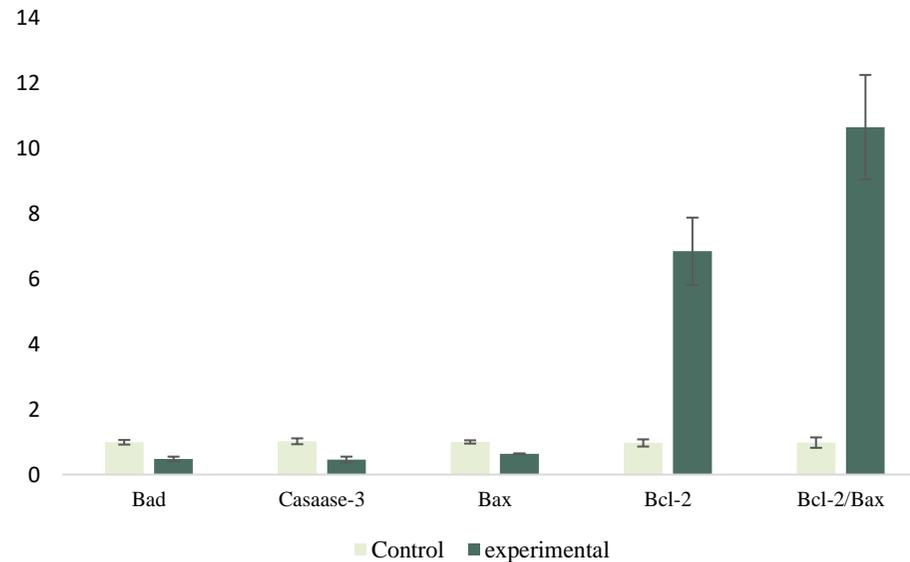
Figure 3: The comparison of relative expression of Bad, Caspase-3, Bax, and Bcl-2 genes to housekeeping genes in both studied groups. *: Indicate significant differences compared with the control group ($P<0.05$).

Table 3: The concentration of 17-β-estradiol (pg mL-1) on Day 7 of culture.

Groups	(Mean% ± SD)
Control	5260.33 ± 1911.83
COQ10	8994.66 ± 1341.41*

COQ10; Coenzyme Q10, *: Indicate significant differences compare with the control group (P<0.05)

The relative expression of pro-apoptotic genes *Caspase*-3, *Bax* and *Bad* was significantly decreased, while the expression ratio of *Bcl-2* and *Bcl-2/Bax* was significantly higher in experimental groups in comparison with the control group (P <0.05).

Discussion

The present study, for the first time, investigated the effect of CoQ_{10} on the expression of apoptosis-related genes in cultured ovarian tissue. Histological examination showed CoQ_{10} decreased degeneration and improved follicular survival rate. Also, CoQ_{10} improved the development of small preantral follicles and increased the number of medium and large preantral follicles by decreasing the rates of degeneration in the primary follicles and small preantral follicles. In addition, it seems that CoQ_{10} preserves the storage of primordial follicles by gradually activating these follicles and turning them into primary follicles.

One of the possible reasons for this effect of CoQ_{10} on the survival and development of ovarian follicles is its antioxidant properties and its ability to scavenge free radicals. Although in the present study we have not analyzed the ROS level in cultured ovaries, in agreement with this suggestion several previous studies have shown that the supplementation of culture media with 50 μM CoQ_{10} decreases ROS production and increases antioxidant capacity which in turn improved follicular survival and development (13, 15). Similarly, Ben Meir *et al.* showed that injection of 22 mg of CoQ_{10} for 12-13 weeks in older mice increased oocyte quality, ovulation rate, and ovarian reserves by increasing the number of primordial, preantral, and antral follicles (18).

CoQ_{10} also improves cytoplasmic maturation by affecting mitochondrial function by improving distribution patterns of mitochondrial, increasing the expression of mitochondrion-associated genes involved in the respiratory chain and metabolism-related genes (16, 18-20, 25). Also it improves nuclear maturation via reduction in DNA fragmentation, restoring spindle assembly,

chromosome alignment, and actin organization (19, 25-27). In this regard, it has been investigated that the presence of 30 μM CoQ_{10} in the maturation media of sheep oocytes increased the gene expression of Growth Differentiation Factor-9 (GDF-9) as well as follicular development (16, 28).

The other part of this study demonstrated that CoQ_{10} stimulate granulosa cell to secret more 17-β-estradiol as follicular functional marker. Similarly, previous studies have shown that CoQ_{10} affects the proliferation of granulosa cells by increasing the expression of genes related to cell proliferation such as *Bone Morphogenetic Protein 15* (*BMP15*) and *Proliferating Cell Nuclear Antigen* (PCNA) (29, 30). On the other hand, Delkhosh *et al.* showed that administration of 22 mg CoQ_{10} in cyclophosphamide-treated mice increased Follicle Stimulating Hormone (FSH) receptor expression levels (29) and FSH stimulates the secretion of estradiol from granulosa cells (31).

Other part of the present study showed that CoQ_{10} decreased the expression of pro-apoptotic genes (*Bax*, *Bad*, *Caspase-3*) and increased the expression of the anti-apoptotic gene (*Bcl-2 and Bcl-2/Bax ratio*). Similarly, Heydarnejad *et al.* demonstrated the supplementation of sheep oocytes maturation media with CoQ_{10} decreased the expression of pro-apoptotic genes such as *caspase*-3 and *Bax* in oocytes and cumulus cells (16). Another in vivo study revealed that injection of CoQ_{10} into aged female mice and pigs reduced cell death and Caspase-3 level and oocytes apoptosis (18, 19, 26). The reduction of pro-apoptotic gene expression in the CoQ_{10}-treated group may be related to reducing to ROS production (13, 15), or inhibition of cytochrome C release and inhibition of Caspase activity (32).

Conclusion

The supplementation of the ovarian culture media with CoQ_{10} improved the follicular development through alteration in expression of the apoptosis-related genes and stimulate the production of estradiol in granulosa cells which in turn promoted follicular development.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

thesis, which was founded and supported by Tabiat Modares University, Tehran, Iran.

References

1. Ferreri J, Fàbregues F, Calafell JM, Solernou R, Borrás A, Saco A, et al. Drug-free in-vitro activation of follicles and fresh tissue autotransplantation as a therapeutic option in patients with primary ovarian insufficiency. Reprod Biomed Online. 2020;40(2):254-60.

2. Devenutto L, Quintana R, Quintana T. In vitro activation of ovarian cortex and autologous transplantation: A novel approach to primary ovarian insufficiency and diminished ovarian reserve. Hum Reprod Open. 2020;2020(4):hoaa046.

3. Lee HN, Chang EM. Primordial follicle activation as new treatment for primary ovarian insufficiency. Clin Exp Reprod Med. 2019;46(2):43-9.

4. Ghezelayagh Z, Abtahi NS, Valojerdi MR, Mehdizadeh A, Ebrahimi B. The combination of basic fibroblast growth factor and kit ligand promotes the proliferation, activity and steroidogenesis of granulosa cells during human ovarian cortical culture. Cryobiology. 2020;96:30-6.

5. Cavalcante BN, Matos-Brito BG, Paulino LR, Silva BR, Aguiar AWM, de Almeida EFM, et al. Effects of melatonin on morphology and development of primordial follicles during in vitro culture of bovine ovarian tissue. Reprod Domest Anim. 2019;54(12):1567-73.

6. Hovatta O, Silye R, Abir R, Krausz T, Winston R. Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. Hum Reprod. 1997;12(5):1032-6.

7. Abedpour N, Salehnia M, Ghorbanmehr N. The Effects of Lysophosphatidic Acid on the incidence of cell death in cultured vitrified and non-vitrified mouse ovarian tissue: separation of necrosis and apoptosis border. Cell J. 2018;20(3):403-11.

8. Mirończuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. Adv Med Sci. 2018;63(1):68-78.

9. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. 2015;5(35):27986-8006.

10. Bhagavan HN, Chopra RK. Coenzyme Q10: absorption, tissue uptake, metabolism and pharmacokinetics. Free Radic Res. 2006;40(5):445-53.

11. Bentinger M, Tekle M, Dallner G. Coenzyme Q–biosynthesis and functions. Biochem Biophys Res Commun. 2010;396(1):74-9.

12. Lee CH, Kang MK, Sohn DH, Kim HM, Yang J, Han SJ. Coenzyme Q10 ameliorates the quality of mouse oocytes during in vitro culture. Zygote. 2022;30(2):249-57.

13. Hosseinzadeh E, Zavareh S, Lashkarbolouki T. Antioxidant properties of coenzyme Q10-pretreated mouse pre-antral follicles derived from vitrified ovaries. J Obstet Gynaecol Res. 2017;43(1):140-8.

14. Ruiz-Conca M, Vendrell M, Sabés-Alsina M, Mogas T, Lopez-Bejar M. Coenzyme Q10 supplementation during in vitro maturation of bovine oocytes (Bos taurus) helps to preserve oocyte integrity after vitrification. Reprod Domest Anim. 2017;52:52-4.

15. Kashka RH, Zavareh S, Lashkarboluki T. The role of Coenzyme Q10 on the Total Antioxidant Capacity of Mouse Vitrified Pre-Antral Follicles. Arch Adv Biosci. 2015;6(3):1-9.

16. Heydarnejad A, Ostadhosseini S, Varnosfaderani SR, Jafarpour F, Moghimi A, Nasr-Esfahani MH. Supplementation of maturation medium with CoQ10 enhances developmental competence of ovine oocytes through improvement of mitochondrial function. Mol Reprod Dev. 2019;86:812-24.

17. Abdulhasan M, Li Q, Dai J, Abu-Soud H, Puscheck E, Rappolee D. CoQ10 increases mitochondrial mass and polarization, ATP and Oct4 potency levels, and bovine oocyte MII during IVM while decreasing AMPK activity and oocyte death. J Assist Reprod Genet. 2017;34(12):1595-607.

18. Ben-Meir A, Burstein E, Borrego-Alvarez A, Chong J, Wong E, Yavorska T, et al. Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell. 2015;14(5):887-95.

19. Zhang M, ShiYang X, Zhang Y, Miao Y, Chen Y, Cui Z, et al. Coenzyme Q10 ameliorates the quality of postovulatory aged oocytes by suppressing DNA damage and apoptosis. Free Radic Biol Med. 2019;143:84-94.

20. Liang S, Niu YJ, Shin K-T, Cui X-S. Protective effects of coenzyme q10 on developmental competence of porcine early embryos. Microsc Microanal. 2017;23(4):849-58.

21. Sinha K, Das J, Pal PB, Sil PC. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol. 2013;87(7): 1157-80.

22. Dehghan M, Shahbazi S, Salehnia M. Lysophosphatidic acid alters the expression of apoptosis related genes and miR-22 in cultured and autotransplanted ovaries. Cell J. 2021;23(5):584.

23. Sadeu J, Adriaenssens T, Smitz J. Expression of growth differentiation factor 9, bone morphogenetic protein 15,

and anti-Mullerian hormone in cultured mouse primary follicles. Reprod. 2008;136(2):195-203.

24. Nasiri M, Saadat M, Karimi MH, Azarpira N, Saadat I. Evaluating mRNA Expression Levels of the TLR4/IRF5 Signaling Axis During Hepatic Ischemia-Reperfusion Injuries. Experimental and clinical transplantation : official journal of the Middle East Society for Organ Transplantation. 2019;17(5):648-52.

25. Ma L, Hu M, Ma X, Cui Y, Liu J. CoQ10 decreases aneuploidy rate and increases mitochondrial mass during in vitro maturation of human immature oocytes. Fertil Steril. 2018;110(4):e312.

26. Casper R, Ben-Meir A, Bentov Y, Esfandiari N, Jurisicova A. Cumulus cell CoQ10 synthesis and mitochondrial function in aged mice: effect of CoQ10 supplementation. Fertil Steril. 2012;98(3):S152.

27. Yang C-X, Liu S, Miao J-K, Mou Q, Liu X-M, Wang P-C, et al. CoQ10 improves meiotic maturation of pig oocytes through enhancing mitochondrial function and suppressing oxidative stress. Theriogenology. 2021;159:77-86.

28. Sharideh H, Zhandi M, Zeinoaldini S, Zaghari M, Sadeghi M, Akhlaghi A, et al. Beneficial effects of dietary coenzyme Q10 on the productive and reproductive variables of broiler breeder hens. Anim Reprod Sci. 2020;213:106256.

29. Delkhosh A, Delashoub M, Tehrani AA, Bahrami AM, Niazi V, Shoorei H, et al. Upregulation of FSHR and PCNA by administration of coenzyme Q10 on cyclophosphamide-induced premature ovarian failure in a mouse model. J Biochem Mol Toxicol. 2019;33(11):e22398.

30. Lee HJ, Park MJ, Joo BS, Joo JK, Kim YH, Yang SW, et al. Effects of coenzyme Q10 on ovarian surface epithelium-derived ovarian stem cells and ovarian function in a 4-vinylcyclohexene diepoxide-induced murine model of ovarian failure. Reprod Biol Endocrinol. 2021;19(1):1-10.

31. Pajokh M, Salehnia M. Evaluation of the Follicular Growth during Mouse Ovarian Culture in Medium Supplemented with Different Doses of Growth Differentiation Factor 9B. Pathobiol Res. 2013;16(3):43-51.

32. Kumari S, Mehta SL, Milledge GZ, Huang X, Li H, Li PA. Ubisol-Q10 prevents glutamate-induced cell death by blocking mitochondrial fragmentation and permeability transition pore opening. Int J Biol Sci. 2016;12(6):688.