Improving the Effects of Omega-3 Fatty Acid on the *In Vitro* Maturation of Oocytes

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(received 27-08-2020; revised 28-10-2020; accepted 10-11-2020)

ABSTRAK

Ghorbani Vahed M, Khanbabaee R, Shariati M, Edalatmanesh MA. 2020. Peningkatan pengaruh asam lemak omega-3 dalam pematangan oosit secara *in vitro*. JITV 25(4): 153-161. DOI:http://dx.doi.org/10.14334/jitv.v25i4.2555

Penelitian ini dilakukan untuk mengetahui pengaruh omega-3 terhadap pematangan oosit secara *in vitro* dan tingkat ekspresi tribbles (gen *TRIB1*, *TRIB2* dan *TRIB3*) pada sel kumulus. Mencit NMRI berumur delapan-sepuluh minggu disuperovulasi menggunakan 7,5 IU serum gonadotropin (PMSG, Intraperitoneal) dari kuda bunting yang kemudian disembelih setelah 44 jam dan ovariumnya diangkat. Oosit digunakan untuk pematangan *in vitro* dan kompleks kumulus-oosit (COC) dilepaskan. Sel kumulus dan oosit dimasukkan ke dalam kontrol, dengan perlakuan etanol dan kelompok yang diberi 10 dan 100 µg/ml omega-3. Sel disiapkan guna melihat tahap pematangan untuk mengevaluasi tingkat ekspresi gen. Data dianalisis secara statistik. Mengekspos oosit dengan omega-3 dosis rendah (10 µg/ml) dan dosis tinggi (100 µg/ml) mengakibatkan penurunan laju oosit stadium GV, penurunan MI-oosit dan peningkatan MII-oosit. Peningkatan kematangan COC juga terdeteksi sebagai respon terhadap omega-3 dosis tinggi (100 µg/ml). Paparan sel kumulus terhadap omega-3 (10 dan 100 µg/ml) menginduksi *TRIB2* dan menghambat tingkat ekspresi gen *TRIB3*; namun, tingkat ekspresi gen *TRIB1* meningkat dan menurun sebagai respon terhadap konsentrasi omega-3 yang rendah (10 µg/ml) dan tinggi (100 µg/ml). Penambahan omega-3 ke dalam lingkungan oosit atau sel kumulus mempengaruhi pematangan oosit dan sel kumulus, yang diikuti oleh ekspresi diferensial dari gen *TRIB*, menunjukkan peran metabolisme asam lemak dalam diferensiasi dan pematangan sel kumulus.

Kata Kunci: Sel Kumulus, Pematangan, Omega-3, Gen *TRIB*

ABSTRACT

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This research was conducted in order to determine the effects of omega-3 on oocyte in vitro maturation and the level of expression of tribbles (*TRIB1*, *TRIB2* and *TRIB3* genes) in cumulus cells. Eight-ten weeks old NMRI mice were super-ovulated using 7.5 IU pregnant mare's serum gonadotropin (PMSG, Intraperitoneal) and they were killed after 44 hours and their ovaries were removed. The oocytes were used for *in vitro* maturation and the cumulus-oocyte complexes (COCs) were released. Cumulus cells and oocytes were assigned into control, ethanol-treated and groups exposed to 10 and 100 µg/ml of omega-3. The cells were prepared to assess the maturation stage in order to evaluate the gene expression level. The data were statistically analyzed. Exposing oocytes to low dose (10 µg/ml) and high dose (100 µg/ml) of omega-3 resulted in a reduced rate of GV-stage oocytes, decreased MI-oocytes and increased MII-oocytes. The enhanced maturity of COCs was also detected in response to a high dose of omega-3 (100 µg/ml). Exposure of cumulus cells to omega-3 (10 and 100 µg/ml) induced *TRIB2* and inhibited *TRIB3* gene expression level; however, *TRIB1* gene expression level increased and decreased in response to low (10 µg/ml) and high (100 µg/ml) concentrations of omega-3, respectively. The addition of omega-3 to the environment of oocytes or cumulus cells affected the maturation of oocytes and cumulus cells, which was followed by the differential expression of *TRIB* genes, suggesting that there was a role of fatty acid metabolism in the differentiation and maturation of cumulus cells.

Key Words: Cumulus Cells, Maturation, Omega-3, Oocyte, *TRIB* Genes

INTRODUCTION

There are many clinical and experimental data demonstrating the health benefits of omega-3 fatty acids in healthy individuals as well as patients with reproductive failures (Jeromson et al. 2015; Cao et al. 2015). *In vivo* and *in vitro* studies represent that many aspects of reproductive system, including oogenesis and spermatogenesis, are influenced by omega-3 fatty acids metabolism (Nehra et al. 2012; Gulliver et al. 2012;

Meher et al. 2013). Omega-3 fatty acids have also a significant role in oocyte maturation as well as oocyte quality (Chiu et al. 2018; Ortiz et al. 2014). *In vivo* experiments have shown that intake of foods and supplements containing omega-3 acids contributes to granulosa cells and oocytes growth and development (Wonnacott et al. 2020). Since ovarian compartments are composed of fatty acids, dietary n-3 fatty acids can modify the ovarian compartments, resulting in improved ovarian structure and function (Zachut et al. 2010). Polyunsaturated fatty acids are major components of the granulosa cells surrounding oocytes and contribute to oocytes maturation and therefore play significant role in female fertility (Khalil et al. 2013; Shaaker et al. 2012). In this condition, *in vitro* studies have indicated that during oocyte maturation, unsaturated lipids are incorporated into the oocyte cytoplasm and influence cellular metabolism and oocyte growth and development (Carro et al. 2013).

Fatty acids involved in maturation of oocytes can regulate expression level of genes associated with oocyte maturation (Veshkini et al. 2016; Virant-Klun et al. 2013). Recently, certain gene expression profiles have been studied and the findings indicate that oocyte maturation and follicular growth involve the expression of a number of genes including *Tribbles* (*TRIB*) genes (Hernández-Montiel et al. 2019; Lussier et al. 2017; Brisard et al. 2014). However, *TRIB* genes have been less studied and the modulation of their expression during oocyte maturation is somehow unclear. The association of Tribbles, *TRIB1*, *TRIB2* and *TRIB3*, with fatty acid metabolism has also been demonstrated in several studies. The findings indicate that *Tribbles* regulate cell proliferation in many tissues partly due to their impact on fatty acid metabolism (Lohan & Keeshan 2013). *TRIB1* has been reported to promote lipid metabolism in human tissues (Legault et al. 2018). However, *TRIB3* prevents fat accumulation in adipocytes (Örd et al. 2015; Lirangi et al. 2012). The association of *in vivo* oocyte maturation and *TRIB1* gene expression level has been reported (Brisard et al. 2014). *TRIB2* gene expression level is also changed in granulosa and cumulus cells during oocyte development (Lussier et al. 2017; Assidi et al. 2010).

Altogether, these observations hypothesized and somehow revealed that omega-3 fatty acids may influence *in vitro* oocyte maturation as a microenvironment. Fatty acids also can alter expression level of *Tribbles* in follicular cells. However, there are not sufficient findings clearly showing the association of omega-3 fatty acids with *Tribbles* expression level alteration during oocyte maturation. In this condition, the purpose of this research was to determine the effects of omega-3 on oocyte maturation *in vitro* and expression level of *Tribbles* (*TRIB1*, *TRIB2* and *TRIB3* genes) in cumulus cells surrounding oocytes.

MATERIALS AND METHODS

Chemicals and reagents

Omega-3-acid ethyl esters capsules were purchased from Pronova Biopharma Norway Pharmacy Company. Ethanol (96%) (as solvent) was added to content of each capsule to prepare the desired solution.

Collection of oocytes

This experimental study was conducted on 100 female NMRI mice, aged 8-10 weeks that were maintained on a 12-12 h light-dark schedule with *ad libitum* access to food and water. According to previous studies (Sirard 2011; Nikseresht 2015) animals were super-ovulated by an intraperitoneal injection of 7.5 IU pregnant mare serum gonadotrophin [PMSG Intervet, UK]. Ether was used to anesthetize the animals and the ovaries were removed into TCM-199 [Sigma] supplemented with 10% fetal bovine serum (FBS). The ovaries were punctured and cumulus-oocyte complexes (COCs) were released under a stereomicroscope. Oocytes were used for *in vitro* maturation. The study protocol was approved by the ethics committee of Islamic Azad University, Shiraz, Iran (Ethical no: IR.IAU.SHIRAZ.16330641322009).

In vitro maturation (IVM)

The oocytes and cumulus cells were cultured in α-MEN culture medium containing 5% FBS, 0.1 IU/mL, LH rh FSH, 7.5 IU/mL and 1% Penicillin-Streptomycin. The cells were assigned into 4 groups: Control (no treatment), Ethanol treated (exposed to ethanol 96%), Experimental 1 and Experimental 2 (exposed to 10 and 100 μg/ml of omega-3 fatty acid, respectively). The cells were incubated for 24h at 38.58˚C under 5% CO_2 in humidified air and prepared for determination of maturation stage and evaluation of gene expression level in cumulus cells.

Assessment of oocyte maturation rate

Oocytes were stripped from cumulus cells by repetitive aspiration-ejection using a Gilson pipette and then immediately fixed in 4% paraformaldehyde phosphate buffered saline (PBS) solution at room temperature for 30 minutes. Hoechst33342 (0.1% in PBS) was used for incubating fixed oocytes for 10 minutes and mounted on a glass slide in Mowiol solution. The maturation quality of oocytes was determined according to previous studies (Brisard et al. 2014). In the case of seeing the telophase-I or metaphase-II stage, the oocytes were considered mature. As a percentage of mature oocyte in a total

Table 1. Specific primers for *TRIB1*, *TRIB2* and *TRIB3* genes

Genes	Parameters		
	Sequence (5'->3')	Annealing (°C)	Product Size (bp)
Trib1-F	CCTCGAATATGGCAGCATTT	60	101
Trib1-R	CGAGTCTCCTCACCCTTGTC		
Trib2-F	TTGGAACAGACCAACCACCT	60	98
Trib2-R	TTTAGCACCCAGGTTTCAGG		
Trib3-F	GGAACCTTCAGAGCGACTTG	60	101
Trib3-R	TCTCCCTTCGGTCAGACTGT		
Rplp0-F	TGCCACACTCCATCATCAAT	60	97
Rplp0-R	AGGAAGGCCTTGACCTTTTC		

F: Forward, R: Reverse

number of live oocytes, the maturation rate was calculated.

Evaluation of *Tribble* expression level

Cumulus cells of COCs at maturation state were seeded in dishes. Twentyfour hours after seeding, the cells were incubated with the ethanol and omega-3 (10 and 100 µg/ml) for 12h. Cells were then centrifuged and washed with ice-cold PBS. Total RNA was extracted using a RNeasy midi kit [Roche, 1 828 665, Germany] and reverse transcribed into cDNA using a Transcriptor First Strand cDNA synthesis kit [Roche,04 379 012 001, Germany]. Quantitative Real-Time PCR (LightCycler-FastStart DNA master SYBR Green I Kit [ABI, 4369016, American] and Light Cycler apparatus [Roche Diagnostics]) was carried out to evaluate the expression level of *TRIB1*, *TRIB2* and *TRIB3* genes using the specific primers (Table 1). RT-PCR reaction was conducted during a program at a temperature of 95° C for 15 minutes and was subjected to 40 cycles compose of three-steps program at 95°C for 10 second, at 60°C for 30 second and at 72°C for 30 second. *Rplp0* gene (as reference gene) was used to normalize the relative expression for interested genes calculated by $2^{-\Delta\Delta CT}$ method. An agarose gel electrophoresis was used to confirm the expected PCR products.

Data analysis

Results are expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA method) followed by post hoc Tukey's multiple comparisons test in SPSS 20 software. Differences were considered significant at the $P<0.05$ level.

RESULTS AND DISCUSSION

Result

Maturation status of oocytes

Oocytes were examined in control, ethanol treated and experimental groups at 24h of IVM. Table 2 indicates the status of oocytes in control and ethanol treated groups and groups exposed to 10 and 100 µg/ml of omega-3 at 24 hours of IVM. Exposure of oocytes to 10 µg/ml of omega-3 resulted in lower degenerated oocytes and to 100 µg/ml of omega-3 led to higher degenerated oocytes compared with control group. The rate of GV-stage oocytes was significantly lower in groups exposed to 10 and 100 µg/ml of omega-3 than control group. Adding omega-3 (10 and 100 µg/ml) to live oocytes environment resulted in significant decrease in oocytes arrested at MI stage and significant increase in the rate of oocytes arrested at MII stage compared to control group.

Maturation status in COCs

Complete maturation was also examined in pre-ovulatory COCs in control, ethanol treated and experimental groups 34–38h after triggering ovulation.

COCs were graded by observational evaluation of morphological features such as the thickness and compactness of the cumulus cells and ooplasm homogeneity. According to previous studies, based on light microscopy, COCs surrounded by several layers of cumulus cells and with evenly granulated ooplasm were considered to have higher developmental competence *in vitro* (complete maturation) than oocytes with irregularly granulated ooplasm and fewer cumulus layers (Gumus et al. 2010).

Reduced maturity was observed in COCs exposed to 10 µg/ml of omega-3; however, exposure of COCs to 100 µg/ml of omega-3 led to an increased maturity rate (Figure 1). The rate of COCs at MII stage risen significantly in the group exposed to 10 µg/ml of omega-3. However, adding 100 µg/ml of omega-3 to COCs environment resulted in decreased COCs at MII stage. GV/MI rate in COCs was significantly lower in the group exposed to 10 µg/ml of omega-3 and was considerably higher in the group exposed to 100 µg/ml of omega-3 compared with control group (Figure 2).

TRIB genes expression level in cumulus cells

TRIB1, *TRIB2* and *TRIB3* genes expression level was evaluated in cumulus cells of COCs at complete maturation stage (Table 3). Exposure of COCs to 10 µg/ml of omega-3 resulted in reduced expression level of *TRIB1* gene and exposure to 100 µg/ml of omega-3 led to significant increase in *TRIB1* gene expression level. *TRIB2* gene expression level significantly increased in groups exposed to 10 and 100 µg/ml of omega-3, however, the expression level was higher in the group exposed to 10 µg/ml of omega-3 than the group exposed to 100 µg/ml of omega-3. *TRIB3* gene expression level significantly decreased in groups exposed to 10 and 100 µg/ml of omega-3. We did not observe significant difference between ethanol treated and control groups in our experiments, indicating that ethanol (as solvent) did not have significant impact on the research data

Discussion

Our findings indicated that exposure of live oocytes to low (10 µg/ml) and high (100 µg/ml) dose of omega-3-acid ethyl ester resulted in reduced rate of GV-stage oocytes, decreased MI-oocytes and increased MII-oocytes, demonstrating the stimulatory effects of omega-3 on maturation of live oocytes *in vitro*. In addition, our results have revealed that adding the low (10 µg/ml) and high (100 µg/ml) dose of omega-3 to COCs environment increased and decreased maturity of COCs, respectively.

In line with our findings numerous studies have reported that unsaturated fatty acids and omega-3 derivatives play significant role in maturation of oocytes and granulosa cells in various species. Indeed, long-chain poly unsaturated fatty acids such as omega-3 and omega-6 are integral component of the membrane lipid bilayer in many types of cells, including reproductive system cells (Gulliver et al. 2012), by which may influence cell function and development. Studies have shown that the dietary supplementation of omega-3 can improve reproductive system function in different species (Kirkup et al. 2010; Safdar et al. 2017). A large body of experimental research has also demonstrated that omega-3 improves female reproductive system function by affecting on female hormones precursors and the genes associated with oogenesis (Gulliver et al. 2012; Cheng et al. 2013; Dirandeh et al. 2015). Dietary n-3 fatty acids have been reported to influence the follicular status and to increase the cleavage rate of oocytes (Zachut et al. 2010). *In vivo* investigations on oocyte and embryo development have revealed that regular intake of unsaturated fatty acids has improving effects on oocyte maturation and embryo development (Fayezi et al. 2018). Fatty acids can also modulate granulosa cell proliferation and steroidogenesis *in vitro* (Maillard et al. 2018), by which may influence cumulus-oocyte complexes (COCs) maturation. The data obtained in recent studies confirm the significant association of unsaturated fatty acids with oocyte maturation and implantation (Mirabi et al. 2017; Oseikria et al. 2016; Mahla et al. 2017).

The cytotoxic and genotoxic effects of unsaturated fatty acids on oocytes and cumulus cells during IVM have been investigated (Nikoloff et al. 2017). The previous studies have suggested that the effect of fatty acids on *in vitro* systems depends in part on the concentration of unsaturated fatty acids and the cell type used in the study (Meng et al. 2013; Zajdel et al. 2013). The use of low concentrations of unsaturated fatty acids such as eicosapentaenoic acid can improve oocyte quality and cumulus expansion, while its high concentrations can induce cytotoxic and genotoxic effects. In the present study, the use of low dose of omega-3 decreased degenerative oocytes and the high dose of omega-3 increased degenerated oocytes, which is consistent with the previous studies (Nikoloff et al. 2017). The relationship between oocytes and cumulus cells is established through a gap junction so that any change made in these cells reduces the quality of oocytes (Zhou et al. 2016). It has been observed that high concentrations of unsaturated fatty acids such as eicosapentaenoic acid can induce apoptosis in cumulus cells. Also, the cytotoxic effects of unsaturated fatty acids at high concentrations can be associated with decreased metabolic activity and decreased mitochondrial activity. The increase in degenerative oocytes in this study can be explained by this possible mechanism that unsaturated fatty acids at high concentrations may be suitable targets for the formation

Table 2. Effect of ethanol and omega-3 on the *in vitro* maturation of oocytes in mice

Parameters	Groups			
	Control	Ethanol-treated	Exp1 (10 µg/ml)	Exp2 (100 µg/ml)
Degenerated (%)	7.6	8.6 ^{ns}	5.8$^{1, *}$	10.8$^{1, **; 2, *}$
GV (%)	13	11.8 ^{ns}	5$^{1, ***}$	8.1$^{1, **; 2, *}$
Metaphase I (%)	15.2	14 ^{ns}	7.9$^{1, ***}$	3.6$^{1, ***; 2, *}$
Metaphase II (%)	64.2	65.6 ^{ns}	81.3$^{1, ***}$	77.5$^{1, ***; 2, *}$
Number of oocytes	150	150	150	150

GV: arrested at germinal vesicle state (nucleus of oocyte is arrested in prophase of meiosis I)
[1] and [2] indicate significant difference compared with control and group exposed to 10 µg/ml of omega-3 at 24h of IVM, respectively; ns: indicates non-significant difference. *: P≤0.05, **: P≤0.01, ***: P≤0.001

Table 3. Effect of ethanol and omega-3 on the relative gene expression level (RQ) of *TRIB1*, *TRIB2* and *TRIB3* in cumulus cells

Genes	Groups			
	Control	Ethanol-treated	Exp1 (10 µg/ml)	Exp2 (100 µg/ml)
TRIB1	1.03±0.02	1.14±0.08 ^{ns}	4.28±0.22$^{1, **}$	0.38±0.03$^{1, ***; 2, ***}$
TRIB2	0.38±0.02	0.44±0.01 ^{ns}	4.85±0.24$^{1, **}$	2.36±0.04$^{1, **; 2, **}$
TRIB3	2.30±0.21	1.94±0.41^{ns}	1.55±0.32$^{1, **}$	0.76±0.06$^{1, **; 2, **}$

Data represents relative gene expression (Target/Rplp0) mean ± SEM of three experiments (n=3). [1] and [2] indicate significant difference compared with control and group exposed to 10 µg/ml of omega-3, respectively; ns: indicates non-significant difference, **: P≤0.01, ***: P≤0.001

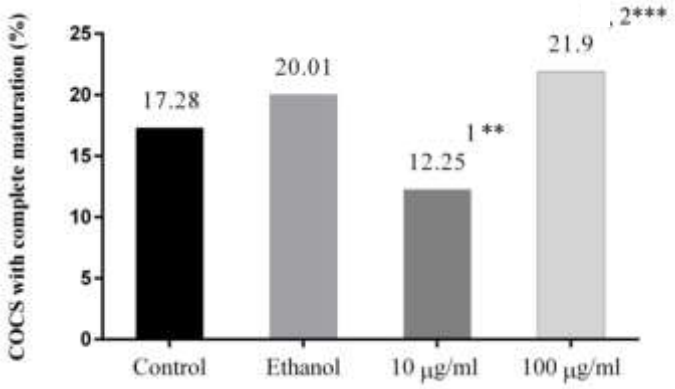

Figure 1. Complete maturation in control, ethanol treated COCs and COCs exposed to 10 and 100 µg/ml of omega-3 at GV/MI stage. [1] and [2] indicate significant difference compared with control group and group exposed to 10 µg/ml of omega-3, respectively (*: P≤0.05, **: P≤0.01, ***: P≤0.001).

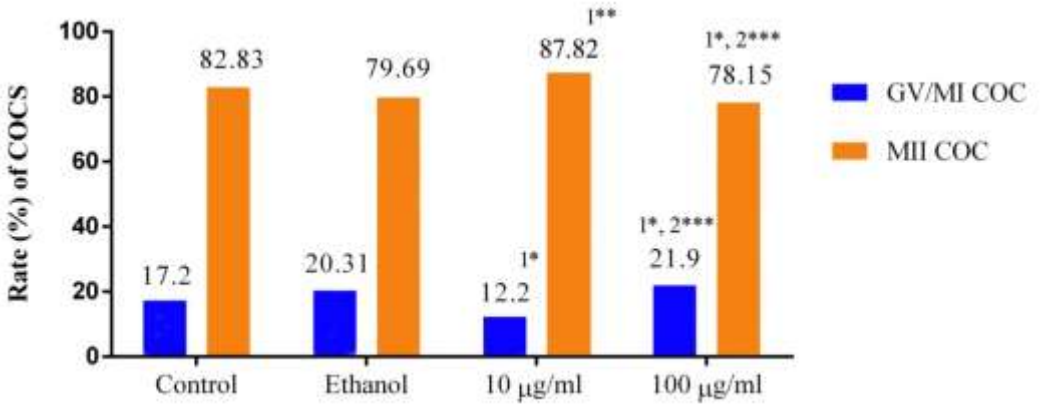

Figure 2. The rate (%) of COCs at GV/MI and MII stages in control and ethanol treated groups and groups exposed to 10 and 100 µg/ml of omega-3. [1] and [2] indicate significant difference compared with control group and group exposed to 10 µg/ml of omega-3, respectively (*: P≤0.05, **: P≤0.01, ***: P≤0.001).

of free radicals. The formation of free radicals after long-chain peroxidation of unsaturated fatty acids can be the main cause of damage to DNA and proteins, resulting in reduced quality and maturation of oocytes (Nikoloff et al. 2017). Therefore, based on the findings of this study, it seems that the high dose of omega-3 has destructive effects on oocyte maturation.

In the present study we found that exposure of COCs to low dose of omega-3 resulted in increased maturation and to high dose of omega-3 led to decreased maturity of COCs. The findings of a recent study have shown that the maturation parameters of COCs were affected by exposure of COCs to different eicosapentaenoic acid concentrations in the IVM medium and higher and lower concentrations of eicosapentaenoic acid have different impacts on COCs maturation (Nikoloff et al. 2017). In contrast to our findings, there are research showing that fatty acids may retard growth and development in oocytes. It has been reported that the exposure of oocytes to an environment high in omega-3 fatty acids led to decreased developmental ability of the blastocyst stage (Dunning et al. 2010). Adding alpha-linolenic fatty acids to oocyte environment also has been shown to increase reactive oxygen species levels, which mediate, at least in part, the inhibitory effect on oocyte maturation (Marei et al. 2012). Higher levels of saturated fatty acids, especially palmitic and stearic acids, were observed in some metabolic contexts to have harmful effects on oocyte maturation (Mirabi et al. 2017).

Results of this study indicated that adding omega-3 (10 and 100 µg/ml) to COCs environment induced *TRIB2* and inhibited *TRIB3* gene expression in cumulus cells. However, *TRIB1* gene expression level increased and decreased in response to low (10 µg/ml) and high (100 µg/ml) concentrations of omega-3, respectively. In line with this findings a research carried out to evaluate *TRIB* genes expression level in pre-ovulatory follicles indicated that *TRIB1*, *TRIB2* and *TRIB3* are expressed in different patterns in cumulus cells surrounding the oocytes from pre-ovulatory follicles (Brisard et al. 2014), and therefore, it is expectable that adding different doses of omega-3 fatty acids to cumulus cells has different effects on *TRIB* genes expression level. Previous studies also have revealed a significant relationship between *TRIB* genes expression level and fatty acid metabolism in cumulus cells during oocyte maturation. It has been shown that *TRIB* genes are involved in the cell-cycle progression during cell division (Dugast et al. 2012), which is accompanied by an increase in cellular metabolism rate including fatty acids metabolism. Indeed, maturation process is associated with significant change in COCs lipid metabolism which at least in part is regulated by *TRIB* genes expression (Bauer et al. 2015). A link between *TRIB1* gene expression level and lipid metabolism has been reported in recent studies (Wang et al. 2015). The findings show that the expression level of *TRIB3* has significant impact on carbohydrate metabolism (Zhang et al. 2016), which in turn, may influence lipid metabolism as well. Although the findings of our research indicated that adding omega-3 to COCs environment upregulates *TRIB2* and downregulates *TRIB3* in cumulus cells, it has been previously shown that *TRIB3* was up-regulated and *TRIB2* was down-regulated during the preovulatory period in cumulus cells (Lussier et al. 2017).

The studies indicate that all the three *TRIB* genes are regulated differently at different times in response to the inhibition of fatty acid peroxidation. This confirms that the three *TRIB* genes are involved in fatty acids metabolism and cumulus cells proliferation and play a key role in the resumption of meiosis and oocyte maturation (Brisard et al. 2014).

In this study, increased expression of *TRIB1* and *TRIB2* and decreased expression of *TRIB3* is associated with increased maturation of the COCs at low dose (10 µg/ml) of omega-3, however, at the maximum dose (100 µg/ml) of omega-3, the expression level of the TRIB genes were significantly reduced compared to the low dose (10 µg/ml) group of omega-3. Based on these findings, omega-3 appears to have a dose-dependent effect on gene expression and maturation of the COCs. Because a few studies are available on the effects of *TRIB* genes expression on the COCs, and the effects of omega-3 on different genes are variable, reducing the expression of *TRIB1* at the maximum dose (100 µg/ml) of omega-3 could be due to the cytotoxic effects of omega-3 at high doses in the form of impaired metabolic activity and the formation of free radicals due to peroxidation of fatty acids (Wu et al. 2015). Decrease in *TRIB1* gene expression at the high dose (100 µg/ml) of omega-3 can also be attributed to an increase in COCs at the GV/MI stage, so that the decrease of oocytes at GV/MI stage is associated with an increase in *TRIB1* expression and its increase is associated with decreased *TRIB1* expression in this study.

Our study only investigated the relationship between omega-3 and expression level of *Tribbles* in cumulus cells *in vitro*; however, to clarify the exact mechanism of omega-3 action on oocytes and cumulus cells, further research are required to reveal the effects of omega-3 on expression level of genes and proteins associated with oocyte and cumulus cells maturation *in vitro* and *in vivo*.

CONCLUSION

In conclusion, the data obtained in the present study suggest that adding omega-3-acid ethyl ester to environment of oocyte and cumulus cells promotes maturation of live oocyte and cumulus cells *in vitro* which is accompanied by increasing of *TRIB2* and decreasing of *TRIB3* gene expression. It is suggested that supplementation of diet with omega-3 may improve the oocyte development in patients suffering failure in oocyte maturation.

ACKNOWLEDGEMENT

This paper has been extracted from the PhD dissertations in developmental biology approved and supported by Shiraz Branch, Islamic Azad University, Shiraz, Iran. We appreciate all who helped us to exert this research.

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