ORIGINAL ARTICLE

Veterinary Research Forum. 2018; 9 (3) 217 - 224
doi: 10.30466/vrf.2018.32085
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Comparative study of the protective effects of chicken embryo amniotic fluid, vitamin C and coenzyme Q10 on cyclophosphamide-induced oxidative stress in mice ovaries

Alireza Kabirian[1], Rooz Ali Batavani[1]*, Siamak Asri-Rezaei[2], Ali Soleimanzadeh[1]

[1] Department of Theriogenology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [2] Department of Internal Medicine and Clinical Pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article Info

Article history:

Received: 28 November 2017
Accepted: 21 February 2018
Available online: 15 September 2018

Key words:

Chicken embryo amniotic fluid
Coenzyme Q10
Cyclophosphamide
Oxidative stress
Vitamin C

Abstract

Cyclophosphamide is a chemotherapy drug for the treatment of cancer. Chicken embryo amniotic fluid, vitamin C and coenzyme Q10 have anti-oxidant properties. Total of 70 adult female mice were selected and divided into seven groups. The first group that received 2 ml kg^{-1} of inactivated amniotic fluid subcutaneously. The second group treated with 75 mg $kg^{-1}$of cyclophosphamide by intraperitoneal injection. Third to fifth groups received 1, 2, and 4 ml kg^{-1} of chicken embryo amniotic fluid, respectively. The sixth group received vitamin C at a dose of 0.2 mg g^{-1} of body weight by oral gavages. Seventh group received 10 mg kg^{-1} coenzyme Q10 intraperitoneally. All cyclophosphamide treated groups (3-7) received 75 mg kg^{-1} of cyclophosphamide intraperitoneal on day 22. The mice were euthanized on day 29 and ovarian tissue antioxidant enzymes including glutathione peroxidase, superoxide dismutase and catalase activities and malondialdehyde (MDA) were evaluated. Activities of above mentioned enzymes in treatment groups (3-7) was significantly higher than patient control group (2). The results also revealed that MDA levels were higher in the control group in comparison to other treatment groups. Therefore, it is concluded that the chick embryo amniotic fluid and coenzyme Q10 can compete with compounds like vitamin C in increasing the anti-oxidant level in ovarian tissue.

بررسی مقایسه ای آثار محافظتی مایع آمنیوتیک جنین جوجه، ویتامین C و کوآنزیم کیو۱۰ بر تنش اکسیداتیو ناشی از سیکلوفسفامید در تخمدان های موش

چکیده

سیکلوفسفامید یک داروی شیمی‌درمانی برای درمان سرطان است. مایع آمنیوتیک جنین جوجه‌ها، ویتامین C و کوآنزیم‌کیو ۱۰ دارای اثرات آنتی اکسیدانتی می باشند. ۷۰ سر موش سوری ماده به هفت گروه تقسیم شدند. گروه اول ۲ میلی لیتر به ازای هر کیلوگرم مایع آمنیوتیک غیر فعال شده دریافت کردند. گروه دوم، ۷۵ میلی گرم به ازای هر کیلوگرم سیکلوفسفامید داخل صفاقی تزریق شد. گروه سوم تا پنجم به ترتیب، ۱ ، ۲، ۴ میلی لیتر به ازای هر کیلوگرم مایع آمنیوتیک جنین جوجه ها دریافت کردند. گروه ششم ۰/۲ میلی گرم به ازای هر گرم ویتامین C بصورت گاواژ دریافت کردند. گروه هفتم ۱۰ میلی گرم به ازای هر کیلوگرم وزن، کوآنزیم‌کیو ۱۰ بصورت داخل صفاقی دریافت کردند. در روز ۲۲ همه گروه های درمان (۳ تا ۷) ۷۵ میلی گرم به ازای هر کیلوگرم وزن سیکلوفسفامید به صورت داخل صفاقی دریافت کردند. موش ها در روز ۲۹ آسان کشی شده و فعالیت آنزیم های آنتی اکسیدانتی شامل گلوتاتیون پراکسیداز، سوپر اکسید دیسموتاز و کاتالاز و مالون‌دی‌آلدئید در بافت تخمدان ارزیابی شدند. سطح فعالیت آنزیم‌های فوق در بافت های تخمدانی در گروه های درمان (۳ تا ۷) به طور معنی داری بیشتر از گروه کنترل بیمار (۲) بود. همچنین نتایج نشان داد که سطح مالون‌دی‌آلدئید در گروه کنترل بیشتر از سایر گروه های درمان بود. لذا از این مطالعه می توان نتیجه گرفت که مایع آمنیوتیک جنین جوجه ها و کوآنزیم‌کیو ۱۰ در افزایش سطح آنتی اکسیدانت در بافت تخمدان، می توانند تا حد قابل توجهی با ترکیباتی همانند ویتامین C رقابت کنند.

واژه های کلیدی: تنش اکسیداتیو، سیکلوفسفامید، کوآنزیم کیو۱۰، مایع آمنیوتیک جنین جوجه، ویتامین C

***Correspondence:**

Rooz Ali Batavani. DVM, DVSc
Department of Theriogenology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
E-mail: ra.batavani@urmia.ac.ir

Introduction

Medications compounds maybe have toxic effects on the ovary.[1] These include alkylated compounds like cyclophosphamide which is used as a chemotherapy drug for the treatment of patients with leukemia and chronic granulomatous lesions and other cancers. Also, as an immunosuppressive compound it has toxic effects on ovary that can lead to infertility. Cyclophosphamide is a widely used drug in treatment of cancer and it works by impairing the growth and proliferation of malignant cells, thus it can increase the life span of affected patients. However, it has toxic effects on organs and tissues such as liver, lungs and ovaries.[2-5] These compounds reduce the number of ovarian follicles and induce primordial, primary, secondary and antral follicles damages. This combination also leads to ovarian atrophy and apoptosis of ovarian cells. Therefore, the number of fertilized eggs decreases. The effects of cyclophosphamide depend on the duration and dosage of drug administration. In mice and rats, the most damages occur in primordial and antral follicles.[5,6]

An important event after the induction of apoptosis in ovary is the oxidative stress reactions in the tissue and antioxidant activities decline such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) reductions. Malondialdehyde (MDA) is the index for lipids peroxidation evaluation.[7-9] There are various biomarkers for oxidative stress assessment in the body. Indicators such as SOD, CAT, GPx, lipid peroxides including MDA, thiobarbituric acid reactive substances, glutarhodoxin, nitric oxide and total antioxidant capacity (TAC) are used in ovary.[10] This study investigated the effects of chick embryo amniotic fluid, vitamin C and coenzyme Q10.

Amniotic fluid is present in the amniotic sac. This fluid builds up in the amniotic sac after passing through the embryonic layer. The compounds of this fluid in various animals include electrolytes, protein, hormone, water, carbohydrates, urea, phospholipids, enzymes and growth factors. It has a nutritional role in addition to protecting the embryo against mechanical damage and thermal shock.[11-13]

Different substances and proteins have been isolated from the animal and human amniotic fluid. The origin of proteins in the amniotic fluid is cells in the amniotic fluid, meconium, maternal uterus and embryonic urine as well as embryonic secretion from the skin.[12] Chickens are considered by researchers today as an important research model. Different materials have been isolated from the chickens amniotic fluid such as nerve growth factor (NGF), transforming growth factor- β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I, IGF-II, 41 amino acids, glucose and alpha-protein.[13-21]

In this regard, it has been noted that the TAC of amniotic fluid in humans is 522µm, which is considerable in comparison with other fluids in the body.[21] It has been described that SOD is one of the antioxidant indices in chickens' amniotic fluid.[22] These are parts of a series of studies on amniotic fluid in chickens and humans.

On the other hand, vitamin C is a water-soluble molecule and a strong antioxidant. Vitamin C reduces the amount of free radicals following tissue damage and its positive effects in wound healing are proven.[14,23] It has been shown that vitamin C reduces the tissue damage and improves tissue function following tissue ischemia-reperfusion. It plays a role in improving the immune system and increasing the synthesis of collagen (wound healing), catecholamine, cortisol and carnitine.[23,24] High levels of vitamin C can prevent small arteries destruction through nitric oxide synthase and nicotine amid adenine dinucleotide phosphate oxidase inhibition. A very important point in relation to vitamin C is that this vitamin also has anti-inflammatory properties due to phospholipase A_2 inhibition; thus, it can contribute to the reduction of tissue-damaging effects of reactive oxygen species (ROS).[25]

Coenzyme Q10 is known as a potent antioxidant for reducing the amount of free radicals in the body.[26] This agent is a lipid-soluble vitamin-like agent that is involved in the mitochondrial respiratory chain. It has been reported that coenzyme Q10 improves fertility and sperm parameters and has effects on oocyte maturation due to the presence in follicular fluid.[27] The presence of this compound is vital for energy activities in the body. This substance plays an important role in the treatment of disorders related to oncology, cardiovascular organs, kidneys and nerves. The useful role of this substance in stabilizing the mucosal calcium channels of membrane, antioxidant defense system and reducing the amount of lipids peroxidation in the body has been proven.[28] Considering the above-mentioned properties and agents in the amniotic fluid, especially its antioxidant properties, this study attempts to evaluate its effects on the improvement of cyclophosphamide-induced oxidative stress in ovarian tissue of mice.

Materials and Methods

Preparation of chicken embryo amniotic fluid. Fertile eggs of white Lohmann chicken were obtained from Zarrin Par incubation center (Urmia, Iran) and incubated in the hatching machine with temperature of 38.00 ± 1.00 ˚C and humidity of 50.00%. Amniotic fluid was taken with a 23-gauge syringe from the egg after breaking it in the Petri dish on different days of hatch (from day 6 to 19). Amniotic fluid was obtained according to the protocols provided previously.[29] The ferric reducing ability of plasma method was used to determine the days

in which the TAC was higher in the amniotic fluid of chickens. After determining the day, amniotic fluid was centrifuged at 500 *g* for 15 min and the supernatant was removed in a sterile 0.22 µm filter.[30,31] Fertile eggs were selected for obtaining amniotic fluid based on the determination of the day of hatching with higher amount of antioxidant and sufficient and easy accessibility of the amniotic fluid.

Animals. Seventy female mice with age of 8-10 weeks and weighing 30-40 g were obtained from Urmia University Animal Resources Center, Urmia, Iran. The animals were kept under constant conditions of light (12 hr light/dark cycles) and temperature (21.00 to 24.00 ˚C). All mice had *ad libitum* access to food and water. The mice were kept in standard metal cages with sawdust bedding and the bedding was changed regularly. Before starting the study, vaginal smears of all mice were taken and mice that were in the same sex cycle were selected. The experiments were performed on animals in accordance with the guidelines of the ethical committee for research on laboratory animals of Urmia University (AECVU-162-2018).

Study groups. The study was conducted in 7 groups (n = 10). The first group was healthy control (CH) and distilled amniotic fluid was used at a dose of 2 mL kg^{-1} (to inactivate the amniotic fluid, it was heated at 56 ˚C for 45 min). In the second group (patient control /CD), 75 mg kg^{-1} of cyclophosphamide was administered intraperitoneally. In the third group (CAF1), mice were treated with 1 mL kg^{-1} chicken amniotic fluid subcutaneously for 5 days per week over a period of three weeks. The day after the end of this protocol, a single dose of cyclophosphamide (75 mg kg^{-1}) was injected intraperitoneally. The fourth (CAF2) and fifth (CAF4) groups were similar to that of the third, except that amniotic fluid at doses of 2 and 4 mL kg^{-1} was used in these two groups, respectively. In the sixth group (Vit C), mice were treated orally with vitamin C at a dose of 0.20 mg g^{-1} of body weight per day over a period of three weeks. Finally, in the seventh group (COQ10), mice were treated with 10 mg kg^{-1} body weight of coenzyme Q10 intraperitoneally for three weeks. Treatment was performed in all groups for 21 days. The time of cyclophosphamide injection in these groups was day 22 and mice were euthanized 7 days later. After euthanasia by intraperitoneal injection of ketamine (45 mg kg^{-1}; Alfasan, Woerden, Netherlands) and xylazine (35 mg kg^{-1}; Alfasan),[32] the ovaries were isolated and placed in a phosphate-buffered saline solution. Oxidative stress profile including the activity of SOD, CAT and GPx and MDA concentration were evaluated in homogenized ovarian tissues of above-mentioned groups.

Biochemical evaluations. The evaluation of GPx and SOD activities was performed using kits (Zellbio GmbH, Ulm, Germany) according to the manufacturer's instructions. Lipid peroxidation was determined by measuring the amount of MDA using thiobarbituric acid as described previously.[33] The CAT activity was measured at 571 nm, based on the amount of hydrogen peroxide consumed by micro-molecules according to previously provided method.[34]

Statistical analysis. Statistical analyses were carried out using a mixed design (within and between group comparisons). The ANOVAs were computed with 95.00% confidence intervals using SPSS software (version 22.0; SPSS Inc., Chicago, USA). All data are presented as means ± SEM and $p < 0.05$ was considered to be statistically significant.

Results

Determination of TAC in chicken amniotic fluid. Analyzing of the amniotic fluid of chicken embryos on different days of hatching revealed that the age increasing led to an increase in TAC of amniotic fluid (Table 1). Despite the increase of antioxidant content in amniotic fluid by day 19, obtaining amniotic fluid was more accessible on day 15.

Table 1. Total antioxidant capacity (TAC; mmol L^{-1}) changes in amniotic fluid of chicken embryo on different hatching days. Data are presented as mean

Days	6	7	8	9	10	11	12
TAC	0.3048	0.3426	0.5448	0.6522	0.7112	0.7362	0.7590
Days	13	14	15	16	17	18	19
TAC	0.8194	0.834	0.9050	1.1606	1.9380	2.1168	2.3652

Evaluation of SOD activity. The SOD activities in the ovary of mice in studied groups are summarized in Figure 1. As shown, the SOD activity in the control group was the lowest in cyclophosphamide treatment, while in other groups, the SOD activity was increased significantly. The increase in SOD in the group treated with coenzyme Q10 is significantly higher than other groups. The difference in activity level of SOD in all groups was significant in comparison with the control group. Meanwhile, the highest effect of amniotic fluid of chicken embryo was observed in increasing SOD activity at a dose of 4 mL kg^{-1}.

The GPx in the ovary. The data obtained from this study about changes in GPx in the studied groups are summarized in Figure 1. The GPx rate is based on U mg^{-1} of protein. As it can be seen, GPx level in the CD group was the lowest and it was the highest in the CH group. The GPx in the COQ10 group was significantly higher than other treatment groups and it was close to the CH group, but the difference between the two groups was significant ($p < 0.05$). Meanwhile, the highest effect of chicken embryo amniotic fluid on increasing the activity of GPx enzyme was observed at a dose of 4 mL kg^{-1}.

The CAT in the ovary. The data from this study regarding CAT changes in the study groups are

summarized in Figure 1. The CAT rate is based on U mg^{-1} of protein. As it can be seen, the level of CAT in the ovary in the CH group was greater than other groups. Interestingly, the CAT level in COQ10 group was close to that of CH group and there was no significant difference between the two groups. The CAF2 and CAF4 groups had significant increased CAT levels in ovarian tissue, so there was no significant difference between the two groups and control and COQ10 groups.

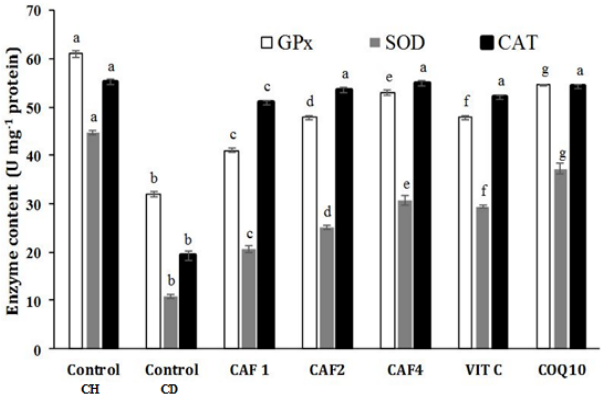

Fig. 1. Comparison of GPx, SOD and CAT content in seven groups: healthy control group (CH), patient control (CD), treatment with amniotic fluid at a dose of 1 mL kg^{-1} (CAF1), treatment with amniotic fluid at a dose of 2 mL kg^{-1} (CAF2), treatment with amniotic fluid at a dose of 4 mL kg^{-1} (CAF4), vitamin C treatment (VIT C) and coenzyme Q10 treatment (COQ10). The significance level of above factors in seven groups is classified as a to g in relation to control group ($p < 0.05$).

Evaluation of MDA. The data obtained regarding the variation of MDA levels are summarized in Figure 2. The MDA levels in the study groups (CAF1/2/4, Vit C and COQ10) were significantly decreased, while the level of MDA in the COQ10 group was not significantly different from that of the CH group, which indicates that the coenzyme Q10 has a more significant effect than the other groups. The MDA level in this group was similar to control group. Meanwhile, the highest effect of amniotic fluid of chicken embryo in reducing the level of MDA was seen at 4 mL kg^{-1} (Fig. 3).

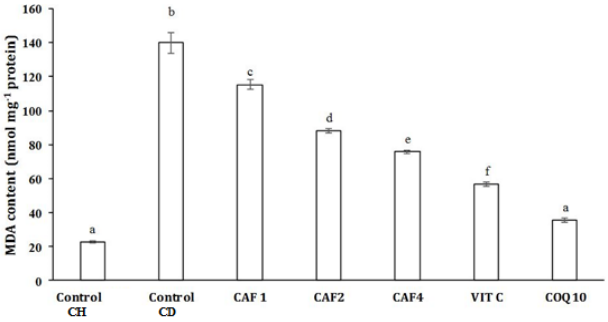

Fig. 2. Comparison of MDA content in seven groups: healthy control group (CH), patient control (CD), treatment with amniotic fluid at a dose of 1 mL kg^{-1} (CAF1), treatment with amniotic fluid at a dose of 2 mL kg^{-1} (CAF2), treatment with amniotic fluid at a dose of 4 mL kg^{-1} (CAF4), vitamin C treatment (VIT C) and coenzyme Q10 treatment (COQ10). The significance level of above factors in seven groups is classified as a to f in relation to control group ($p < 0.05$).

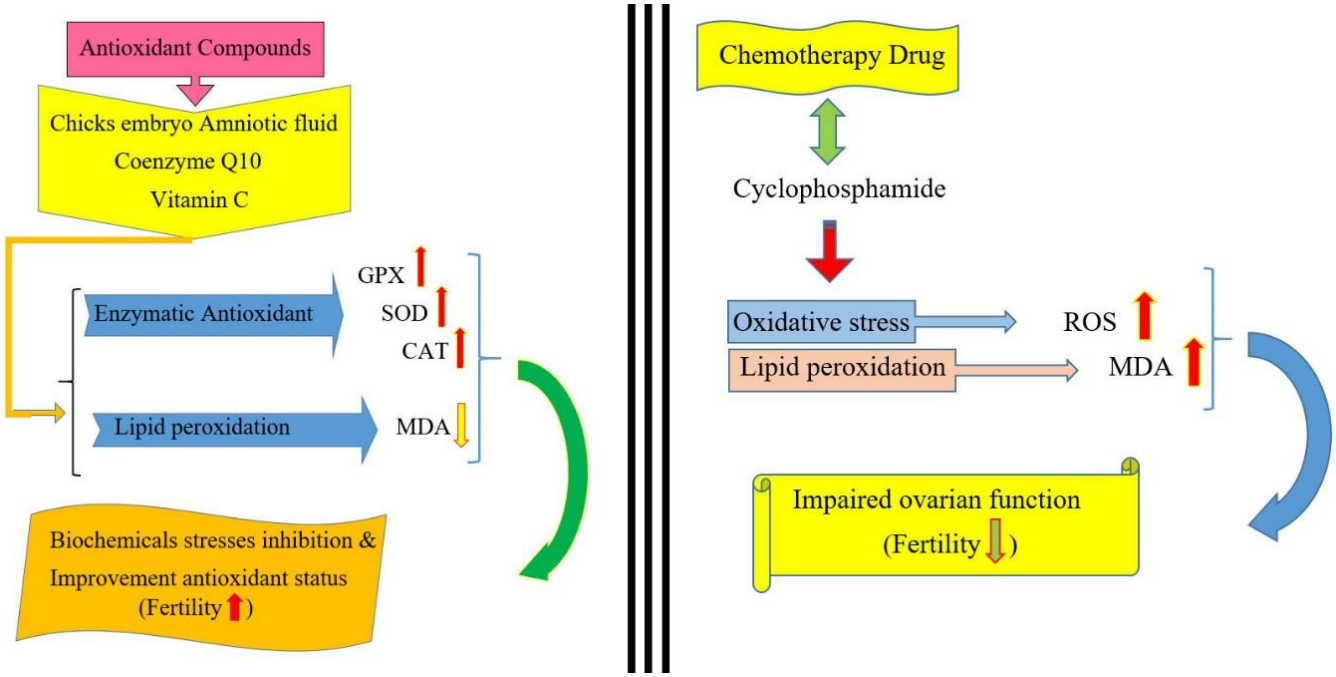

Fig. 3. Cyclophosphamide induced ovarian toxicity. This medication increased lipid peroxidation and decreased GPx, SOD and CAT activities. Antioxidant compounds like vitamin C, coenzyme Q10 and chicks' embryo amniotic fluid can increase antioxidant status in ovarian tissue and cause fertility improvement.

Discussion

Aims of this study were to elucidate whether chick's amniotic fluid has antioxidant effects and is this fluid effective in reducing ROS induced damages in ovaries, like vitamin C and coenzyme Q10. It has been reported that the TAC level of human amniotic fluid is 522 µm.[22] This study proves the presence of a significant level of TAC in the embryonic fluid of fertile eggs. It seems that various factors could affect the level of TAC in embryonic fluids of chicken including the diet of farms, environmental stresses and management issues in poultry farms.[35]

Cyclophosphamide is one of the cytotoxic drugs metabolized by the liver enzymes to its active metabolites such as phosphoramide mustard and acrolein. Phospharamide mustard is responsible for the anti-cancer effects, but acrolein interacts with the antioxidant defense system of the tissues and generates a large amount of oxygen free radicals with mutagenic properties. This can be easily combined with other molecules including enzymes, receptors and ion pumps, which can directly inhibit their normal function. The increase in synthesis of phosphoramid mustard in the body results in toxic damage to the ovary. The antioxidant system plays an important role in preventing the destructive effects of drug after induction of ROS.[34,36,37] As seen, in the patient control group receiving only cyclophosphamide, the increase in MDA levels and the reduction of SOD, GPx and CAT levels were significant, and this is consistent with the incidence of cyclophosphamide in the body and induction of oxidative stress in tissues in accordance with the mentioned mechanism.

In the oxidative stress state, with the increase in the level of ROS and the suppression of the antioxidant balance of the body, the process of lipid peroxidation increases, which suggests that MDA is an indicator of lipid peroxidation in the body.[38] As noted in the results, MDA levels in CD group were the highest and the lowest MDA level was found in CH group. Among the treated groups, the COQ10 group had more effect on decreasing the MDA level. In the Vit C group, MDA levels were decreased significantly, but there was a significant difference between the groups with CH and COQ10 groups in terms of MDA level. The effect of vitamin C on reducing the amount of MDA following oxidative stress induction in ovary using rhodamine B has been reported previously.[39] Also, positive effects of vitamin C on decreasing MDA levels in depot-medroxyprogesterone acetate induced oxidative stress in ovarian tissue have been demonstrated.[40] Beneficial effects of vitamin C on the reduction of MDA levels following the induction of oxidative stress in the ovary have been proven. This study showed that COQ10 with the aforementioned dose could be more effective than vitamin C in reducing the level of MDA following oxidative stress induction by cyclophosphamide. Moreover, the beneficial effects of COQ10 and alpha-lipoic acid on reducing the amount of MDA following induction of oxidative stress in an experimental ischemia-reperfusion in ovarian tissue have been indicated previously.[41] Similarly, Ozler *et al*. have reported the same beneficial effects of COQ10.[42] However, due to the limited research in relation to chicken amniotic fluid, it would be better to compare the results of this study in reducing MDA levels with vitamin C and COQ10. It seems that chicken amniotic fluid at a dose of 4 mL kg^{-1} compared to a dose of 1 mL kg^{-1} is more effective in reducing MDA. Therefore, there is a significant correlation in the CAF4 group in relation to decreasing MDA in comparison with the CAF1 group. There is no significant relationship between CAF4 and CAF2 groups. In fact, it can be concluded that these two groups reduced approximately the same amount of MDA in ovarian tissue. However, none of these groups could work as efficient as vitamin C and COQ10 in reducing the oxidative stress-induced damages in ovarian tissue.

The SOD, GPx, CAT and non-enzymatic antioxidants play an important role in neutralizing ROS and protecting the egg and the fetus. The SOD is responsible for destruction of O_2 to H_2O_2 and oxygen. The CAT and GPx convert H_2O_2 into water and oxygen and stop the tissue damage caused by ROS.[43] In this study, the lowest level of GPx was observed in CD group and the highest GPx values were observed in COQ10 group. However, the Vit C group also seems to be successful in increasing the amount of GPx in ovarian tissue as much as COQ10. Chicken embryo's amniotic fluid had relatively good performance in increasing the amount of GPx in the ovarian tissue. The best performance was for CAF2 and CAF4 groups. The CAF2 was approximately as effective as Vit C group. The CAF4 group had better performance in increasing the GPx levels in the ovary than all amniotic fluid groups. Sekhon *et al*. have considered the use of antioxidant supplements to counteract the effects of ROS in the body and increase the amount of GPx and SOD. Results obtained from this study were consistent with Sekhon *et al.* reports.[44]

In relation to SOD values in the ovary, the lowest level was seen in CD group, as in the case of GPx, and since the CD group had the highest MDA level, the ROS significantly increased in this group. This is normal, since no treatment has been performed in this group. Laloraya *et al*. have stated that the presence of various SOD isoforms involved in the process of folliculogenesis, steroidogenesis and retention of fertility.[45] Matzuk *et al*. have also stated that mice with poor fertility have lower pre-ovulatory follicles and luteal tissue. Thus, it is important to maintain a proper level of SOD in ovarian follicles, theca and granulosa cells to maintain fertility in animals with high ROS levels.[46] The highest SOD was seen in CH group. The CAF2 group received cyclophosphamide to induce oxidative stress in ovarian tissue. As you can see, the increase in antioxidant

enzymes in the CAF2 was about 15 to 20 units in comparison with CD group. Therefore, in the CH, antioxidant enzymes in ovarian tissue are expected to be higher than these values in the absence of antioxidant compounds in the amniotic fluid. Secondly, in similar reports provided by other researchers you will find that the level of antioxidant enzymes in the control group was consistent with the current study, for example, Dai *et al*. have used melamine to induce oxidative stress in ovarian tissue and antioxidant values in the control group were approximately the same as the present study.[43] However, with the increase in amniotic fluid doses, the SOD significantly increased in the treated groups. The highest levels of SOD were observed in vitamin C and COQ10 receiving groups and this seems to be normal so far as their antioxidant effects have been substantiated. However, CAF4 was successful in increasing the level of SOD in ovarian tissue following cyclophosphamide administration.

The CAT is another enzyme that protects the cell from free radicals. The CAT was first isolated in 1975 by immunohistochemistry from the ovarian tissue. Increased CAT activity is observed consistently with follicular growth in granulosa and theca cells. Of course, the level of CAT in a follicular fluid in follicles of different sizes is not the same.[47,48] In fact, CAT plays an important role in maintaining oxidative balance in tissue and producing ovarian steroids. Evaluation of this enzyme in tissue following the use of chemotherapy drugs can help to maintain fertility. The CAT levels in CAF2, CAF4 and COQ10 groups increased almost equally, so there is no statistically significant difference between them. The Vitamin C also significantly increased the CAT range. Previous studies have shown that increasing oxidative stress in the body following a disease or the use of certain substances and drugs reduces the level of CAT in the body and increases the amount of cell damage. For example, Skrajnowska *et al*. have considered that breast carcinogenicity in rats due to use of 7,12-dimethyl-1,2-benz[a]anthracene (DMBA) can reduce CAT levels. Interestingly, the use of chemotherapy drugs such as cyclophosphamide can also lead to induction of oxidative stress and its possible damages.[49] In this study, in the CD group, the level of antioxidant enzymes was minimal and MDA levels were maximal.

It is true that compounds such as vitamin C contribute to increase antioxidant enzymes in tissue, but this study revealed that the use of COQ10 could significantly compete with vitamin C. Presence of substances such as VEGF, IGF, protein, NGF, antioxidant, TGF-β and other agents has been proven in the chick embryo's amniotic fluid. Perhaps this fluid has substances that have harmful effects on the tissues and can increase the amount of free radicals in the blood and tissue, which, according to the results of this study, seems to be the effect of beneficial compounds in the amniotic fluid that were successful in increasing the level of antioxidant enzymes in the ovarian tissue towards potentially harmful compounds. For example, Farjah and Fazli have used amniotic fluid of chickens in the process of sciatic nerve regeneration and have reported significant effects on the recovery of neural tissue.[29] Since this fluid can be introduced into the mouse body and its potential beneficial effects can be evaluated without causing an abnormal body reaction and given the beneficial ingredients present in the fluid and the function of each of them, it is possible to conduct further studies on the amniotic fluid of the chicken. Farjah and Fazli have also found no abnormal reactions following amniotic fluid injection.[29]

In conclusion, it can be concluded that Chicks' embryo amniotic fluid and coenzyme Q10 can compete with vitamin C in increasing the antioxidant levels in ovarian tissue following oxidative stress induction. Therefore, it seems that it is possible to perform further studies on fetal fluids and their possible effects on fertility and reproductive technologies.

Acknowledgments

The authors would like to sincerely thank the Faculty of Veterinary Medicine and Urmia University Research Council for the approval and support of this research. The authors appreciate Dr. Mehdi Kian and Dr. Sajjad Moradi for their cooperation and help in the course of the study.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Hoyer PB, Sipes IG. Assessment of follicle destruction in chemical-induced ovarian toxicity. Annu Rev Pharmacol Toxicol 1996; 36: 307-331.
2. Jarrell JF, Bodo L, YoungLai EV, et al. The short-term reproductive toxicity of cyclophosphamide in the female rat. Reprod Toxicol 1991; 5(6): 481-485.
3. Tomao F, Spinelli GP, Panici PB, et al. Ovarian function, reproduction and strategies for fertility preservation after breast cancer. Crit Rev Oncol Hematol 2010; 76(1): 1-12.
4. Jiang Y, Zhao J, Qi H, et al. Accelerated ovarian aging in mice by treatment of busulfan and cyclophosphamide. J Zhejiang Univ Sci B 2013; 14(4): 318-324.
5. Plowchalk M, Mattison DR. Reproductive toxicity of cyclophosphamide in the C57BL/6N mouse: 1. Effects on ovarian structure and function. Reprod Toxicol 1992 ; 6(5): 411-421.
6. Eman A, Kishk F, Mona HM. Effect of a gonadotropin-releasing hormone analogue on cyclophosphamide

-induced ovarian toxicity in adult mice. Arch Gynecol Obstet 2013; 287(5): 1023-1029.
7. Tsai-Turton M, Luong BT, Tan Y, et al. Cyclo-phosphamide-induced apoptosis in COV434 human granulosa cells involves oxidative stress and glutathione depletion. Toxicol Sci 2007; 98(1): 216-230.
8. Fujii J, Iuchi Y, Okada F. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reprod Biol Endocrinol 2005; 3: 43.
9. Tilly JL, Tilly KL. Inhibitors of oxidative stress mimic the ability of follicle-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. Endocrinology 1995; 136(1): 242-252.
10. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005; 3(28): 1-29.
11. Larsen WJ. Human embryology. 3rd ed. Philadelphia, USA: Churchill Livingstone 2001; 490.
12. Burdett P, Lizana J, Eneroth P, et al. Proteins of human amniotic fluid. II. Mapping by two-dimensional electrophoresis. Clin Chem 1982; 28:935-940.
13. Batavani RA, Ghasemzadeh M, Kheradmand A. Changes of enzyme activities in ovine fetal fluids and maternal blood serum with gestational age. Comp Clin Pathol 2008; 17: 105-109.
14. Kaya M, Ozkan A, Eroz R, et al. Effect of combined anti-inflammatory and antioxidant therapy on ischemia-reperfusion injury in rat ovary: An experimental study. Int J Clin Exp Med 2016; 9(3): 6246-6254.
15. Mashayekhi F, Dianati E, Masomi Moghadam L. Quantitative analysis of nerve growth factor in the amniotic fluid during chick embryonic development. Saudi J Biol Sci 2011; 18(2): 209-212.
16. Mirzajani E, Dejhagah S, Mashayekhi F, et al. Amniotic fluid TGF-β1 concentration during chick embryonic development. Ann Biol Res 2011; 2(5): 185-190.
17. Mashayekhi F, Zahiri S, Mirzajani E, et al. Developmental changes in amniotic fluid vascular endothelial growth factor levels of chick embryos. Ann Biol Res 2011; 2(1): 94-99.
18. Karcher DM, McMurtry JP, Applegate TJ. Developmental changes in amniotic and allantoic fluid insulin-like growth factor (IGF)-I and -II concentrations of avian embryos. Comp Biochem Physiol A Mol Integr Physiol 2005;142(4):404-409.
19. Busch M, Milakofsky L, Hare T, et al. Regulation of substances in allantoic and amniotic fluid of the chicken embryo. Comp Biochem Physiol 1997; 116(2): 131-136.
20. Pressman EK, Thornburg LL, Glantz JC, et al. Inflammatory cytokines and antioxidants in midtrimester amniotic fluid: Correlation with pregnancy outcome. Am J Obstet Gynecol 2011; 204(2): 155.e1-7.
21. Bartosz G. Total antioxidant capacity. Adv Clin Chem 2003; 37: 219-292.
22. Sarala P, Suneetha S, Parthasarathy PR. Detection of superoxide dismutase isozymes in normal and X-irradiated chick amniotic fluid by using isoelectric focusing. Indian J Exp Biol 1996; 34(9): 901-904.
23. May JM. How does ascorbic acid prevent endothelial dysfunction? Free Radic Biol Med 2000; 28: 1421-1429.
24. Gasparetto C, Malinverno A, Gulacciati D, et al. Antioxidant vitamins reduce oxidative stress and ventricular remodeling in patients with acute myocardial infarction. Int J Immunopathol Pharmacol 2005; 18(3): 487-496.
25. Mohamed R, Dharmappa KK, Tarannum S, et al. Chemical modification of ascorbic acid and evaluation of its lipophilic derivatives as inhibitors of secretory phospholipase A2 with anti-inflammatory activity. Mol Cell Biochem 2010; 345(1-2): 69-76.
26. Hosseinzadeh E, Zavareh S, Lashkarboluki T. Coenzyme Q10 improves developmental competence of mice pre-antral follicle derived from vitrified ovary. J Paramed Sci 2015; 6(2): 65-71.
27. Turi A, Giannubilo SR, Brugè F, et al. Coenzyme Q10 content in follicular fluid and its relationship with oocyte fertilization and embryo grading. Arch Gynecol Obstet 2012; 285(4): 1173-1176.
28. Bonakdar RA, Guarneri E. Coenzyme Q10. Am Fam Physician 2005; 72(6):1065-1070.
29. Farjah GH, Fazli F. The effect of chick embryo amniotic fluid on sciatic nerve regeneration of rats. Iran J Vet Res 2015; 16(2): 167-171.
30. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. 1951.Dev Dyn 1992; 195(4): 231-272
31. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay. Anal Biochem 1996; 239(1): 70-76.
32. Jalali AS, Najafi G, Hosseinchi M, et al. Royal jelly alleviates sperm toxicity and improves *in vitro* fertilization outcome in stanozolol-treated mice. Iran J Reprod Med 2015; 13(1): 15-22.
33. Yapca OE ,Turan MI, Borekci B, et al. Bilateral ovarian ischemia/reperfusion injury and treatment options in rats with an induced model of diabetes. Iran J of Basic Med Sci 2014; 17(4): 294-302.
34. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 2016; 6: 194-199.
35. Surai PF. Antioxidant systems in poultry biology: Superoxide dismutase. J Anim Res Nutr 2015; 1:8. doi: 10.21767/2572-5459.100008.
36. Senthilkumar S, Yogeeta SK, Subashini R, et al. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem-bioloint 2006; 160(3): 252-260.

37. Mohammadi T, Khaksary Mahabadi M, Najaf-ZadeVarzi H, et al. Protective effect of concurrent administration of mesna and galbanum oil on histological structure of rat placenta against cyclophosphamide toxicity. J Ardabil Uni Med Sci 2016; 16(2): 211-222.
38. Yener NA, Sinanoglu O, Ilter E, et al. Effects of spirulina on cyclophosphamide-induced ovarian toxicity in rats: Biochemical and histomorphometric evaluation of the ovary. Biochem Res Int 2013; 2013: http://dx.doi.org/10.1155/2013/764262.
39. Laili AN, Ananingsih L, Wiyasa WA, et al. Protective effect of combined vitamin C and E against ovarian and endometrial toxicity in rats that receiving oral rhodamine B. Biomarkers Genomic Med 2015; 7(4): 154-158.
40. Ismiyati A, Wiyasa WA, Hidayati DYN. Protective effect of vitamins C and E on depot-medroxyprogesterone acetate-induced ovarian oxidative stress *in vivo*. J Toxicol 2016; doi: 10.1155/2016/3134105.
41. Tuncer AA, Bozkurt MF, Koken T, et al. The protective effects of alpha-lipoic acid and coenzyme Q10 combination on ovarian ischemia-reperfusion injury: An experimental study. Adv Med. 2016;2016: doi: 10.1155/2016/3415046.
42. Ozler A, Turgut A, Görük NY, et al. Evaluation of the protective effects of CoQ_{10} on ovarian I/R injury: An experimental study. Obstet Gynecol Sci Investigation 2013; 76(2): 100-106.
43. Dai XX, Duan X, Cui X-S, et al. Melamine induces oxidative stress in mouse ovary. J PLoS One 2015; 10(11):1-9.
44. Sekhon L, Gupta S, Kim Y, et al. Female infertility and antioxidants. Curr Womens Health Rev 2010; 6(2): 85-95.
45. Laloraya M, Kumar GP, Laloraya MM. Histochemical study of superoxide dismutase in the ovary of the rat during the oestrous cycle. J Reprod Fertil 1989; 86(2): 583-587.
46. Matzuk MM, Dionne L, Guo Q, et al. Ovarian function in superoxide dismutase 1 and 2 knockout mice. Endocrinology 1998; 139(9): 4008-4011.
47. Neill JD, Smith MS. Pituitary-ovarian interrelationships in the rat. Curr Top Exp Endocrinol 1974; 2: 73-106.
48. Enders AC, Nelson DM. Pinocytotic activity of the uterus of the rat. Dev Dyn J 1973; 138(3): 277-299.
49. Skrajnowska D, Bobrowska-Korczak B, Tokarz A, et al. Copper and resveratrol attenuates serum catalase, glutathione peroxidase, and element values in rats with DMBA-induced mammary carcinogenesis. Biol Trace Elem Res 2013; 156(1-3): 271-278.