Original Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health Published online Jan 20, 2020
https://doi.org/10.5534/wjmh.190145

The World Journal of MEN's HEALTH

Coenzyme Q10 Improves Sperm Parameters, Oxidative Stress Markers and Sperm DNA Fragmentation in Infertile Patients with Idiopathic Oligoasthenozoospermia

Ahmed T. Alahmar[1], Aldo E. Calogero[2], Pallav Sengupta[3], Sulagna Dutta[4]

[1]College of Science, University of Sumer, Thi-Qar, Iraq, [2]University Teaching Hospital Policlinico-Vittorio Emanuele, University of Catania, Catania, Italy, [3]Faculty of Medicine, Bioscience and Nursing, Mahsa University, [4]Faculty of Dentistry, Bioscience and Nursing, Mahsa University, Kuala Lumpur, Malaysia

Purpose: Oxidative stress and sperm DNA fragmentation (SDF) are potential contributing factors for idiopathic male infertility. Coenzyme Q10 (CoQ10) have been reported to be effective in the treatment of idiopathic male infertility, in general, owing to its antioxidant properties. Thus, the present study intends to investigate the effects of CoQ10 therapy on semen parameters, oxidative stress markers and SDF in infertile men, specifically with idiopathic oligoasthenozoospermia (OA).

Materials and Methods: In this case-control study, sixty-five infertile patients with idiopathic OA and forty fertile men (control) were included. All participants underwent semen analysis based on the World Health Organization guidelines (5th edition, 2010). Patients received CoQ10 at the dose of 200 mg/d orally for three months. Seminal plasma CoQ10, total antioxidant capacity (TAC), total reactive oxygen species (ROS), glutathione peroxidase (GPx), and SDF levels were measured in controls (baseline) and infertile patients pre- and post-CoQ10 treatment.

Results: CoQ10 treatment for three months significantly improved sperm concentration ($p<0.05$), progressive motility ($p<0.05$), total motility ($p<0.01$), seminal fluid CoQ10 concentration ($p<0.001$), TAC ($p<0.001$), and GPx ($p<0.001$) levels in infertile men with OA. Further, ROS level ($p<0.05$) and SDF percentage ($p<0.001$) were reduced in OA patients as compared to the baseline. CoQ10 levels also correlated positively with sperm concentration ($r=0.48$, $p=0.01$) and total motility ($r=0.59$, $p=0.003$) while a negative correlation was recorded between SDF and sperm motility ($r=-0.54$, $p=0.006$).

Conclusions: CoQ10 supplementation for three months could improve semen parameters, oxidative stress markers and reduce SDF in infertile men with idiopathic OA.

Keywords: Coenzyme Q10; DNA fragmentation; Male infertility; Oxidative stress; Sperm

INTRODUCTION

World Health Organization (WHO) defines infertility as the inability to conceive after at least 12 months of regular unprotected sexual intercourse [1]. According to WHO, about 60 to 80 million couples suffer from infer-

Received: Nov 3, 2019 **Revised:** Dec 24, 2019 **Accepted:** Dec 30, 2019 **Published online** Jan 20, 2020
Correspondence to: Ahmed T. Alahmar https://orcid.org/0000-0003-2100-5807
College of Science, University of Sumer, Thi-Qar 00964, Iraq.
Tel: +964-7808180900, **E-mail:** ahmed.t.alahmar@gmail.com

tility globally [2]. Men's are found to be solely responsible for 20% to 30% of infertility cases and contribute to 50% of cases overall [3]. Male infertility could be attributed to varicocele, genital infections, developmental abnormalities, endocrine disruptions, genetic, immunological, and systemic diseases as well as environmental factors [4].

In approximately 30% of male infertility cases, the underlying causes are unknown (idiopathic infertility) [5]. One of the factors that have been proven to cause idiopathic male infertility is oxidative stress (OS) which is a key responsible factor for causing about 30% to 80% of men subfertility [6]. OS occurs due to the exhaustion of seminal antioxidant capacity caused by imbalance between pro-oxidants and antioxidants in the seminal plasma [7]. Reactive oxygen species (ROS) are required for certain physiological processes such as sperm capacitation, acrosome reaction and fertilization [8,9]. However, excessive generation of ROS can damage sperm plasma membrane by lipid peroxidation and can also cause sperm DNA fragmentation (SDF). This may decrease sperm membrane fluidity, affecting sperm motility, vitality and ultimately resulting in altered fertilizing potential of sperm [9].

Oligoasthenozoospermia (OA) is defined as sperm concentration lower than 15 million per mL and sperm progressive motility and total motility lower than 32% and 40% respectively according to WHO 2010 guidelines [10]. It has been reported that, in approximately one third of infertile patients the cause of OA is unknown and, therefore, it is called idiopathic OA [5].

One of the mechanisms proposed for idiopathic OA is SDF. The increased OS causes DNA nicks and breaks that need repair [11]. The faulty repair of the DNA owing to decreased protamination can result in DNA damage. Other factors that can lead to sperm DNA damage are replication errors, ionized radiations, activation of endogenous endonucleases and caspases, exposure to environmental toxins and ultraviolet (UV) rays [12]. DNA fragmentation is an irreversible process that can alter sperm function leading to infertility and several tests have been established for detecting SDF [13].

Coenzyme Q10 (CoQ10), also termed as ubiquinone, is a respiratory chain component and acts as an antioxidant molecule. It is present in the human seminal fluid where it is involved in several antioxidant, mitochondrial bioenergetics and metabolic processes [14]. CoQ10 deficiency can lead to sperm damage, lower sperm motility and sperm count. Studies have shown that the supplementation with CoQ10 can improve the reproductive outcomes in men with fertility problems [15]. In addition, seminal fluid CoQ10 concentrations correlate positively with sperm motility and count. Accordingly, patients who have been treated with CoQ10 have a significant higher sperm count and a better sperm morphology compared with those who did not receive CoQ10 [16].

On the other hand, endogenous antioxidants, such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), normally maintain the scavenging potential in the seminal fluid and gonads [17]. However, studies reporting the impacts of CoQ10 in the treatment of idiopathic OA are scanty. Therefore, the aim of this study was to evaluate the effects of CoQ10 (200 mg/d) therapy on conventional sperm parameters, OS markers and SDF in infertile patients with idiopathic OA.

MATERIALS AND METHODS

1. Patients

Sixty-five infertile patients with idiopathic OA (mean age, 29.1±10.2 years; mean duration of infertility 6.3±4.1 years) and forty fertile healthy men who served as controls (mean age, 31.4±11.3 years) were enrolled in the study. The patients were recruited from the Fertility Clinic, Babyl, Iraq, during the period from May to September 2018. Data were collected using a questionnaire designed for this study. Medical history, physical examination as well as laboratory and radiological tests were performed to ascertain the presence of a known cause of OA. Patients with infertility of 12 months or more despite regular unprotected intercourse were included in the study. Patients were considered to have OA when their semen analysis showed abnormal sperm concentration (<15 million/mL), progressive motility (<32%), and total motility (<40%) as defined by the WHO 2010 criteria for semen analysis [10]. Patients with azoospermia, anatomical abnormalities of men genital tract, varicocele, genital infection, scrotal surgery, systemic diseases, smoking, women factor, and recent (within the last six months) antioxidant and selective serotonin reuptake inhibitors intake were excluded from the study. All the participants underwent baseline seminal fluid analysis (WHO 2010 guidelines),

seminal antioxidant measurement (total antioxidant capacity [TAC], GPx, and ROS), seminal fluid CoQ10 concentration and evaluation of the percentage of spermatozoa with DNA fragmentation. Each patient received CoQ10 200 mg/d orally (one a day) for three months and their conventional sperm parameters, TAC measurements, seminal fluid CoQ10 level and SDF were compared before and after CoQ10 treatment.

2. Ethics statement

The study design was approved by the University of Sumer local research ethical committee (EC/2018/8876) and adhered to the Declaration of Helsinki. An informed consent for participation in the study was obtained from each patient and control enrolled in this study.

3. Semen analysis

Semen was collected by masturbation after an abstinence of 2 to 3 days. It was collected into a sterile wide mouth plastic container, held at 37℃ until liquefaction was achieved and then analyzed within one hour from ejaculation using the methods suggested in the WHO manual WHO guidelines (5th edition) [10] for all semen parameters but sperm morphology which was evaluated according to the WHO guidelines (4th edition) [18]. Semen analysis was performed by the same researcher to ensure data consistency. Two semen analyses were performed before and after CoQ10 administration and the mean values were recorded and used for statistical evaluation.

4. Seminal total antioxidant capacity

Semen samples were centrifuged at 3,000 rpm for 5 minutes and seminal plasma was collected and stored at -20℃ until further testing. A colorimetric method was applied to measure TAC using the total antioxidant capacity assay kit (#E-BC-K136; Elabscience, Houston, TX, USA). Antioxidants can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline. TAC was measured using an absorbance at 520 nm as suggested by the manufacturer protocol.

5. Glutathione peroxidase activity

GPx was measured by a colorimetric method using the GPx assay kit (#E-BC-K096; Elabscience). The test is based on the principle that the enzyme promotes the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione (GSH) to produce H_2O and oxidized GSH. The activity of GSH can be calculated by measuring the consumption of reduced GSH. H_2O_2 and reduced GSH can react without catalysis of GPx, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH can react with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which shows a stable yellow color. TAC was calculated by measuring the absorbance at 412 nm as suggested by the manufacturer protocol.

6. Seminal reactive oxygen species measurement

ROS were measured manually using the method previously described by Venkatesh et al [19]. To 400 μL of liquefied neat semen, 10 μL of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma-Aldrich, St. Louis, MO, USA), prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. Ten microlitres of 5 mM luminol in DMSO served as blank. Twenty-five microlitres H_2O_2 with 10 μL luminol was used as a positive control. The levels of ROS were assessed by measuring the luminol-dependent chemiluminescence.

7. Measurement of seminal coenzyme Q10 concentrations

The seminal plasma concentrations of CoQ10 were measured by high-performance liquid chromatography (HPLC) method using a UV detector at 275 nm and calculated using the method described previously by Li et al [20]. The principle of this method is based on reversed-phase HPLC method with UV detection using coenzyme Q9 as the internal standard.

8. Sperm chromatin dispersion test

SCD test was performed using the Halosperm kit (Halotech DNA, S.L., Madrid, Spain). The test is based on the principle that spermatozoa with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in spermatozoa with non-fragmented DNA, following acid denaturation and removal of nuclear proteins. Intact non-fixed spermatozoa were immersed in an inert agarose microgel on pre-treated slide. Initial acid treatment denatured DNA molecules in those spermatozoa with fragmented DNA. Following this, the lysis solution removed most of the nuclear proteins, and in the absence of massive DNA breakage produced nucleoids with large halos of

spreading DNA loops, emerging from a central core. The nucleoids from spermatozoa with fragmented DNA either did not show a dispersion halo or the halo was minimal. Bright field microscopy with Diff-Quik staining was used to assess the halos. SDF, defined as the percentage ratio of fragmented *versus* total spermatozoa, was calculated for each sample and recorded [21].

9. Statistical analysis

Statistical analysis was performed using IBM SPSS software ver. 24 (IBM Corp., Armonk, NY, USA). The results are shown as mean±standard deviation. Normality of data was assessed by Kolmogorov–Smirnov test. Paired Student's t-test was used to compare means before and after CoQ10 administration. Correlations between sperm parameters and TAC, CAT, and SOD were analyzed using the Pearson correlation coefficient (r). A p-value lower than 0.05 was considered statistically significant.

RESULTS

CoQ10 treatment for three-months improved sperm concentration (p<0.05), progressive motility (p<0.05), and total motility (p<0.01) compared to the baseline (Table 1).

The seminal antioxidant status in the infertile patients were significantly lower (CoQ10 [p<0.05], TAC [p<0.01], and GPx [p<0.001]), while total ROS level was significantly higher (p<0.001) as compared to the controls (Table 2). CoQ10 therapy significantly improved seminal CoQ10 level (p<0.001), TAC (p<0.01), and GPx (p<0.001) levels, whereas it descreased the total ROS levels (p<0.05) in patients with idiopathic OA, as compared with pre-treatment values. SDF was significantly higher (p<0.01) in patients with idiopathic OA compared to the fertile controls, and treatment with CoQ10 significantly decreased the percentage of SDF (p<0.01). CoQ10 levels have been found to be positively correlated with sperm concentration (r=0.48, p=0.01) and motility (r=0.59, p=0.003) (Table 3). Moreover, SDF correlated negatively with sperm motility (r=-0.54, p=0.006). Seminal fluid CoQ10 levels did not show and correlation with sperm concentration or morphology.

Table 1. Semen parameters in fertile and infertile men before and after administration of CoQ10

Semen parameter	Fertile men (n=40)	Infertile patient (n=65)	
		Before CoQ10	After CoQ10
Age (y)	31.4±11.3	29.1±10.2	-
Infertility duration (y)	-	6.3±4.1	-
Volume (mL)	2.9±0.8	2.88±1.4	3.1±1.2
Concentration (million/mL)	51.1±29.4	9.4±5.4[c]	11.5±5.3[a,c]
Progressive motility (%)	47.8±10.8	22.3±9.3[c]	27.1±13.6[a,c]
Total motility (%)	66.4±13.4	30.1±9.8[c]	37.1±15.1[b,c]
Normal morphology (%)	43.4±9.1	42.3±8.8	40.5±9.6

Values are presented as mean±standard deviation.
CoQ10: coenzyme Q10.
[a]*vs.* patients baseline, p<0.05; [b]*vs.* patients baseline, p<0.01; [c]*vs.* fertile men, p<0.001.

DISCUSSION

CoQ10 follows *de-novo* pathway for its synthesis during normal physiological processes in most of the tis-

Table 2. Seminal plasma CoQ10, oxidative stress markers and SDF levels in fertile and infertile men before and after administration of CoQ10

	Fertile men (n=40)	Infertile patient (n=65)	
		Before CoQ10	After CoQ10
CoQ10 level (ng/mL)	63.8±42.38	46.2±33.8[a]	85.8±29.9***[,b]
ROS (×10^4 RLU/min/20 million spermatozoa)	0.11±0.08	4.6±1.95[c]	3.8±1.6*[,c]
TAC (mmol/L)	1.87±0.26	1.03±0.65[c]	1.32±0.59**[,c]
GPx (U/mL)	0.67±0.06	0.22±0.03[c]	0.39±0.05***[,c]
SDF (%)	15.8±4.5	35.6±7.1[c]	30.9±8.3**[,c]

Values are presented as mean±standard deviation.
CoQ10: coenzyme Q10, SDF: sperm DNA fragmentation, ROS: reactive oxygen species, RLU: relative light unit, TAC: total antioxidant capacity, GPx: glutathione peroxidase.
*Significant difference from patients baseline, p<0.05; **Significant difference from patients baseline, p<0.01; ***Significant difference from patients baseline, p<0.001.
[a]Significant difference from control, p<0.05; [b]Significant difference from control, p<0.01; [c]Significant difference from control p<0.001.

Table 3. Correlation between SDF, CoQ10 level and semen parameters in infertile men post-CoQ10 treatment

	Concentration	Total motility	Normal morphology
SDF	-0.14 (0.28)	-0.54 (0.006)	-0.27 (0.09)
CoQ10	0.48 (0.01)	0.59 (0.003)	0.2 (0.15)

Values are presented as r (p-value).
SDF: sperm DNA fragmentation, CoQ10: coenzyme Q10.

sues [22]. Thus, the exogenous supply of this coenzyme is not required in those tissues. However, in conditions of elevated OS, the endogenous supply might not be enough to fulfil the demands for CoQ10 [23].

It has been reported to be increased in blood and seminal plasma following its supplementation and is well proven to improve semen quality. Balercia et al [16] evaluated the concentration of CoQ10 in the seminal fluid and found increased CoQ10 levels in the patients after supplementation. A study also concluded that CoQ10 levels in the blood as well as in the seminal fluid increases after three or six months of supplementation [24].

An *in-vitro* study with CoQ10 supplementation has shown that it increases sperm motility significantly in asthenozoospermic patients [15]. A placebo-controlled, randomized study reported a significant improvement of sperm concentration, total sperm count, and sperm motility after CoQ10 supplementation to infertile men [25]. Mancini et al [26] have also reported similar observations in idiopathic OA men. By quantifying CoQ10 levels in the seminal plasma from 77 patients, they have recorded that reduced CoQ10 levels are associated with abnormal sperm morphology and motility. Based on their findings, they have concluded that patients with idiopathic OA can benefit from CoQ10 supplementation [26]. Recent studies have found that some of these effects of exogenously administered CoQ10 are due to the modulation of gene expression [22]. A randomized, placebo-controlled study, performed in patients with idiopathic OA showed a reduction in the OS after a three-month long CoQ10 supplementation, which has reported similar findings to the present results [27].

In the state of increased OS, endogenous enzymatic antioxidants also alter their activities to cope with the condition. The present study also showed a significant increase in TAC and GPx after CoQ10 administration. GSH has been found to be associated with men fertility due to its scavenger activity. The seminal TAC plays a significant role in the protection of spermatozoa when there is an elevation of seminal OS. Our study reported an improvement in seminal TAC after the administration of oral antioxidants. In contrast, few studies have reported no relationship between CoQ10 and TAC levels [28].

Studies have shown that men fertility can be compromised by excessive SDF [29]. The present study also focusses on the decrease of SDF index after CoQ10 administration in the infertile patients with idiopathic OA. A significant positive difference in sperm morphology following CoQ10 treatment also indicates that CoQ10 improves sperm structure. Results resembling our studies were obtained thus strengthening the fact that CoQ10 has positive effect on the sperm damage [30].

CONCLUSIONS

Seminal fluid of infertile patients with idiopathic OA is accompanied with high levels of ROS, which induces OS mediated disruptions of sperm functions and sperm DNA integrity. CoQ10 can attenuate ROS effects and enhance sperm functions owing to its antioxidant activity. The study affirms that supplementation with CoQ10 for the duration of least three months decreases SDF and improves semen quality and seminal antioxidant capacity in OA men. Thus, CoQ10 finds potential to be screened further for its application in the management of OA male infertility patients.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: ATA. Data curation: ATA. Formal analysis: ATA. Investigation: ATA. Methodology: ATA. Validation: ATA. Writing – original draft: ATA. Writing – review & editing: AEC, PS, SD.

Data Sharing Statement

The data required to reproduce these findings cannot be shared at this time due to legal and ethical reasons.

REFERENCES

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al.; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary on ART terminology, 2009. Hum Reprod 2009;24:2683-7.
2. Rutstein SO, Shah IH. Infecundity, infertility, and childless-

ness in developing countries. Calverton and Geneva: ORC Macro and World Health Organization; 2004.
3. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
4. Ilacqua A, Izzo G, Emerenziani GP, Baldari C, Aversa A. Lifestyle and fertility: the influence of stress and quality of life on male fertility. Reprod Biol Endocrinol 2018;16:115.
5. Tadros NN, Sabanegh ES. Empiric medical therapy with hormonal agents for idiopathic male infertility. Indian J Urol 2017;33:194-8.
6. Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, et al. Reactive oxygen species and male reproductive hormones. Reprod Biol Endocrinol 2018;16:87.
7. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003;79:829-43.
8. Alahmar AT. Role of oxidative stress in male infertility: an updated review. J Hum Reprod Sci 2019;12:4-18.
9. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: a systematic review on evaluation and management. Arab J Urol 2019;17:87-97.
10. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
11. Ozmen B, Koutlaki N, Youssry M, Diedrich K, Al-Hasani S. DNA damage of human spermatozoa in assisted reproduction: origins, diagnosis, impacts and safety. Reprod Biomed Online 2007;14:384-95.
12. Panerselvam M, Sengupta P, Agarwal A. Sperm DNA fragmentation and male infertility. In: Arafa M, Elbardisi H, Majzoub A, Agarwal A, editors. Genetics of male infertility: a case-based guide for clinicians. Cham: Elsevier; 2020.
13. Panner Selvam MK, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab J Urol 2018;16:65-76.
14. Ghanbarzadeh S, Garjani A, Ziaee M, Khorrami A. CoQ10 and L-carnitine attenuate the effect of high LDL and oxidized LDL on spermatogenesis in male rats. Drug Res (Stuttg) 2014;64:510-5.
15. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Mol Aspects Med 1997;18 Suppl:S213-9.
16. Balercia G, Mancini A, Paggi F, Tiano L, Pontecorvi A, Boscaro M, et al. Coenzyme Q10 and male infertility. J Endocrinol Invest 2009;32:626-32.
17. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci 1996;1:e78-86.
18. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
19. Venkatesh S, Shamsi MB, Dudeja S, Kumar R, Dada R. Reactive oxygen species measurement in neat and washed semen: comparative analysis and its significance in male infertility assessment. Arch Gynecol Obstet 2011;283:121-6.
20. Li K, Shi Y, Chen S, Li W, Shang X, Huang Y. Determination of coenzyme Q10 in human seminal plasma by high-performance liquid chromatography and its clinical application. Biomed Chromatogr 2006;20:1082-6.
21. Zaazaa A, Adel A, Fahmy I, Elkhiat Y, Awaad AA, Mostafa T. Effect of varicocelectomy and/or mast cells stabilizer on sperm DNA fragmentation in infertile patients with varicocele. Andrology 2018;6:146-50.
22. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: an evidence based review. Int J Reprod Biomed (Yazd) 2016;14:729-36.
23. Bhagavan HN, Chopra RK. Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations. Mitochondrion 2007;7 Suppl:S78-88.
24. Balercia G, Mosca F, Mantero F, Boscaro M, Mancini A, Ricciardo-Lamonica G, et al. Coenzyme Q(10) supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study. Fertil Steril 2004;81:93-8.
25. Safarinejad MR. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. J Urol 2009;182:237-48.
26. Mancini A, De Marinis L, Oradei A, Hallgass ME, Conte G, Pozza D, et al. Coenzyme Q10 concentrations in normal and pathological human seminal fluid. J Androl 1994;15:591-4.
27. Bhagavan HN, Chopra RK. Coenzyme Q10: absorption, tissue uptake, metabolism and pharmacokinetics. Free Radic Res 2006;40:445-53.
28. Eroglu M, Sahin S, Durukan B, Ozakpinar OB, Erdinc N, Turkgeldi L, et al. Blood serum and seminal plasma selenium, total antioxidant capacity and coenzyme Q10 levels in relation to semen parameters in men with idiopathic infertility. Biol Trace Elem Res 2014;159:46-51.
29. Carrell DT, Liu L, Peterson CM, Jones KP, Hatasaka HH, Erickson L, et al. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 2003;49:49-55.
30. Kobori Y, Ota S, Sato R, Yagi H, Soh S, Arai G, et al. Antioxidant cosupplementation therapy with vitamin C, vitamin E, and coenzyme Q10 in patients with oligoasthenozoospermia. Arch Ital Urol Androl 2014;86:1-4.