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The effect of free radicals on sperm DNA and antioxidant protective role; an assessment and review

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ABSTRACT

Oxidative stress is an imbalance between the level of ROS and antioxidants. Sperm membranes are rich in polyunsaturated fatty acids and are very sensitive to increased free radicals. Increasing the level of ROS can lead to an increase in lipid peroxidation in sperm membrane, decrease in their flexibility, and ultimately a decrease in sperm motility. Also, oxidative stress may impair sperm axonemal and mitochondrial function, as well as DNA integrity, RNA and protein synthesis. Several studies have shown that antioxidants are beneficial to human health and are used to prevent cancer and coronary artery disease. Based on the present review, there are more documents in protective effects of antioxidants on sperm cells. Studies showed adding in vitro antioxidants to sperm samples can increase sperm parameters such as sperm motility, morphology and decrease sperm membrane lipid peroxidation and sperm DNA damage. Also in vivo or oral antioxidants supplementation has protective effects on sperm from ROS negative effects. Since the causes of infertility are various, the lack of antioxidants action in reducing the sperm DNA damage, the level of antioxidant activity depends on the type, dose and duration of intake, so more studies are needed to determine the appropriate antioxidant type, the dosage and timing of administration in vivo and in vitro.

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Introduction

Sperm contains half of the genetic material of the fetus, and its integrity is essential for successful fertilization and embryo development (1). Although there are many articles on sperm DNA damage in infertile men, it has also been reported in lower levels in fertile men (2). Factors influencing sperm DNA damage can be inside or outside the testicles (1).

Internal factors are include genetic abnormalities, cryptorchidism, age and idiopathic abnormalities (2). Several studies have shown an association between aging and decreased sperm parameters (3, 4). In several studies, a decrease in the quality of semen and an increase in sperm DNA damage have been reported due to external factors such as alcohol consumption, smoking, cancer, inflammation and infection of reproductive organs (7, 8, 9). These factors often lead to decreased motility, morphology, sperm concentration, and increased DNA degradation in sperm by increasing the level of Reactive Oxygen Species (ROS) in the semen (7, 8, 9).

Fever leads to an increase in DNA damage and increase in the ratio of histone to protamine in sperm chromatin by increasing the temperature in the testicles. (5). Occupations such as bakery, welding, long-term driving, as well as behaviors such as excessive use of saunas, hot baths, and laptops by increasing the temperature in the testicles, and the level of ROS can lead to sperm DNA

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damage (6, 7).

Exposure to pesticides and metals such as lead, cadmium and phthalates is associated with increased levels of oxidative stress and sperm DNA degradation.(8-10). There are also studies showing an association between varicocele and high levels of oxidative stress and spermDNA damage (11, 12).

Literature Review

Article collection for the present narrative review searched by male reproductive oxidative stress, antioxidant, sperm, and sperm DNA damage key words in the PubMed database. We only focused on the clinical trials related to the male reproductive oxidative stress and the effects of antioxidants on the male reproductive system. All studies including in vitro and in vivo included in this present study.

ROS is a term defined as all oxygen contains with high reactive molecules. In the biology this species named free radicals. Hydroxyl radical, anion superoxide radical, hydrogen peroxide, oxygen radical, nitric oxide (NO), hypochlorite and various lipid peroxides are the main types of ROS. Free radicals and ROS in normal levels have progressive special effects in human biological activities. On the other hand they were not harmful in normal level and when the level of ROS increase and system balance disrupted, they are harmful. ROS are the main intracellular messenger and the regulation of gene expression. Consequently, ROS in normal level is an important factor for progress cell function (13).

interestingly ROS in high level are dangerous for human body, because they can generate harmful effects on all parts of cells including cell membrane, cytoplasmic organelles, enzymes and proteins, genetic substance and nucleic acids (11, 14).

Pathological role of oxidative stress on sperm

Oxidative stress is an imbalance between the level of ROS and antioxidants. Sperm membranes are rich in polyunsaturated fatty acids and are very sensitive to increased free radicals. Increasing the level of ROS can lead to an increase in lipid peroxidation in sperm membrane, decrease in their flexibility, and ultimately a decrease in sperm motility. Also, oxidative stress may impair sperm axonemal and mitochondrial function, as well as DNA integrity, RNA and protein synthesis (9, 15).

It can evoke single stranded (ss), double stranded (ds) DNA breaks and small chromosomal deletions (15). Recent studies have reported high levels of free radicals in 30 to 80 percent of infertile men (16).

Sperm motility is an important factor to move through the female reproductive tract and successful fertilization. A negative correlation was shown between increase the level of ROS and reduce of sperm concentration, morphology and motility (17, 18). The residual cytoplasm in immature spermatozoa contains high level of glucose-6-phosphate dehydrogenase (G-6-PDH), which catalyzes NADPH generation and NADPH is involved in the production of ROS (19).

Antioxidants

Oxidation is a phenomenon that plays a role in the production of free radicals, and antioxidants are compounds that inhibit the process of oxidation. Antioxidants can be endogenous or exogenous. Endogenous antioxidants play an important role in maintaining proper cellular function, but when oxidative stress occurs, they are usually insufficient and require exogenous antioxidants. Antioxidants are exogenous or endogenous. They can also be enzymatic or non-enzymatic. Glutathione peroxidase, catalase and superoxide dismutase are the most important enzymatic antioxidants. Vitamin E, C, melatonin, carotenoids and natural flavonoids are also non-enzymatic antioxidants (20). Several studies have shown that antioxidants are beneficial to human health and are used to prevent cancer and coronary artery disease (21-23). In addition, antioxidants are used in food preservatives, cosmetics and anti-corrosion materials for metals (24).

Seminal fluid antioxidants

One of the main sources of antioxidant in male reproductive system is seminal fluid. The seminal fluid is containing many different enzymatic and non-enzymatic antioxidants. Some antioxidants in the seminal fluid are vitamins E and C, superoxide dismutase, glutathione, lycopene and thioredoxin (25). These antioxidants can protect sperm cells from ROS activities (26, 27). There were some documents that showed each disruption in seminal fluid antioxidant level can create dysfunction in sperm cells, sperm DNA damages, decrease quality of sperm and finally male infertility happened (28, 29). The high level of oxidative stress in seminal fluid is harmful because can create peroxidation in the sperm plasma membrane and disturb DNA integrity (30). There is usually a balance between the amount of ROS and the oxidative stress elimination system. One of the natural ways to neutralize the effect of oxidative stress is to increase the stress-removal system in the seminal plasma (31). Researchers have tested different antioxidants in different compositions with different dosage in several time periods and consequently by this enormous study, with various design and control of individuals, there was not a definite conclusion in this regard. Because

the most important achievement of fertilization (pregnancy) has been reported only in quantitative studies (32). (Table 1)

Antioxidants and sperm parameters

Antioxidants can play an important role to protect sperm cells from ROS negative effects. The studies showed vitamin E can increase sperm morphology and motility (33, 34). Another antioxidants such as vitamin C and Q-10 coenzyme are high protective factor and prevent sperms motility from oxidative stress (35). Studies reported in patients with defects in sperm parameters, in vitro incubation of sperm in rich antioxidant medium for a day can increase sperm motility in comparison with control group (36, 37).

More studies reported 90 days oral antioxidant supplementation were effective for improvement of sperm parameters including sperm motility and morphology (38, 39). The results of a study of stress-induced albino rats showed that oral administration of moderate (20 mg/kg/day) to high doses (30 mg/kg/day) of vitamin C significantly increased sperm count and motility (40). (Table 1)

Antioxidants and sperm membrane lipid peroxidation

Sperm membrane is containing fatty acids and oxidative stress has negative effects on sperm membrane. Many factors such as high temperature, medications, disease and ageing can elevate sperm membrane lipid peroxidation. (41, 42). In last decay more studies plane to assess several methods to prevent sperm membrane from ROS effects. The results of studies showed in vitro incubation of sperm cells in superoxide dismutase (SOD) or vitamin E is effective for decrease sperm lipid peroxidation (43, 44). (Table 1)

Antioxidants and sperm DNA

Many studies have reported the role of antioxidants in reducing the fragmentation of DNA from oxidative stress. (45, 46). In a double blind randomized controlled clinical trial, 115 infertile men with varicocele participated, and after surgery, they were given 250 mg of vitamin C for three months. The vitamin C treatment group provided better sperm motility and morphology and DNA integrity statistically than their placebo group (47).

Supplementation of selenium – vitamin E on 690 infertile men showed on improve in semen quality and pregnancy rate (48).

In a study, 20 infertile men diagnosed with terato-asthenozoospermia received a pill containing of creatinine, vitamin E, C, B9, B12, quinidine Q10 and selenium for three months daily. Their results showed improve in sperm parameters and DNA integrity. Also DNA fragmentation was decreased in study group. So they suggested prescribing these antioxidants before assisted reproductive techniques (ART) (49).

Greco et al. found that daily administration of vitamin E and C for two months in infertile men with high levels of DNA Fragmentation (≥ 15%) reduced the rate of DNA Fragmentation in 76% of patients. They also showed that these antioxidants improved intra-cytoplasmic sperm injection (ICSI) outcome in these patients (50). Another study revealed that oral administration of zinc sulfate to infertile men, significantly improves sperm DNA integrity in this patients (51).

Infertile men with high levels of oxidative stress, ROS, and sperm DNA damage received capsules containing of vitamin E, C, zinc, selenium and folate daily for three months. The researchers found that sperm DNA damage, protein deficiency, ROS and sperm apoptosis were reduced. But they did not see any clear statistical changes in the improvement of sperm parameters (52).

Gual - Frau et al. administered a combination of several supplements including selenium, coenzyme Q10, L-carnitine, vitamins C, E, B9, Zinc B12, to twenty patients with grade 1 varicocele for three months orally. These patients usually show a high level of sperm DNA damage. After three months, they showed a 22.1 percent reduction in sperm DNA damage (53).

In an in vitro study, sperm samples taken from twelve infertile men were subjected to oxidative stress with hydrogen peroxide. In the experimental group, 2 hours before induction of stress, the samples were incubated with 5 micromoles of lycopene. The results showed that in the experimental group, lycopene was able to protect the patient's sperm from DNA damage, but did not improve sperm motility (54).

Antioxidants are able to protect sperm DNA from free radicals endogenous (55-57) and exogenous(58, 59).

Sperm washing raises the level of ROS, which in turn can have a negative effect on sperm DNA (60). Also, sperm freezing and thawing may result in over generation of ROS which lead to DNA fragmentation. E. Tvrda et al. found that lycopene can decrease the oxidative sperm DNA damage in treated group compared to control group (61). In another study, supplementation with methionine, inositole and carnitine before the cryopreservation protected the sperm DNA integrity against the cryodamage (62). (Table 1)

Trolox is a derivative of vitamin E with potent of antioxidant property. Supplementation of Trolox for two weeks significantly improved the sperm motility in post – thawed human spermatozoa (63).

Table 1. Summary of studies about antioxidants and sperm cell

	Reference	Conclusion
Seminal fluid antioxidants	Dzyuba (2016) (25)	Seminal fluid is containing many different enzymatic and non-enzymatic antioxidants.
	Atig (2017) (26)	Antioxidants can protect sperm cells from ROS activities.
	Esmilzade (2017) (31)	Seminal plasma can remove effect of oxidative stress by the stress-removal system.
Antioxidants and sperm parameters	Gvozdjakova (2015) (35)	Vitamin C and Q-10 coenzyme can prevent sperms motility from oxidative stress.
	Biagi (2019) (37)	In vitro incubation of sperm in rich antioxidant medium can increase sperm motility.
	Sabeti (2017) (38)	oral antioxidant supplementation were effective for improvement of sperm parameters.
	Vijayprasad (2014) (40)	Vitamin C significantly increased sperm count and motility.
Antioxidants and sperm membrane lipid peroxidation	Kobayashi (1991) (43)	Incubation of sperm cells in superoxide dismutase (SOD) can decrease sperm lipid peroxidation.
	Rolf (1999) (44)	In vitro incubation of sperm cells in vitamin E is effective for decrease sperm lipid peroxidation.
Antioxidants and sperm DNA	Cyrus (2015) (47)	Vitamin C treatment has positive effects on sperm DNA integrity.
	Moslemi (2011) (48)	Selenium – vitamin E can improve in semen quality and pregnancy rate.
	Abad (2013) (49)	Creatinine, vitamin E, C, B9, B12, quinidine Q10 and selenium for three months can improve in sperm parameters and DNA integrity.
	Raigani (2014) (51)	Oral administration of zinc sulfate to infertile men, significantly improves sperm DNA integrity.
	Tunc (2009) (52)	Vitamin E, C, zinc, selenium and folate can decrease sperm DNA fragmentation.
	Gual-Frau (2015) (53)	Selenium, coenzyme Q10, L-carnitine, vitamins C, E, B9, zinc and B12 can decrease high level of DNA damage.

Overall, the evidence suggests that the use of antioxidants in cryoprotectants may protect the spermatozoa from cryodamage but it is important to know that the method of freezing and the type of cryoprotectants are important (64).

In addition, oxidative stress is one of the causes of male infertility, and these compounds may only be useful in cases where the cause of DNA damage is oxidative stress (65, 66).

Conclusion

Based on the present review, there are more documents in protective effects of antioxidants on sperm cells. Studies showed adding in vitro antioxidants to sperm samples can increase sperm parameters such as sperm motility, morphology and decrease sperm membrane lipid peroxidation and sperm DNA damage. Also in vivo or oral antioxidants supplementation has protective effects on sperm from ROS negative effects. Since the causes of infertility are various, the lack of antioxidants action in reducing the sperm DNA damage, the level of antioxidant activity depends on the type, dose and duration of intake, so more studies are needed to determine the appropriate antioxidant type, the dosage and timing of administration in vivo and in vitro.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Hosseini A, Zare S, Borzouei Z, Ghaderi Pakdel F. Cyclophosphamide-induced testicular toxicity ameliorate by American ginseng treatment: An experimental study. Int J Reprod Biomed. 2018;16:711-718.
2. Ghuman N, Ramalingam M. Male infertility. Obstetrics, Gynaecology & Reproductive Medicine. 2018;28:7-14.
3. Deenadayal Mettler A, Govindarajan M, Srinivas S, Mithraprabhu S, Evenson D, Mahendran T. Male age is associated with sperm DNA/chromatin integrity. Aging Male. 2019;9:1-8.
4. Veron GL, Tissera AD, Bello R, et al. Impact of age, clinical conditions, and lifestyle on routine semen parameters and sperm kinematics. Fertil Steril. 2018;110:68-75.
5. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. Reprod Biomed Online. 2015;30:14-27.
6. El Salam MAA. Obesity, An Enemy of Male Fertility: A Mini Review. Oman Med J. 2018;33:3-6.
7. Hayden RP, Flannigan R, Schlegel PN. The Role of Lifestyle in Male Infertility: Diet, Physical Activity, and Body Habitus. Curr Urol Rep. 2018;19:56.
8. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nature Reviews Urology. 2017;14:470.
9. Bisht S, Dada R. Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies. Front Biosci. 2017;9:420-447.
10. Pourmasumi S, Sabeti P, Rahiminia T, Mangoli E, Tabibnejad N, Talebi AR. The etiologies of DNA abnormalities in male infertility: An assessment and review. Int J Reprod Biomed. 2017;15:331-344.
11. Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. Etiologies of sperm oxidative stress. Int J Reprod Biomed. 2016;14:231-240.
12. Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl. 2016;18:186-193.
13. Weidinger A, Kozlov AV. Biological Activities of Reactive Oxygen and Nitrogen Species: Oxidative Stress versus Signal Transduction. Biomolecules. 2015;5:472-484.
14. Hamilton TR, de Castro LS, Delgado Jde C, et al. Induced lipid peroxidation in ram sperm: semen profile, DNA fragmentation and antioxidant status. Reproduction. 2016;151:379-

390.
15. Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Dev. 2017;84:1039-1052.
16. Ko EY, Sabanegh ES Jr, Agarwal A. Male infertility testing: reactive oxygen species and antioxidant capacity. Fertil Steril. 2014;102:1518-1527.
17. Pourmasumi S, Khoradmehr A, Rahiminia T, Sabeti P, Talebi AR, Ghasemzadeh J. Evaluation of Sperm Chromatin Integrity Using Aniline Blue and Toluidine Blue Staining in Infertile and Normozoospermic Men. J Reprod Infertil. 2019;20:95-101.
18. Moazamian R, Polhemus A, Connaughton H, et al. Oxidative stress and human spermatozoa: diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. Mol Hum Reprod. 2015;21:502-515.
19. Nowicka-Bauer K, Nixon B. Molecular Changes Induced by Oxidative Stress that Impair Human Sperm Motility. Antioxidants. 2020;9:134.
20. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal. 2016;15(1):71.
21. Leopold JA. Antioxidants and coronary artery disease: from pathophysiology to preventive therapy. Coron Artery Dis. 2015;26:176-183.
22. Yadav A, Kumari R, Yadav A, Mishra J, Srivatva S, Prabha S. Antioxidants and its functions in human body-A Review. Res Environ Life Sci. 2016;9:1328-1331.
23. Niki E. Oxidative stress and antioxidants: Distress or eustress? Arch Biochem Biophys. 2016;595:19-24.
24. Nitschke M, Silva SSE. Recent food applications of microbial surfactants. 2018;58:631-638.
25. Dzyuba V, Cosson J, Dzyuba B, Yamaner G, Rodina M, Linhart O. The antioxidant system of seminal fluid during in vitro storage of sterlet Acipenser ruthenus sperm. Fish physiology and biochemistry. 2016;42:563-568.
26. Atig F, Kerkeni A, Saad A, Ajina M. Effects of reduced seminal enzymatic antioxidants on sperm DNA fragmentation and semen quality of Tunisian infertile men. Journal of assisted reproduction and genetics. 2017;34:373-381.
27. Agarwal A, Roychoudhury S, Bjugstad KB, Cho C-L. Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? Therapeutic Advances in Urology. 2016;8:302-18.
28. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: An evidence based review. Int J Reprod Biomed. 2016;14:729-736.
29. Liu Q, Wang X, Wang W, et al. Effect of the addition of six antioxidants on sperm motility, membrane integrity and mitochondrial function in red seabream (Pagrus major) sperm cryopreservation. Fish physiology and biochemistry. 2015;41:413-422.
30. Pourmasumi S, Nazari A, Fagheirelahee N, Sabeti P. Cytochemical tests to investigate sperm DNA damage: Assessment and review. Journal of Family Medicine and Primary Care. 2019;8:1533.
31. Esmailzade M, Sadeghi A. Chromium nanoparticles affect antioxidant activity, hormones and sperm parameters in heat-stressed male rats: a model of mammals. Journal of Livestock Science. 2017;8:1-7.
32. Pourmasumi S, Ghasemi N, Talebi AR, Mehrabani M, Sabeti P. The effect of vitamin E and selenium on sperm chromatin quality in couples with recurrent miscarriage. International Journal of Medical Laboratory. 2018;5:1-10.
33. Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review. J Clin Diagn Res. 2017;11:1.
34. Mortazavi M, Salehi I, Alizadeh Z, Vahabian M, Roushandeh AM. Protective Effects of Antioxidants on Sperm Parameters and Seminiferous Tubules Epithelium in High Fat-fed Rats. J Reprod Infertil. 2014;15:22-8.
35. Gvozdjakova A, Kucharska J, Dubravicky J, Mojto V, Singh RB. Coenzyme Q(1)(0), alpha-tocopherol, and oxidative stress could be important metabolic biomarkers of male infertility. Dis Markers. 2015;827941:25.
36. Chi HJ, Kim JH, Ryu CS, et al. Protective effect of antioxidant supplementation in sperm-preparation medium against oxidative stress in human spermatozoa. Hum Reprod. 2008;23:1023-1028.
37. Biagi M, Noto D. Antioxidant Effect of the Castanea sativa Mill. Leaf Extract on Oxidative Stress Induced upon Human Spermatozoa. 2019;2019:8926075.
38. Sabeti P, Amidi F, Kalantar SM, et al. Evaluation of intracellular anion superoxide level, heat shock protein A2 and protamine positive spermatozoa percentages in teratoasthenozoospermia. Int J Reprod Biomed. 2017;15:279-286.
39. Imamovic Kumalic S, Pinter B. Review of clinical trials on effects of oral antioxidants on basic semen and other parameters in idiopathic oligoasthenoteratozoospermia. Biomed Res Int. 2014;426951:31.
40. Vijayprasad S, Bb G, Bb N. Effect of vitamin C on male fertility in rats subjected to forced swimming stress. J Clin Diagn Res. 2014;8(7):Hc05-8.
41. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod. 1989;41:183-1897.
42. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev. 2008;1:15-24.
43. Kobayashi T, Miyazaki T, Natori M, Nozawa S. Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod. 1991;6:987-991.
44. Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum Reprod. 1999;14:1028-1033.
45. Alahmar AT. The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia. Clin Exp Reprod Med. 2018;45:57-66.
46. Safarinejad MR, Safarinejad S, Shafiei N. Effects of the reduced form of coenzyme Q10 (ubiquinol) on semen parameters in men with idiopathic infertility: a double-blind, placebo controlled, randomized study. J Urol. 2012;188:526-531.
47. Cyrus A, Kabir A, Goodarzi D, Moghimi M. The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: a double blind placebo controlled clinical trial. International braz j urol : official journal of the Brazilian Society of Urology. 2015;41:230-238.
48. Moslemi MK, Tavanbakhsh S. Selenium-vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. International journal of general medicine. 2011;4:99-104.
49. Abad C, Amengual MJ, Gosalvez J, et al. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. Andrologia. 2013;45:211-216.
50. Greco E, Romano S, Iacobelli M, Ferrero S, Baroni E, Minasi MG, et al. ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. Hum Reprod. 2005;20:2590-2594.
51. Raigani M, Yaghmaei B, Amirjannti N, et al. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. Andrologia. 2014;46:956-962.
52. Tunc O, Thompson J, Tremellen K. Improvement in sperm DNA quality using an oral antioxidant therapy. Reprod Biomed Online. 2009;18:761-768.
53. Gual-Frau J, Abad C, Amengual MJ, et al. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. Human fertility. 2015;18:225-229.
54. Zini A, San Gabriel M, Libman J. Lycopene supplementation in vitro can protect human sperm deoxyribonucleic acid from oxidative damage. Fertil Steril. 2010;94:1033-1036.
55. Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. Reprod Biomed Online. 2014;28:684-703.
56. Arias ME, Sanchez-Villalba E, Delgado A, Felmer R. Effect of transfection and co-incubation of bovine sperm with

exogenous DNA on sperm quality and functional parameters for its use in sperm-mediated gene transfer. Zygote. 2017;25:85-97.
57. Barbato V, Talevi R, Braun S, et al. Supplementation of sperm media with zinc, D-aspartate and co-enzyme Q10 protects bull sperm against exogenous oxidative stress and improves their ability to support embryo development. Zygote. 2017;25:168-175.
58. Bejarano I, Monllor F, Marchena AM, et al. Exogenous melatonin supplementation prevents oxidative stress-evoked DNA damage in human spermatozoa. Journal of pineal research. 2014;57:333-339.
59. Fatehi D, Mohammadi M, Shekarchi B, Shabani A, Seify M, Rostamzadeh A. Radioprotective effects of Silymarin on the sperm parameters of NMRI mice irradiated with gamma-rays. J Photochem Photobiol B. 2018;178:489-495.
60. Moein MR, Vahidi S, Ghasemzadeh J, Tabibnejad N. Comparison of reactive oxygen species in neat and washed semen of infertile men. Iranian journal of reproductive medicine. 2014;12:301-306.
61. Tvrda E, Mackovich A, Greifova H, Hashim F, Lukac N. Antioxidant effects of lycopene on bovine sperm survival and oxidative profile following cryopreservation. Veterinární medicína. 2017;62:429-436.
62. Bucak MN, Tuncer PB, Sariozkan S, et al. Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: antioxidants protect DNA integrity against cryodamage. Cryobiology. 2010;61:248-253.
63. Minaei MB, Barbarestani M, Nekoonam S, et al. Effect of Trolox addition to cryopreservation media on human sperm motility. Iranian journal of reproductive medicine. 2012;10:99-104.
64. Taylor K, Roberts P, Sanders K, Burton P. Effect of antioxidant supplementation of cryopreservation medium on post-thaw integrity of human spermatozoa. Reprod Biomed Online. 2009;18:184-189.
65. Osipova VP, Berberova NT, Gazzaeva RA, Kudryavtsev KV. Application of new phenolic antioxidants for cryopreservation of sturgeon sperm. Cryobiology. 2016;72:112-118.
66. Kedechi S, Zribi N, Louati N, et al. Antioxidant effect of hydroxytyrosol on human sperm quality during in vitro incubation. Andrologia. 2017;49:e12595.