Impact of Four Week Swimming Exercise with Alpha-Tocopherol Supplementation on Fertility Potential in Healthy Rats

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Purpose: The aim of this study was to evaluate the effect of 4 week intensive swimming exercise and alpha-tocopherol supplementation on testicular oxidative stress and spermatogenesis in rats.

Materials and Methods: 40 male rats were randomly assigned to Control (C), Sham (S), Exercise (E) and Exercise + supplement (ES) groups. Exercise training performed for 4 weeks (1session/day, 6days/week). Each session included 180 minutes of swimming. In ES group, alpha-ocopherol was injected at a dose of 50 mg/kg/day. 48 hours after last training session, all rats were killed and gonads of them were removed from their body for histological and biochemical assays. All statistical analysis was performed by SPSS 16. *P* values less than 0.05 were considered as statistically significant.

Results: Total testicular antioxidant capacity increased significantly in E ($P = .003$) and ES groups ($P = .001$) whereas there was no significant difference between C and E group in testicle Malondialdehyde (a lipid peroxidation marker) level ($P = .999$) and spermatogenesis quality ($P = .381$). Testicle Malondialdehyde level decreased ($P = .009$) and spermatogenesis quality was improved significantly in ES group ($P = .001$).

Conclusion: Alpha-tocopherol supplementation is effective in order to improve spermatogenesis process in athletes who exercise with high intensity.

Key words: alpha-tocopherol; fertility; free radical; spermatogenesis; swimming

INTRODUCTION

Nowadays, male infertility is one of the most important medical challenges. Several factors are related to increased risk of infertility such as: reproductive diseases, cancers, genetic anomalies, long-term use of some medications, and oxidative stress.[1-3] Oxidative stress is the result of Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) overproduction or body antioxidant defense weakness. It has negative effects on sperm quality and motility and also causes sperm DNA damage and lipid peroxidation of sperm membrane.[2] Doing exercise training with high intensity is one of the conditions which can lead to oxidative stress occurrence. It has been reported that intensive exercise develops free radical production and, simultaneously, reduced spermatogenesis quality and male fertility.[4] Since it is estimated that semen ROS level is higher than normal range in approximately 25% of infertile men,[5] oxidative stress due to intensive exercise can be suggested as a main reason for reduced fertility in high trained men. Some antioxidants like alpha-tocopherol (vitamin E) are strong ROS and RNS detergents and using them may have positive effects on male reproductive function.[6] So in current study, it was assumed that vitamin E intake during intensive training may prevent negative effects of exercise on spermatogenesis process via antioxidant defense improvement.

MATERIALS AND METHODS

40 Speragues Dawley rats were selected as subjects. General specifications of the rats are given in **Table 1**. They were housed at temperature 22 ± 2°c and 12-hour light/dark cycle with full access to food and water, in animal lab of Arak University of Medical Sciences. The subjects were randomly classified into four experimental groups:

1. Control group (C): Rats of this group did not perform any exercise and no substance was injected to them.
2. Exercise group (E): During research, rats of this group were performing aerobic swimming exercise at 10 A.M
3. Exercise + Supplement group (ES): During research, rats of this group were performing aerobic swimming exercise at 10 A.M, after intraperitoneal (I.P.) injection of 50mg/kg vitamin E.
4. Sham group (S): In order to investigate stress of injection and floatation in water, rats of this group were put in water at 10 A.M (15min/day) after injection of normal saline solution. Water depth was 10 cm, therefore they could not swim in it.

Health deputy approved codes for working with laboratory animals were respected at all stages of the experiment.

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Table 1. General characteristics of rats.

GROUP	QUANTITY	AGE (month)	WEIGHT[a] (gr)
C	10	3-4	250.7 (± 5)
S	10	3-4	250.4 (± 5.4)
E	10	3-4	250.6 (± 5.3)
ES	10	3-4	250.5 (± 5.1)

Abbreviations: C, control group; S, sham group; E, exercise group; ES, supplement group.
[a] Weight of rats is presented as Mean (±SD)

Aerobic exercise program

Exercise program was aerobic swimming (1 session/day, 6 days/week) that was performed in two stages: Stage1 (one week preparation): During this stage, rats of E and ES groups swam in a pool (150*50*50 cm) containing water with 32°c temperature . Swimming duration was 60 minutes in first session which was increased gradually (20min/session) until reached up to 160 minutes.
Stage 2 (main exercise): During this stage, the rats swam in the pool for 4 weeks. Exercise duration was 180 minutes in each session. This program is a mode of intense aerobic training.[7]
Providing gonad tissue samples
48 hours after last training session, all rats were anesthetized with I.P. injection of ketamine (70mg/kg) combined with Xylazine (4mg/kg). Then gonads of each rat were removed from its body and one of them was put in Bohen solution for histological assay and the other one was put in an ice container for biochemical analyzing.

Evaluation of testicular biochemical parameters
Lipid peroxidation of testicles

One of the most important productions of lipid peroxidation is Malondialdehyde (MDA).[8] Therefore, its level was determined in tissue samples with Ohkawa method. Results were expressed as nmol/Gkw (Table 2). Details of this procedure have been explained completely in previous studies.[9]

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP test uses antioxidants as reductants. In samples, reductants reduce ferric tripyridyl-triazine complex (Fe3+- TPTZ) in stoichiometric excess to a blue ferrous form (Fe2+) with an increase in absorbance at 593 nm.[10] The absorbance values were read at 593 nm immediately and 4 min later using a spectrophotometer UV-1018 (**Table 2**). Details of this procedure have been explained completely in previous studies.[11]

Histological assay

Left gonad of each rat was divided into two equal pieces with a longitudinal cutting. In order to fix, each of these pieces was submersed in Bohen liquid for 24 hours. After fixation, tissue preparation was done by the following method: Fixed tissue samples were put in Xylene and then in Paraffin for transparency. So paraffin blocks including samples were produced. 12 sections with 5 micron thickness were prepared from each block. Tissue slices were investigated by microscope after painting with Hematoxyline and Eosin (H & E) method and spermatogenesis was scored based on modified Johnsen score.[12] In this classification system, spermatogenesis is rated from1 (only sertoli cell existence in seminiferous tubules) to10 (normal condition).
Then scores are classified into three groups:
Scores of 1–3: poor spermatogenesis
Scores of 4–7: moderate spermatogenesis
Scores of 8–10: good spermatogenesis.
For each sample, all tissue sections were investigated by microscope and an overall score was calculated (**Table 3**).

Statistical analysis

Data from histological and biochemical assessment was collected and Kolmogorov - Smirnov test was applied to check the normality of data distribution. Comparisons were carried out using one way analysis of variance (ANOVA) followed by post hoc Tukey test. All statistical analysis was performed by SPSS 16. *P* values less than 0.05 were considered as statistically significant.

RESULTS

There was no significant difference between C and S groups in FRAP level (*P* = .986), MDA level (*P* = .990) and Spermatogenesis quality (*P* = .882). Also, there was no significant difference in MDA level of tissue samples between E and C groups (*P* = .999) But FRAP level of E group was significantly more than C group (*P* = .003). In ES group, FRAP level were significantly higher (*P* = .001) and MDA level was significantly lower (*P* = .009) than C group. Spermatogenesis quality of ES group was significantly higher than C group (*P* = .001) but difference between E and C group was not significant (*P* = .381).

DISCUSSION

Large numbers of athletes, especially professional athletes, have heavy exercise programs. These programs impose great stresses on their body and consequently, body homeostasis is disturbed. This condition may mistune some organs or systems, for example reproductive system. In some studies, defect in male sexual hormones, sperm parameters and reproductive system function have been reported following intensive exercises.[13,14] Etiology of these disorders are not clear exactly but oxidative stress has been introduced as one

Table 2. Mean (±SD) of biochemical assessment data.

GROUP	FRAP (µM)	*P* value [a]	MDA(nmol/Gkw)	*P* value [b]
C	.76 (± .02)	_	.11 (± .01)	_
S	.76 (± .03)	.986	.1 (± .01)	.990
E	.81 (± .03)	.003	.11 (± .01)	.999
ES	.87 (± .02)	.001	.09 (± .01)	.009

Abbreviations: C, control group; S, sham group; E, exercise group; ES, supplement group.
[a] P value for FRAP compared with control group
[b] P value for MDA compared with control group

Table 3. Mean (±SD) of Spermatogenesis scores.

GROUP	SPERMATOGENESIS SCORE	*P* value [a]
C	8.6 (± .33)	_
S	8.5 (± .33)	.882
E	8.4 (± .35)	.381
ES	9.4 (± .15)	.001

Abbreviations: C, control group; S, sham group; E, exercise group; ES, supplement group.
[a] P value for spermatogenesis score compared with control group

of the possible mechanisms.[15] Oxidative stress occurs due to overproduction of free radicals or body disability for decrease their destructive effects optimally.[2] It's estimated that in normal metabolism state of the body, within oxygen consumption, 2-5 percent of electrons are used in free radical generation. Since oxygen consumption increases up to 20 times during intense aerobic exercises, free radical production is developed vigorously in these practices too.[16] Furthermore, exercise trainings can lead to overproduction of free radicals through some other pathways such as: energy charge deficit in skeletal muscles, elevated catecholamine secretion and oxidation, Nitric-oxide production, visceral ischemia, released Fe union from red blood cells, blood flow reduction and reflux in cells and etc.[17,18] Our body confronts with these free radicals by its antioxidant enzymes like Superoxide dismutase (SOD), Catalase and Glutathione-S-Transferase (GST). Suitable recovery after exercise sessions increases generation of these enzymes and therefore elevates body antioxidant capacity in athletes. But following high intensity exercise sessions which are repeated over and over, free radical production may overcome body antioxidant power and oxidative stress may occur.[19] This condition can impose some destructive effects on spermatogenesis process because testicular and sperm cell membrane are very vulnerable to attack by free radicals since they are rich in polyunsaturated fatty acids. Sperm motility can be affected by increased lipid peroxidation and altered membrane function. Also, epididymis is the site of sperm capacitation and maturation and also has an essential role in sperm motility. Oxidative stress results harmful effects in the Epididymis. Furthermore, high concentration of free radicals causes sperm DNA damage and reduces spermatogenesis quality.[20] However, results of current study showed that 4 weeks of intense aerobic exercise had no destructive effect on spermatogenesis quality in male rats. Our results do not match with some previous studies.[14,21-23] This contradiction is perhaps pertaining to duration of research and shows that body antioxidant system, at least in short term training programs, is probably able to frustrate harmful effects of free radical overproduction. If period of our study was longer, harmful effects might be created.

Existence of antioxidants like Selenium, vitamin C and alpha-tocoferol (vitamin E) in diet, enhances body antioxidant capacity. Vitamin E is a powerful antioxidant that because of its lipophilic characteristic can locate itself in the interior of cell membrane, react with fatty acid peroxyl radicals and terminate the chain reactions. Therefore it is able to alleviate wrecking functions of free radicals on male reproductive performance.[24] In the current study it was observed that testicular antioxidant capacity was elevated, testicle MDA level was decreased and simultaneously, spermatogenesis quality was improved significantly in ES group. These findings confirm beneficial effect of vitamin E supplementation on spermatogenesis process in male athletes and are supported by some previous studies.[25-27] However, it must be noted that free radicals also have some beneficial effects on male reproductive system. They have important roles in several processes like sperm maturation, Capacitation and the hyper activation. They also control Acrosome reaction and sperm-oocyte fusion.[28] Therefore dose of vitamin E or other antioxidants for supplementation should be selected accurately because over suppressing free radicals may weaken their advantageous efficacy.

CONCLUSIONS

Alpha-tocopherol supplementation is useful to attenuate destructive effects of free radicals on spermatogenesis process and improves male fertility potential in athletes who have intensive exercise programs.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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