

The role of Vitamin E – Selenium - Folic Acid Supplementation in Improving Sperm Parameters After Varicocele: A Randomized Clinical Trial

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Purpose: In this study, we aimed to evaluate the effects of antioxidants including Vitamin E-Selenium-Folic acid (Vit E -Se- FA) on semen parameters following varicocele (VCT).

Materials and Methods: Sixty patients from 64 infertile male patients diagnosed with varicocele (VC) who underwent sub-inguinal VCT were included in the study. Following sub-inguinal VCT, the patients were randomized into two groups: 30 receiving Vit E-Se-FA supplementation for six months, and 30 as the control group with supplemental treatment. The post-operative semen parameters of Vit E-Se-FA receiving group were compared with control group at the end of experiment. The sperm count, percentage of motile and abnormal sperms were considered.

Results: There were statistically significant differences in terms of count ($P = .031$) and motility ($P = .01$) of sperm after six months of receiving Vit E-Se-FA supplementation comparing with control group.

Conclusion: Vit E-Se-FA supplementation can improve sperm parameters (count and motility of sperm) after VCT.

Keywords: antioxidants; sperm parameters; infertility; varicocele

INTRODUCTION

Infertility, with a prevalence of 15%, is known as the inability to conceive after at least one year of regular and unprotected sexual intercourse, in which male related infertility is defined as unique or contributing factors in about half of the recorded cases.^(1,2) Several male factors of infertility have been recognized⁽³⁾ in which the exact cause of infertility could not be detected. Varicocele (VC) as a vascular defect in the internal spermatic vein is a most common cause of male infertility. This defect is a source of primary infertility in 35–50% of cases, as well as up to 81% of secondary infertility.^(4,5) Although, the pathophysiology behind VC-induced infertility has been extensively investigated, the mechanisms for VC related impaired spermatogenesis have not been well established.⁽⁶⁾ However, the important mechanisms are scrotal hyperthermia, oxidative stress (OS), hormonal disturbances, testicular hypoperfusion and renal and adrenal metabolites reflux.⁽⁷⁾ OS due to an excess of reactive oxygen species (ROS), is now recognized as a major factor in infertility.^(8,9) Over the last few years, the relation and a directly correlation between semen ROS levels and the grade of VC has been well recorded.^(7,10,11) Thus, removing the OS using different approaches may repair VC-related infertility. Multi-faceted therapeutic approach including nutrition-

al programs such as antioxidants in combination with operational procedures and assisted reproductive technology (ART) can be performed for management of infertility. Varicocele (VCT) as a main procedure for treatment of patients with VC is associated with various complications including testicular atrophy.⁽¹²⁾ Recent studies showed that surgical VCT reduced the seminal OS in infertile men.^(13,14) Even when seminal analysis do not improve after VCT, this surgical procedure enhances the rates of pregnancy and live birth following intrauterine insemination (IUI).⁽¹⁵⁾ Although, studies reported improvement of semen parameters after VC repair, there are still conflicting opinions as to whether a VCT improves antioxidant defences.^(16,17) While antioxidants has been indicated as an approach to increase the antioxidant capacity in the seminal plasma,⁽¹⁸⁻¹⁹⁾ their actual clinical value is unclear. Vitamin E (Vit E) as a fat-soluble antioxidant neutralizes free radicals and protects the cellular membrane.⁽²⁰⁾ Vit E suppressed the production of ROS in infertile male.⁽²¹⁾ As well, Selenium (Se) is known as an antioxidant and necessary trace element in biosynthesis of testosterone and generation of sperm.^(22,23) At least 25 selenoproteins have been detected which can help maintain cellular integrity of sperm.⁽²⁴⁾ Additionally, folic acid (FA), the synthetic form of folate, can effectively scavenge free radicals and has been introduced as a factor to reduce

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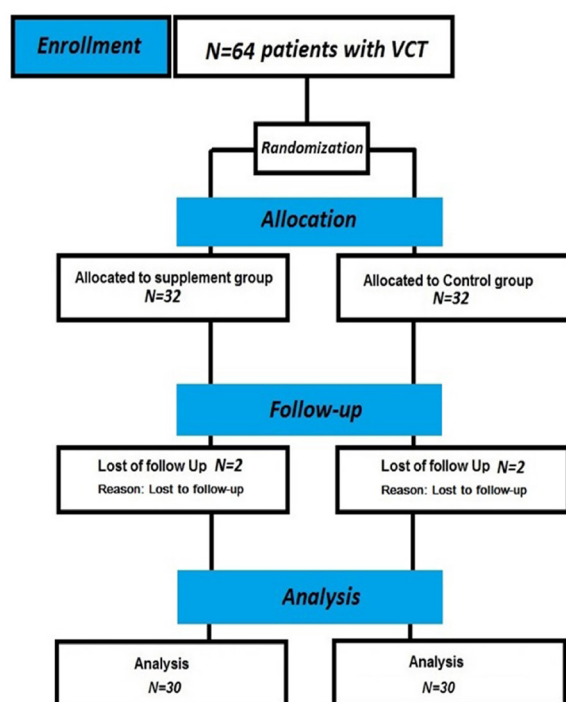


Figure 1. Flow chart of study randomization.

OS in the seminal plasma.⁽²⁵⁾ This antioxidant has been utilized in combination with zinc following VCT to improve semen parameters.⁽⁶⁾

Although, using antioxidants for sub-fertile patients was suggested in several studies, these approaches were rarely considered after VCT. To the best of our knowledge in this study, the combination of Vitamin E-Selenium-Folic acid (Vit E -Se- FA) was used for the first time to improve the semen parameters following VCT. The aim of the present study was to investigate the Vitamin E-Selenium-Folic acid (Vit E -Se- FA) supplementation with potential benefit on basic semen parameter results for management of male infertility in patients underwent VCT.

MATERIALS AND METHODS

Study Population

In a randomized, single blind clinical trial, between January 2015 and December 2017, 64 infertile patients with VC who underwent sub-inguinal VCT at Kosar Hospital, were included. This investigation was designed to study the influence of Vit E-Se-FA supplementation on fertility of subjects who underwent sub-inguinal VCT. VC was proven by physical examination in a warm room after applying the Valsalva maneuver in the standing position.⁽²⁶⁾ The abnormalities in sperm parameters including count, morphology and motility of sperm were evaluated in two separate semen analyses and patients with VC diagnosis and abnormal sperm parameters were planned for VCT. Exclusion criteria were usage of supplements, vitamins or alcohol, tobacco smoking, addiction to opium or using the opium during the follow-up period, diabetes mellitus, peptic ulcer history, hormonal disorders (based on clinical history and medical examination), chronic or active genitourinary infection (according to the history, med-

ical examination, urine and semen analysis) and previous reaction to FA, Se or Vit E. As well, patients with missed follow-up, incorrect usage of drugs, presenting side effects, and delayed complications of VCT including recurrent VC, hydrocele or testicular atrophy were excluded from the study. During the study, four patients (n=4) were excluded due to lost to follow-up and thus, the data of 60 patients were evaluated.

Ethics

The aim of research and the methods were explained for each patient. All subjects were aware of receiving VitE-Se-FA supplementation. Before entering to the study, the informed written consent form was signed by all subjects and it was explained that they can exit from study whenever they wanted. The code number of trial was IRCT2015091223855N2 and it was registered in Iranian Registry of Clinical Trials (IRCT) site (www.irct.ir).

Study design

After performing sub-inguinal VCT, the patients were allocated into two groups; supplementation group received daily oral FA (5 mg, Iran Daru Co.), Se (200 ug, Nature Made®) and Vit E (400 iu, Zahravi Co.) for six months, while the remaining 30 subjects with no supplemental treatment during same time as control group. The background, results of physical examination and semen analyses of the patients were investigated to confirm the fertility status.

Randomization

After obtaining informed consent, patients were assigned into two groups to receive supplements or nothing using random block design. In this study, permuted block randomization was used to allocate interventions in a completely random manner to the two treatment groups. Six blocks of 4 were defined. Structure of each block was four-way combination of two methods of intervention in a perfectly balanced way. Random digits table was used for random assignment of blocks to each group. Accordingly, a list was prepared. Eligible participants were enrolled in the study according to the list, respectively. Additional matching did not take place. Laboratory specialist and statistic consultant were blinded to treatment assignment (**Figure 1**).

Surgical procedure

In all patients, sub-inguinal VCT was performed under general or regional anesthesia using optical magnification (HEINE Cx2.3 binocular loop; Dusseldorf, Germany) to preserve arteries and lymphatic in the supine position. In each case, an approximately 2 to 3 cm incision was made. The spermatic cord was elevated and placed on a penrose drain. While preserving the arterial and lymphatic vessels, the dilated veins were ligated. In the follow-up period after surgery, semen analysis was done again.

Semen analysis

After 3 or 4 days of sexual abstinence, the semen was obtained from the patients via masturbation. Sperm count, motility and morphology were assessed at two points in time before the VCT and six months after the VCT. Semen samples were produced via masturbation into polypropylene containers. Within half an hour after sample collection, the samples were liquefied and analyzed according to World Health Organization (WHO) Manual for Semen Analysis (2010).⁽²⁷⁾ All laboratory analyses were performed by specialists blinded to study

Table 1. Comparing the pre-study and after treatment data based on groups of study in infertile patients who underwent varicocelectomy at Kosar Hospital.

Variables	Groups		P-value
	Supplement Mean $\pm$ SD	Control Mean $\pm$ SD	
Age (year)	30.27 $\pm$ 4.67	30.47 $\pm$ 6.09	0.772
Pre-study data			
I	5 (8.33)	1 (1.66)	0.171
II	14 (23.33)	14 (23.33)	
III	11 (18.33)	15 (25)	
Sperm count (10 ⁶ /ml)	35.92 $\pm$ 23.14	30.77 $\pm$ 22.14	0.069
Sperm motility (%)	46.45 $\pm$ 16.02	44.17 $\pm$ 16.89	0.056
Sperm morphology (%)	39.34 $\pm$ 18.97	40.69 $\pm$ 17.43	0.340
After treatment data			
Sperm count (10 ⁶ /ml)	41.26 $\pm$ 24.52	35.83 $\pm$ 23.21	0.031
Sperm motility (%)	50.29 $\pm$ 15.14	46.40 $\pm$ 16.51	0.01
Sperm morphology (%)	43.15 $\pm$ 12.56	41.59 $\pm$ 12.55	0.315

Abbreviations: SD: standard deviation; N: number

protocol. Sperm parameters were defined as below:
Sperm count: The number of sperms in 1 ml of semen, determined using hemocytometer method.

Sperm motility: The percentage of progressive sperms in examined samples, determined using a light microscope.

Sperm Morphology: The percentage of normal featured sperms in examined samples determined using a light microscope.

Statistical analysis

Data analyzed using SPSS-22 software. Numeric variables were summarized using mean $\pm$ standard deviation (SD). The distribution of the data was evaluated using the Kolmogorov-Smirnov test. Comparisons were performed using parametric test of Student's *t*-test or non-parametric test of Mann-Whitney. *P*-value less than 0.05 was considered as significant level.

RESULTS

In this study, 60/64 infertile patients who undergone VCT at Kosar Hospital, affiliated to Semnan University of Medical Sciences, were enrolled to this study from January 2015 to December 2017. Patients were randomly evaluated in two supplement (*n* = 30) and control groups (*n* = 30). In **Table 1**, the pre-study data related to basic variables of patients (age, semen analysis and VC grade) were summarized based on control and supplementation groups. According to Table 1, there were not statistically significant differences in the pre-study data of two groups, which showed equal characteristics

of the two groups and adequacy of randomization. According to **Table 1**, there were statistically significant differences in the sperm count (*P* = .031) and motility (*P* = .01) of patients in two groups of study after treatment with supplementation. As well, the pre-op and post-op data based on groups of study were compared and showed that there were no significant differences in the pre and post-op data of control group for sperm count (*P* = .084), motility (*P* = .091) and morphology (*P* = .441)(**Table 2**). However, significant differences were recorded for sperm count (*P* = .021) and motility (*P* = .003) of pre and post-op data in supplement group.

DISCUSSION

In this study, we evaluated the effects of antioxidants including Vit E-Se-FA on semen parameters six months following VCT. Although, VC has been suggested to be associated with infertility, its pathogenesis is not completely understood, yet. Many theories explained that developing VC is associated with disrupted venous stasis in testes at the presence of impaired venous valves and disrupted hydrostatic pressure difference in the testis at puberty which enhances the venous capacity, resulting in venous varicose.^(28,29) Consequently, increasing in the venous pressure and reducing in arterial blood flow resulting in hypoxia which leads to ROS distribution. Recently, it has been recommended that infertility caused by VC had an association with the presence of OS.^(30,31)

Failing to restore fertility in patients after VCT brings

Table 2. Comparing the pre-op and post-op data based on groups of study in infertile patients who underwent varicocelectomy at Kosar Hospital.

Variables	Groups	
	Supplement (Mean $\pm$ SD)	Control (Mean $\pm$ SD)
Pre-op Sperm count (10 ⁶ /ml)	35.92 $\pm$ 23.14	30.77 $\pm$ 22.14
Post-op Sperm count (10 ⁶ /ml)	41.26 $\pm$ 24.52	35.83 $\pm$ 23.21
P-value	0.021	0.084
Pre-op Sperm motility (%)	46.45 $\pm$ 16.02	44.17 $\pm$ 16.89
Post-op Sperm motility (%)	50.29 $\pm$ 15.14	46.40 $\pm$ 16.51
P-value	0.003	0.091
Pre-op Sperm morphology (%)	39.34 $\pm$ 18.97	40.69 $\pm$ 17.43
Post-op Sperm morphology (%)	43.15 $\pm$ 12.56	41.59 $\pm$ 12.55
P-value	0.195	0.441

Abbreviations: SD: Standard deviation

up the question of the necessity of a supplemental treatment. For improving the sperm characteristics, Wirleitner et al. (2012) demonstrated that antioxidants, with no adverse impacts, might be taken by sub-fertile subjects⁽³²⁾. However, Raigani et al. (2014) showed no effect of micronutrient supplements, zinc sulphate and FA, on sperm characteristics, despite their antioxidant effects.⁽³³⁾ Based on the results of present study, using Vit E-Se-FA supplements for six month could improve sperm count and motility. Although, application of antioxidants for sub-fertile patients was suggested in several studies, using these antioxidants after VCT were rarely considered according to the literature. As well, there were no reports to show the combined using of Vit E-Se-FA antioxidants after VCT. Nematollahi-Mahani et al. (2014) conducted a study to evaluate the impacts of FA and zinc sulphate on seminal antioxidant level and endocrine parameters three and six months after surgical repair of varicocele. Their results showed a significant increase in peripheral blood inhibin B and improvement in superoxide dismutase (SOD) activity in the zinc sulphate/FA group after six months.⁽⁶⁾ In a similar study, Azizollahi et al. (2013) investigated the effects of zinc sulfate, FA and zinc sulfate/FA on protamine content, acrosomal integrity and sperm quality following VCT. They demonstrated that administration of FA increased sperm count. Zinc sulfate (ZS) improved the sperm morphology. In both ZS and FA groups, protamine content and halo formation rate significantly enhanced.⁽³⁴⁾ In a placebo-controlled and double-blind trial, Lu et al. (2018) indicated that administration of melatonin as a strong antioxidant three month after varicocelelectomy added extra benefit by improving sperm parameters, total antioxidant capacity and hormonal profile.⁽³⁵⁾ In our study, both sperm count and sperm motility were improved six month after receiving supplementation following VCT. These findings showed that the agents with antioxidant capacity can improve the sperm parameters by reducing the oxidative stress and regulating hormone production. Vit E-Se-FA antioxidants can help to improve the efficiency of sperms by removing the ROS from the environment. It has been shown that Vit E alone and in combination with other antioxidants has positive impacts on sperm fertility and testis. Studies demonstrated that the supplemental prescriptions containing Vit E alone can repair the functions of spermatozoa by reducing OS impairment.^(36,37) No similar studies have done to show the beneficial effects of Vit E following VCT. However, Moslemi et al. (2011) evaluated 690 infertile men with idiopathic asthenoteratospermia who received supplement of Vit E (400 IU/daily) in combination with Se (200 µg/daily) for at least 100 days and reported 362 cases (52.6%) with total improvement in sperm morphology and/or motility as well, 75 cases (10.8%) with spontaneous pregnancy comparing with control group.⁽³⁸⁾ Gerco et al. (2005) conducted a study to evaluate the effects of a combination of Vit E (1 gr) and Vit C (1 gr) on infertile men and demonstrated that the level of DNA damage was reduced after two months.⁽³⁹⁾ As well, Mortazavi et al. (2014) suggested that supplementation including astaxanthin, Vit A and Vit E reduced cholesterol levels and serum triglyceride, and improved the semen parameters in obese cases with infertility or sub-fertility.⁽⁴⁰⁾ In contrast with studies that show beneficial effects of

Vit E analogues on sperm parameters, it has been presented that Vit E and Vit C have toxic impacts and may act as a pro-oxidant instead of an antioxidant, specially when utilized in high doses. In a study evaluating the results of antioxidant supplementation on DNA of human sperm integrity during preparation of Percoll, Hughes et al. (1998) showed that the combination of acetyl cysteine or ascorbate and alpha tocopherol induced further DNA damage.⁽⁴¹⁾ The safe dosages of 100 or 300 mg/daily Vit E are recommended, and the dose can be safely raised up to 1000 mg/daily when it is essential. Therefore, further investigations are needed to determine the optimal Vit E dose in infertile men. In this study, we used 400 IU/daily dose of Vit E.

To show the impacts of Se on semen parameters, Placebo-controlled clinical trial were carried out in Iran and Tunisia. Their results demonstrated that Se supplementation improved sperm counts, motility and morphology as well as sperm concentration in infertile men.^(42,43) Safarinejad et al. (2009) investigated the combined effects of Se and N-acetyl-cysteine on 468 infertile men with idiopathic oligo-asthenoteratospermia in a 30 weeks treatment period. In response to therapy, the levels of Inhibin B and serum testosterone enhanced, but serum follicle-stimulating hormone reduced. Furthermore, all semen parameters improved with Se and N-acetyl-cysteine treatment.⁽⁴²⁾

In the previous studies, FA is also used as adjuvant therapies to enhance the sperm parameters in combination with other antioxidants.^(6,34) Azizollahi et al. (2013) randomized 112 infertile patients after VCT used to repair VC with clinical grade III into four groups including 32 receiving ZS (66 mg/daily) alone, 26 receiving FA (5 mg /daily) alone, 29 receiving ZS (66 mg /daily)+ FA (5 mg /daily), and 25 receiving placebo for six months starting immediately after surgery. Patients receiving ZS therapy showed improvement in only sperm morphology while those receiving FA showed improvement only in sperm concentration. Patients receiving combination therapy showed improved sperm concentration, morphology, and motility at the end of six-month treatment. Furthermore, increasing the blood Inhibin B levels was reported after combination therapy. Improvement in seminal SOD activity was recorded in both receiving zinc alone and combination therapy.⁽³⁷⁾ This study was conducted in a limited population. So, we recommended to perform the present study in a large scale population.

CONCLUSIONS

In conclusion, Vit E-Se-FA supplementation could improve the sperm parameters including sperm count and motility after VCT; however, further studies including larger number of samples are needed to make a proper decision on Vit E-Se-FA supplementation after VCT.

CONFLICT ON INTEREST

Authors declared no conflict of interest.

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