Effects of Selenium on Various Sperm Parameters in Varicocele Rats

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Abstract

Aim: Varicocele is an abnormal enlargement of the pampiniform venous plexus and blood blockage in the spermatic cord. Among major effects on male fertility is oxidative stress and metabolites reflex to testicles, which in turn have a significant part in producing free radicals (reactive oxygen species =ROS). As a micronutrient, selenium has an antioxidant characteristic to normal spermatogenesis and combats oxidative stress. The present study aimed at examining selenium effects, as an antioxidant element) on sperm parameters (sperm count, motility, morphology) in varicocele rats.

Methods: This study was a case-control. It was done in 2017 in the Qazvin University of Medical Sciences on 24 male Rats that were divided randomly to four 6-subject groups of control, sham, varicocele, and treated varicocele. A daily 0.2-mg selenium diet was injected intraperitoneally to the treated varicocele group. After four weeks, sperm indices were examined among all the groups and the data was analyzed by one-way ANOVA.

Results: All the indices in this study (sperm count, motility and morphology) had a significant decline in the varicocele group compared to the control group ($p<0.05$). Results also indicated an effect of selenium as an antioxidant on the number of sperm as well as their motility. This led to improvement of these parameters having no effects on sperm morphology among varicocele rats.

Conclusion: Results showed that, as an antioxidant, selenium eliminated free radicals in varicocele rats indicating its effectiveness on varicocele-dependent infertility.

Key words: varicocele, spermatozoa, sperm count, sperm motility, sperm morphology, Rat

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Introduction

Infertility is a prevailing problem among couples who have over a one-year struggle to have a child. About 50% of infertility relates to male factors [1]. Male infertility may have various causes among which are genetic mutations, changes in chromosome counts or morphology, infectious diseases, varicocele, chemotherapy, radiotherapy, ejaculation disorders. Varicocele is paid much attention as a cause of infertility [2-5]. Varicocele is an abnormal enlargement, as well as blood blockage, in the plexus of vein (pampiniform) within the spermatic cord that drains the blood through testicles. Its occurrence rate among fertile men is 15-20%; 30-50% among primary infertile men; and 50-80% among infertile men. The mechanism involving varicocele leading to male infertility has not been identified yet. However, theories have identified factors like reflection of poisonous metabolites from kidneys or adrenals, rise in testicle temperature, testicular hypoxia resulting from venous stasis and malfunction of hypothalamus or hypophysis in varicocele. Recent studies report that the main influencing factors on infertility include oxidative stress and reflection of metabolites to testicles. In this regard, ROS plays a major part [6]. Oxidative stress is a result of imbalance in production of ROS and antioxidants defence resulting in damage to DNA integrity in sperm nucleus, leading to many changes in parameters, especially sperm motility, within 90% of cases and decline in sperm count parameter up to 20 million in 60% of cases. ROS production is a physiologic process, which can cause sex cells' death, dysfunction of Sertoli–Leydig cells and spermatogenesis disorder. High levels of ROS in semen was reported in 80% of varicocele patients [7-8]. Selenium acts as a micronutrient and is required in mammals' diets like all other vital elements such as zinc, taking a crucial part in normal sperm spermatogenesis to protect sperm against oxidative substances. Protective effects of selenium are shown through building structures called selenoproteins that are part of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase that are important for cellular health protection against oxidants and oxidative stressors like ROS. Recent studies show that defects in selenium production can lead to smaller testicles and, in the long run, to sperm impairment through spermatogenesis and sperm maturity processes [9-11]. Because expression of selenium (as an oxidant) is of great importance for normal spermatogenesis in sperm, and to defend against oxidative stress, the present study was initiated with the aim of investigating selenium effects on various sperm parameters in varicocele rats.

Materials and Method

This was a case-control study done in Qazvin University of Medical Sciences in 2017 according to the moral code of IR.QUMS.REC.1394.213. The sample of the study consisted of 22 mature male rats or mice with 250-350 gram weight. The rats were kept in 12 hours of light and 12 hours of darkness and 20°C temperature, then divided randomly to four 6-rat groups. These groups included the control group (with no surgery), the Sham group (varicocele was induced on them without any renal vein blockage), varicocele group, treated varicocele group (underwent surgery to induce varicocele with a daily selenium diet of 0.2mg during 8 weeks based on Koksal et al method) [12]. Eight weeks after the surgery, rats from varicocele, sham and control groups were killed by chloroform. In order to examine the sperm indices (count, motility, morphology), testicular fascia was torn by sterile scissors. After observing the left epididymis (the coiled tube) its tail was cut to be kept in Hams_F10 medium. Epididymis was divided into various parts and placed in the incubator for 15 minutes in order for the sperm to be released from the testicles into the petri dish.

To count the sperm, a Neobar light was employed. There were two sections for counting. Each section was a large 3×3mm box fragmented to 9 smaller curved 1×1mm boxes. The Neobar lam was covered with a special lamel which produced a 0.1mm when put on the lam. The semen was diluted with 1:2 proportion and all the squares (1 to 9) were counted. Only the sperm with head and tail (a complete sperm) were counted [13]. The border of each of the 9 boxes had three lines. If sperm head was located inside the box or on its internal margin, the sperm was counted, but if it was on the two exterior lines, it was not counted. To identify the percent of motile sperm, a droplet of the sample was placed on the lam. This parameter was examined by a 40-magnification microscope based on WHO criterion [13]. To determine the morphology of sperm, Papanicolaou staining was used. In this type of staining, the acrosome part of a sperm turned light blue and it was dark blue in the rear part of the acrosome. The neck was a little reddish, the tail blue and the cytoplasmic parts around the neck were pink. After that, using a microscope with 400×magnification, at least 200 sperm were examined [13]. In order to evaluate the viability of sperm, eosin-nigrosine staining procedure was employed, which is based on solubility level of eosin-nigrosine to cell membrane of the damaged cells as well as on insolubility of the colour in healthy cells [13]. A dead sperm is of red or pink colour; the living ones are light pink to white. If the neck is pink but the remainder of head doesn't absorb colour, this sperm is alive too showing that the membrane is permeable in neck. After staining, 100 sperm were evaluated from each sample, by CX31 microscope with 100×magnification. SPSS 16 and two-way ANOVA were employed to analyze the data.

Result

All sperm parameters (motility, normal morphology, the number of viable sperm) had a significant decrease in the varicocele group compared to the control group and selenium diet improved these parameters (see Table 1).

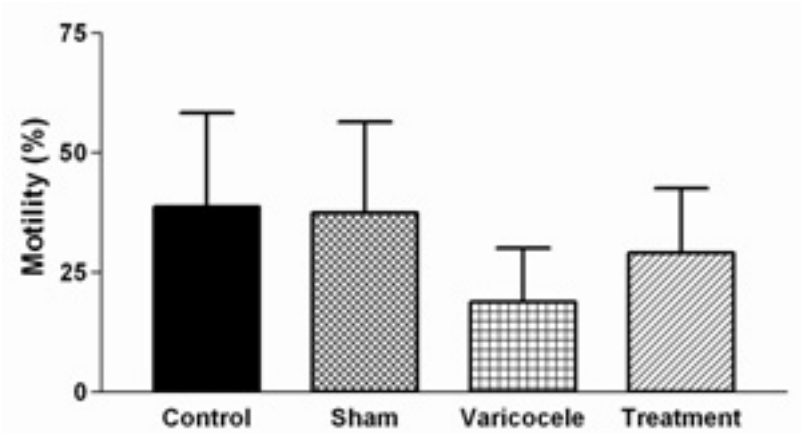
The percentage of sperm with good motility was significantly higher in the control, sham and treated groups than the varicocele group ($p<0.05$). The percentage of sperm with good motility was significantly different in the treated group from that of the varicocele group ($p<0.05$) (Diagram 1).

Table 1: Mean of sperm parameters among the different groups

Group / Sperm Parameters	Control	Sham	Varicocele	Treated
Mobility (%)	33.2±4.5	29.8±3.2	24.2±5.2	**27.1±2.5
Count	145±3.2	144±4.2	75±3.1	**129±4.1
Normal Morphology	44±1.2	41.3±3.2	37±1.8	40.1±3.1
Dead Sperm (%)	16.1±4.2	15.8±3.2	29.8±1.8	**20.1±2.2

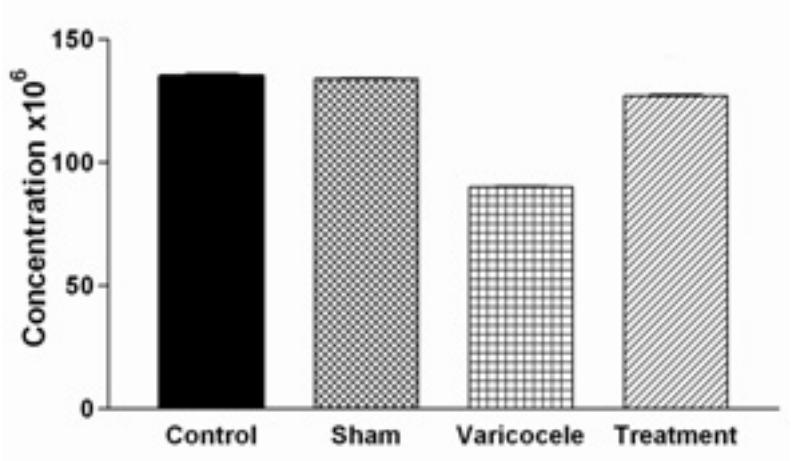
** significant difference between the varicocele and the treated groups

Diagram 1. Comparing the mobility of sperm among the mentioned groups



The number of sperm was significantly higher in sham, control and treated groups than the varicocele group ($p<0.05$). On the other hand, sperm count of the treated group had a significant increase compared to the varicocele group ($p<0.05$) indicating the positive effect of selenium on the sperm count in varicocele rats (Diagram 2).

Diagram 2. Comparison of sperm counts among the groups



The percent of sperm with normal morphology in sham, control, and treated groups was significantly higher than the varicocele group ($p<0.05$); however, in the treated group, no significant difference was found between the treated group and the varicocele regarding normal morphology ($p>0.05$) showing that selenium had no effects on sperm morphology (Diagram 3 - next page).

Percent of dead sperm in sham, control, and treated groups was significantly lower than the varicocele group ($p<0.05$) but this parameter declined significantly in the treated group compared to the varicocele group ($p<0.0$(, showing a positive effect of selenium on the mentioned parameter in varicocele rats' sperm (Diagram 4 - next page).

Diagram 3. Normal morphology of sperm among different groups of the study

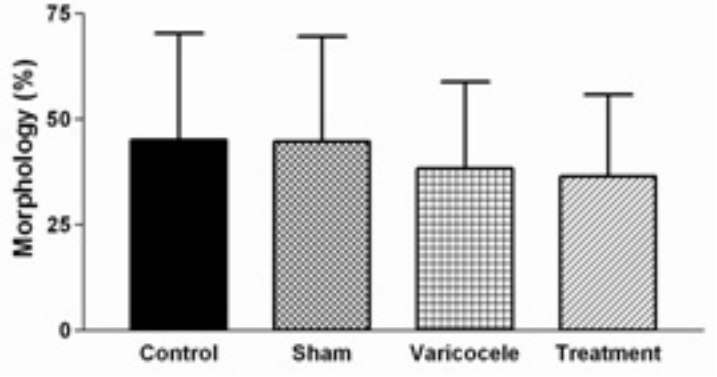
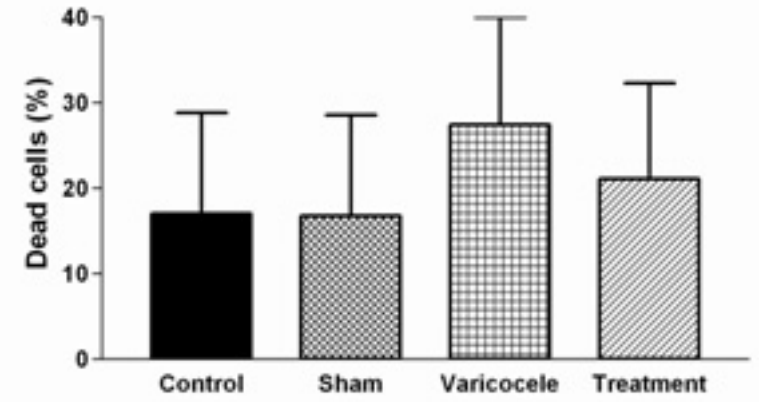


Diagram 3. Normal morphology of sperm among different groups of the study



Discussion

It was reported in this study that selenium could improve the parameters of sperm count and the percent of dead cells in varicocele rats. A study by Camejo et al on infertile men reported that a decline in selenium in semen significantly decreased sperm count, motility as well as good morphology [14]. Another study (Molina et al) reported that lower levels of selenium would lead to higher levels of oxidative stress in testicles. They also showed that a diet of 0.1 – 0.3 selenium per one kilo of antioxidant is required for a good production of sperm in animals. This study was consistent with the present study regarding the effect of antioxidants on male infertility resulting from varicocele [15]. It was reported in a study (2016) that selenium, along with carnitine, improved sperm parameters like motility, count, viability and normal morphology [16]. Sobhani et al (2015) examined the effects of antioxidants on sperm parameters and found a significant difference in sperm morphology between the control group and the case group, which was inconsistent with the present work [17]. Amidi et al reported that saffron had a significant positive effect on sperm morphology. Unlike Amidi et al, in the present study, selenium improved sperm morphology but no significant difference was found [18]. This study found significant higher sperm counts in the treated group than in the varicocele group. Testai et al (2016) investigated the relationship between oxidative stress and damage to mitochondrial membrane which reported that, because internal mitochondrial membrane contain cytochrome c, mitochondrial collapse by oxidative stress would result in cytochrome release from mitochondrial membrane, which in turn leads to cell death. This death might lead to exacerbate negative effects of the stressors' activities like collapse of DNA [19]. Based on this study, significant decline in sperm counts in varicocele group compared to other groups is justified. Mostafaeroglue et al conducted a study on men suffering from varicocele. They showed that selenium expression improved sperm parameters including sperm counts, which was consistent with the present study, although inconsistent regarding the positive effect of selenium on normal morphology and mobility of sperm [20]. Seminal leucocytes and abnormal sperm are the main sources of free radicals in infertile people. Protective factors like antioxidants, act as a treatment in infertile men. Investigating creation of oxidative stress by ROS production, Alaa Hamada et al concluded that, as an antioxidant, selenium could control oxidative tension in sperm of infertile people [21].

Conclusion

Generally speaking, selenium is an antioxidant that can have a positive effect on sperm counts and viability in varicocele rats. Further study is needed to better demonstrate effects of antioxidants like selenium because oxidative stress involves different parts of the body.

References

1. O'Brien KLF, Varghese AC, Agarwal A.The genetic causes of male factor infertility: a review. Fertility and sterility 2010;93(1): 1-12.
2. Foresta C, Moro E, Ferlin A.Y Chromosome microdeletions and alterations of spermatogenesis. Endocrine reviews 2001;22(2):. 226-239.
3. Dowsing AT. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. The Lancet 1999;354(9179): 640-643.
4. Bashamboo A. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor. The American Journal of Human Genetics 2010;87(4): 505-512.
5. Foresta C. Role of hormones, genes, and environment in human cryptorchidism. Endocrine reviews 2008;29(5): 560-580.
6. Chen, Shiou-Sheng; Chen, Li-Kuei. "Risk Factors for Progressive Deterioration of Semen Quality in Patients with Varicocele". Urology 2012; 79 (1): 128–32
7. Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. Etiologies of sperm oxidative stress. Int J Reprod Biomed (Yazd) 2016;14(4):231-40.
8. Azenabor A, Ekun AO, Akinloye O. Impact of Inflammation on Male Reproductive Tract. J Reprod Infertil. 2015;16(3):123-9.
9. Brown DG, Burk RF. Selenium retention in tissues and sperm of rats fed a Torula yeast diet. J Nutr 1973; 103 (1): 102-8
10. Kehr S, Malinouski M, Finney L, et al. X-ray fluorescence microscopy reveals the role of selenium in spermatogenesis. J Mol Biol 2009 26; 389 (5): 808-18
11. Chavarro JE, Toth TL, Wright DL, et al. Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. Fertil Steril 2010 1; 93 (7): 2222-31.
12. Lovercamp KW, Stewart KR, LinX, Flowers WL. Effect of dietary selenium on boar sperm quality. Anim Reprod Sci 2013; 138 (3-4): 268-75.
13. Heidary M, Vahhabi S, Reza Nejadi J, et al. Effect of saffron on semen parameters of infertile men. Urol J 2008; 5 (4): 255-9.Biol Trace Elem Res. doi: 10.1007/s12011-011-8957-5. Epub 2011 Jan 15.
14. Camejo MI, Abdala L, Vivas-Acevedo G, Lozano-Hernández R, Angeli-Greaves M, Greaves ED. Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. Biol Trace Elem Res 2011;143(3):1247-54.
15. Molina RI, Martini AC, Tissera A, et al. Semen quality and aging: analysis of 9.168 samples in Cordoba. Argentina. Arch Esp Urol 2010; 63 (3): 214-22
16. Pajovic B1, Dimitrovski A, Radojevic N, Vukovic M. A correlation between selenium and carnitine levels with hypo-osmotic swelling test for sperm membrane in low-grade varicocele patients. Eur Rev Med Pharmacol Sci. 2016;20(4):598-604.
17. Sobhani A, et al. Antioxidant Effects of Brown Algae Sargassum on Sperm Parameters: CONSORT-Compliant Article. Medicine 2015; 94(52): 1938.
18. F. Amidi S, Ebrahimi M, Abbasi M, Yazdani S, Ghasemi A. Effects of saffron extract on sperm parameters in rats with experimentally induced varicocele. JQUMS 2014;18(5): 4-11.
19. Testai L, Marino A, Piano I, Brancaleone V, Tomita K, Di Cesare Mannelli L, Martelli A, Citi V, Breschi MC, Levi R, Gargini C, Bucci M, Cirino G, Ghelardini C, Calderone V. The novel H₂S-donor 4-carboxyphenyl isothiocyanate promotes cardioprotective effects against ischemia/reperfusion injury through activation of mitoK_{ATP} channels and reduction of oxidative stress. Pharmacol Res. 2016 9;113(Pt A):290-299.
20. Mustafa Eroglu,Sadik Sahin, Birol Durukan, Ozlem Bingol Ozakpinar, Nese Erdinc, Lale Turkgeldi, Kenan Sofuoglu, Ates Karateke. Blood Serum and Seminal Plasma Selenium, Total Antioxidant Capacity and Coenzyme Q10 Levels in Relation to Semen Parameters in Men with Idiopathic Infertility. Biological Trace Element Research 2014 159;(1): 46–51.
21. Hamada A, Sandro C, Nizza M, Agarwal A. Unexplained Male infertility: diagnosis and Management. Int braz j urol. 201238(5) 35-41.