Multidisciplinary

Investigation of the role of selenium on some fertility and sex hormones in infertile men

Ahmed Sadeq Habeeb AL-Adban[1*], Muthanna Shareef Tahibul Alradhi[2] and Jabbar Hamed Fezza[1]

[1] Department of Chemistry, Graduate School of Natural and Applied Sciences, Cankiri Karatekin University, Cankiri, Turkey

[2] Department of Biology, Graduate School of Natural and Applied Sciences, Çankiri Karatekin University, Cankiri, Turkey

[3] Department of Biology, University of Thi-Qar, Nasiriyah, Iraq

Correspondence Author: Ahmed Sadeq Habeeb AL-Adban

Received 5 March 2024; Accepted 12 April 2024; Published 22 Apr 2024

Abstract

Infertility continues to be one of the biggest problems of human history, and the desire of humans to exist in future generations is due to emotional reasons. In the last 50 years, about 10-15% of couples have infertility.

The male factor is effective in 30-40% of all infertile couples, and the female factor is effective in 40-50%. In 15% of the couples, no cause of infertility can be determined as a result of all diagnostic tests, and it has been defined as unexplained infertility.

Based on these considerations, it was aimed to investigate the element levels important in male fertilization and to determine the relationship between spermiogram parameters and trace element (se) levels in infertile men, and serum and semen anti-Müllerian, Follicle stimulating hormone (FSH), luteinizing hormone in fertile and infertile men. (LH) testosterone was evaluated as spermatogenesis markers.

Our study was carried out in Al-Hussein Education and Research Hospital in Thi-Qar Province. 100 infertile male patients were included in our study by forming an experimental group, taking into account various criteria. For hormone analysis, all blood was taken in patients on an empty stomach in the morning. Folliculstimulant hormone (FSH), LH, and total testosterone determinations were made. Being over 18 years old. - - Not using any medication that will affect the study results, not having any other disease. - Not receiving cancer treatment, chemotherapy and radiotherapy treatment. - 96 hours of sexual abstinence prior to sample collection. Semen analysis was performed according to the analysis criteria specified by WHO.

FSH, LH, and testosterone levels were measured in serum and semen of all cases. Serum and seminal plasma levels of anti-Müllerian hormone were found to be significantly lower in patients with azoospermia compared to controls. Decreased blood plasma anti-Müllerian hormone levels were positively correlated with seminal plasma testosterone levels.

Selenium level is significantly lower in oligozoospermic individuals (66.51 ± 10.26 µg / L) compared to normozoospermic (296.49 36.088 µg / L) individuals. In conclusion, the concentrations of trace elements known to have roles in fertilization in seminal plasma indicate that they have toxic effects and effects on various biological pathways that cause infertility. The fact that the excess of the elements can be an indicator of infertilization is another finding that can be said in line with the relationship of these elements with the spermiogram results. It is thought that the obtained data will contribute to further studies.

Keywords: Male infertility, Selenium, Treatment, Sperm Count, Sperm Morphology, FSH, LH, Testosteron

1. Introduction

Up to 15 percent of couples are infertile. This means that despite having frequent unprotected sex for a year or more, they are unable to conceive a child. Male infertility plays a role in more than a third of these couples. Male infertility is caused by low sperm production, abnormal sperm function, or blockages that prevent sperm from being delivered. Diseases, injuries, chronic health problems, lifestyle choices, and other factors can play a role in causing male infertility. Infertility is defined clinically in men and women who cannot conceive after 1 year of sexual intercourse without using birth control, and in women who have had two or more unsuccessful pregnancies. Studies show that after 1 year of unprotected sex, 15% of couples are unable to conceive, and after 2 years 10% of couples still do not have a successful pregnancy. In generally healthy couples younger than 30 years of age, 20% to 37% can get pregnant in the first 3 months(Alizadeh *et al*. 2018) [2]. Many different medical conditions and other factors can contribute to fertility problems, and an individual case may have a single cause, several causes, or - in some cases - no identifiable cause. Overall, a third of infertility cases are due to male reproductive issues, a third to female reproductive issues, and a third to both male and female reproductive issues or unknown factors. To conceive a child, a man's sperm must combine with a woman's egg. The testicles produce and store sperm ejaculated by the penis to deliver sperm to the female reproductive tract during intercourse. The most common problems that lead to male infertility are those that affect the way the testicles work. Other problems are hormone imbalances or blockages in the male reproductive organs. In about 50% of cases, the cause of male infertility cannot be determined. About 10% to 15% of men who are infertile experience a complete lack of sperm. Hormone imbalance or blockage of sperm movement can cause sperm deficiency. In some cases of infertility, a man produces less sperm than normal. The most common cause of this condition is varicocele (pronounced VAR-i-koh-seel), which is an enlarged vein in the testis. Varicocele is present in approximately 40% of men with infertility problems (Abid *et al*. 2008) [1].

Prolactin (PRL) is important because of its affinity for luteinizing hormone (LH) receptors in Leydig cells in the testis and indirectly affects testosterone production (Abid *et al*. 2008) [1]. However, the effect of PRL on spermatogenesis and sperm functions has not been fully determined yet. While many studies have shown a positive relationship between PRL and semen characteristics, some studies have failed to show such a relationship. Selenium: It has no effect on semen parameters alone, but it has been reported that it provides significant improvement in semen parameters (motility and concentration) in cases used with N-acetyl Cysteine (Sigman *et al*. 2006, Iwanier and Zachara 1995) [16]. In the study of Keskes Ammar *et al*, it was reported that with the use of Vit E (400 mg/g) + Selenium (225 µg/day), MDA concentration decreased and sperm motility increased significantly (Keskes Ammar *et al*. 2003) [10].

The aim of this study is to examine the role of selenium on some fertility and sex hormones in infertile men.

2. Materials and methods

2.1 Selection of patients

Our study was carried out at Al-Hussein Training and Research Hospital in the Tikar Province of Iraq. 100 infertile male patients were included in our study by forming an experimental group considering various criteria. These criteria are:

- be over 18 years old.
- To have applied to the Urology Polyclinic of Al-Hussein Training and Research Hospital and to have been diagnosed with azoospermia and/or oligospermia by the clinic.
- Not to use any medication that will affect the study results, not to have any other disease.
- Cancer treatment, - Not receiving chemotherapy and radiotherapy treatment.
- To practice sexual abstinence for 96 hours before sample collection.

The control group created for our study consists of 60 healthy men. A number of criteria were also taken into account in the formation of the control group. These criteria are:

- be over 18 years old.
- Not having been diagnosed with azoospermia or oligospermia in the control examinations performed by the Urology Polyclinic of Al-Hussein Training and Research Hospital.
- Not using any medication or having a different disease that may affect the study.
- Cancer treatment, - Not receiving chemotherapy and radiotherapy treatment.
- To practice sexual abstinence for 96 hours before sample collection.

At least two semen analyzes were performed for each patient, and the spermiogram was not repeated because there was no significant difference between the two analyses. Participants and patients were selected from those who had not supplemented with folic acid, glutathione, vitamins E and C, selenium, and zinc for the past three months. Each patient and each participant was subjected to spermiogram after 96 hours of sexual abstinence. Spermiograms were evaluated by the same laboratory team in the biochemistry laboratory of Al-Husseyin Training and Research Hospital.

Semen analysis was performed according to the analysis criteria specified by WHO (Tórtora-Pérez 2010). Semen characteristics were determined by taking the averages (sperm count, morphology and motility). Patients with azoospermic, hypogonadotropic and systemic diseases such as diabetes were excluded from this study, since the etiology may be hypergonadotropic hypogonadism (primary testicular failure), obstruction or hypogonadotropic hypogonadism in azoospermic patients, and PRL is not of primary importance in hypogonadotropic patients.

2.2 Materials used

All blood samples for hormone analysis were taken in the morning on an empty stomach. Follicle stimulating hormone (FSH), LH, PRL and total testosterone determinations were made. The tools and materials to be used for these analyzes are as follows:

- Deep freeze (PLATILAB, 550 H-RE, LOT. LS07835, England),
- Centrifuge (KOKUSAN H-19F, LOT. 139113, Japonya),
- ELISA plate reader (ELX800, BioTek Instruments, USA),
- ELISA plate Washer (ELX50, BioTek Instruments, USA),
- MDA ELISA kiti, Biosource, (Biosource, ABD), Cat.No: MBS2548471.
- Pure Water Device (Raypa, Model: 700 700, SN: 840.110.110.784, European union),
- Automatic pipettes (Eppendorf, Hamburg, Germany),
- Refrigerator: temperature 4 – 8 C˚, Royal, Model: BC-ROY200, CHINA),
- Incubator (HERAEUS, LOT. 26.061.010, Germany),
- Microscope (NOVEL, XSZ-N107, Lot No: 003375)
- Testo ELISA kiti; Bioactiva diagnostica Testesterone EASIA Kit (DIAsource
- ImmunuAssays S.A. Belgium; Catalog No:BDTT37-BA, bioactive diagnostica,USA).
- FSH ELISA kiti; Accu-Bind FSH ELISA kiti (Follicle Stimulating Hormone) Kit; CatNo: 425-300 Monobind Inc. USA.
- LH ELISA kiti; Accu-Bind LH ELISA kiti (Follicle Stimulating Hormone) Document No: 0640; Ürün Kodu: 625-300 Monobind Inc. USA.
- SELENIUM, Selenyum Tablet, 200 mcg, SOLGAR,
- AAS Spectrophotometer Device, TERMOFISHER, USA, Model No:ICE 3300.

Fig 1: ELISA device

2.3 Experiment

The experiments within the scope of our study were carried out between 19/1/2020 - 29/3/2020. Before the semen analysis, face-to-face interviews were conducted with the patients and information was obtained about their medical history, family medical history, socioeconomic status, smoking, alcohol and drug use. It was determined that none of the patients had a factor that would affect the study results.

Within the scope of our study, 100 infertile men whose semen samples were taken at the beginning were treated with capsules containing 200 mcg Selenium, once a day, until the last semen sample collection. MDA was determined by calorimetric reaction with TBA, which gave a pink color, which could be determined by spectrophotometer in the Biochemistry Laboratory of Al-Hussein Hospital. These reaction conditions are 90 – 100 oC and pH = 2-3 for 15 minutes (El Kissi *et al*. 2013) [5]. Semen (GSH) was determined by the method described by Tietz (1999). At this stage, the Elleman procedure was applied. Hormonal analyzes were performed using the ELISA method. In addition, zinc and magnesium concentrations in semen were measured by atomic absorption spectrophotometer (Safarinejad 2012) [15].

2.4 Statistical analysis

Windows SPSS 21.0 program was used for statistical analysis. Statistical significance between the groups was carried out with student-t test, and correlations between parameters were investigated with Spearman rank correlation analysis. It was also evaluated as $p<0.005$.

3. Result

Infertile men treated with selenium during the study period showed increased and improved sperm count, motility, and normal morphology. Details are in the table below.

Table 1: Changes in semen parameters before and after treatment with selenium supplementation

Variable	Before Selenium Treatment	After Selenium Treatment	Increase (%)*
Sperm Count (million/mL)	37.50 ± 30.50	60.40 ± 20.50	61.06
Sperm Mobility (%)	21.25 ± 12.2	49.50 ± 16,80	132
Sperm Vitality (%)	31.40 ± 10.80	59.00 ± 15,00	89
Normal Sperm Morphology (%)	63 ± 7.60	84.50 ± 8.00	34.12
Ejaculate Volume (mL)	2.4 ± 0.50	3.50 ± 0.35	45.83

$$* Increase = \frac{(Last\ Measured - First\ Measured)x100}{First\ Measured}$$

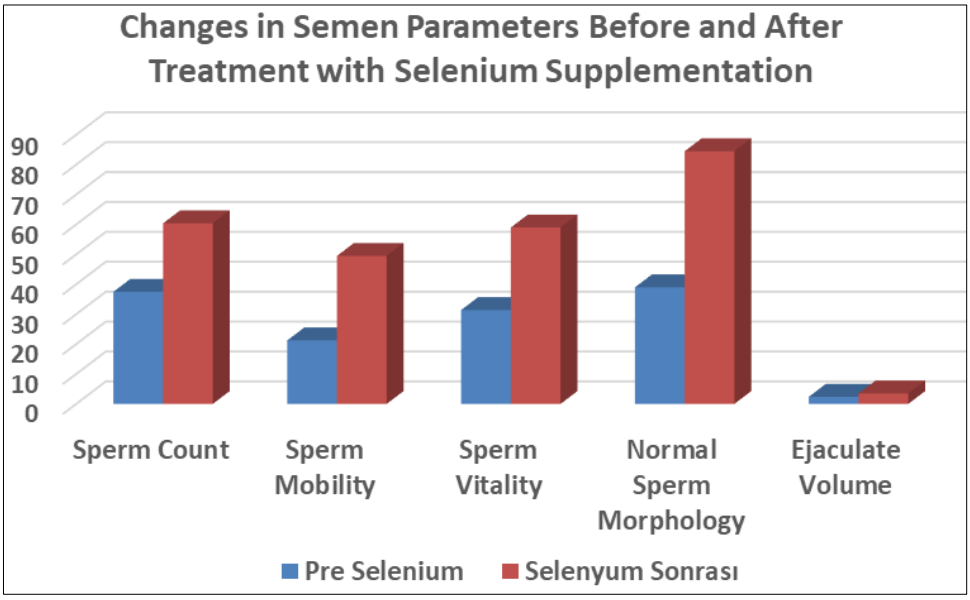

Fig 2: Changes in semen parameters before and after treatment with selenium supplementation

In our study, it was determined that there is a relationship between low intake of Selenium, an antioxidant, and sperm motility, sperm count, sperm viability, normal sperm morphology and ejaculate volume in semen. With selenium

supplementation, an increase of 61.06% in sperm count, 132% in sperm motility, 89% in sperm viability, 34.12% in normal sperm morphology and 45.83% in ejaculate volume was observed. However, it should be noted that the changes seen in these issues may have an effect on the habits, lifestyle and nutrition of the person.

In our study, it was determined that 64 of 100 infertile men had oligospermia and 36 had oligospermia, and this finding was compared with the clinical finding and the consistency of the determinations was checked. It was determined that all of the participants in the control group did not have infertility problems. Selenium concentrations in the serum and seminal plasma of the patients were evaluated by atomic absorption spectrophotometry.

The above-mentioned first results obtained as a result of the study were associated with spermatogram and hormonal levels in order to determine their relationship with male infertility. Mean serum concentrations of selenium were significantly higher in oligospermia cases than in azoospermia cases, and a significant inverse correlation was observed between selenium levels and sperm count. In addition, it has been determined that seminal plasma selenium levels are associated with sperm motility, viability and morphology. However, selenium has been observed to significantly increase sperm motility in infertile men with reduced sperm motility. It seems possible to state that low selenium levels in the testis are associated with male infertility and that selenium is necessary for spermatogenesis. It is also known that selenium deficiency increases oxidative stress, which negatively affects spermatogenesis (Zorn *et al*. 2007) [18].

Table 2: Changes in seminal plasma elements as a result of treatment with selenium

Variable	Before selenium treatment	After selenium treatment	Increase (%)*
Zinc (µg/dL)	39.24 ± 5.5	63.2 ± 5.5	%61.06
Magnezyum (µg/dL)	18.75 ± 4.5	32.5 ± 2.5	%73.33
MDA (µmol/dL)	5.7 ± 0.25	2.575 ± 0.27	% -54.82
Glutathione (µmol/dL)	8.50 ± 1.20	12.4 ± 1.2	%45.88

$$* Increase = \frac{(Last\ Measured - First\ Measured)x100}{First\ Measured}$$

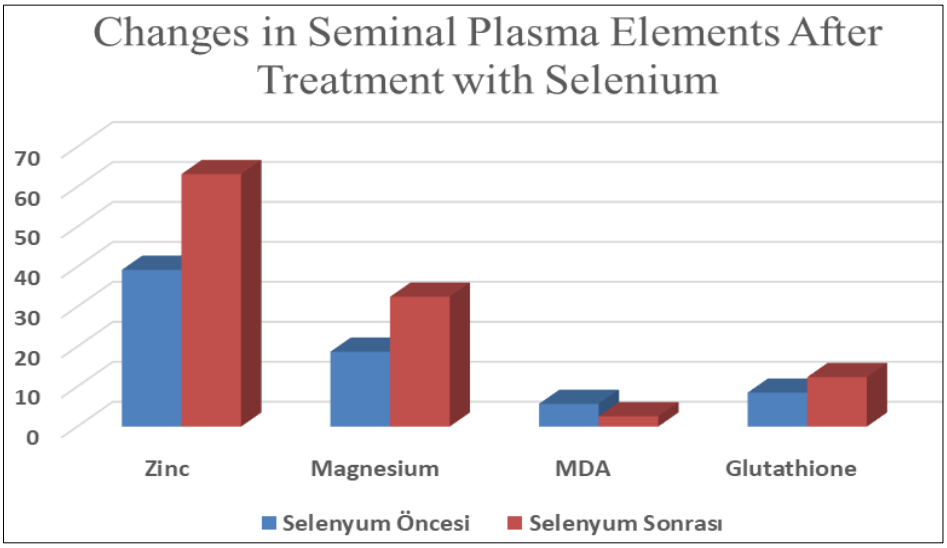

Fig 3: Changes in seminal plasma elements after treatment with selenium

It is seen that the results obtained with this experiment show parallelism with previous studies (Wiersemav 2006) [17]. In infertile patients treated with selenium supplementation, it was observed that the zinc level in the seminal plasma increased by 61.06%, the magnesium level increased by 73.33%, the malondialdehyde (MDA) concentration decreased by 54.82%, and the glutathione concentration increased by 45.88%. Here, it is determined that selenium supplementation significantly decreases MDA and increases glutathione concentration, which is also supported by previous studies (Chaudhury *et al*. 2005) [4]. When the serum concentrations of the same components are examined, it is seen that the result is different.

Table 3: Changes in serum elements as a result of treatment with selenium

Variable	Before selenium treatment	After selenium treatment	Increase (%)*
Zinc (µg/dL)	0.54 ± 0.15	0.75 ± 0.12	%38.8
Magnesium (µg/dL)	14.5 ± 1.49	18.30 ± 1.83	%26.20
MDA (µmol/dL)	4.9 ± 0.8	2.45 ± 0.4	-%50

Glutathione (µmol/dL)	6.8 ± 0.4	10.2 ± 0.83	%50

$$* Increase = \frac{(Last\ Measured - First\ Measured)x100}{First\ Measured}$$

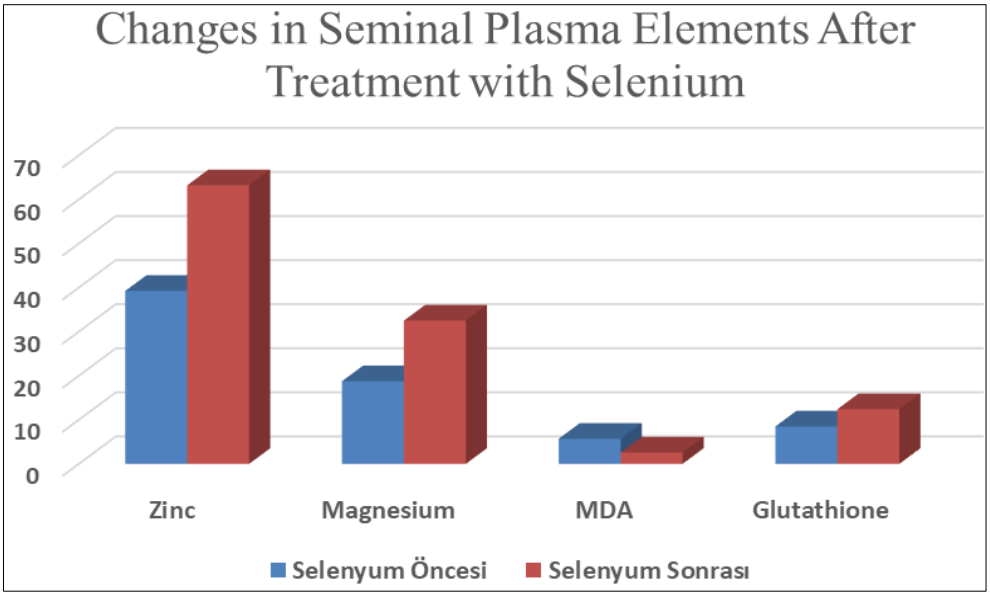

Fig 4: Changes in serum elements after treatment with selenium

In infertile patients treated with selenium supplementation, serum zinc level increased by 38.8%, magnesium level increased by 26.20%, malondialdehyde (MDA) concentration decreased by 50%, and glutathione concentration increased by 50%. Here, it is determined that selenium supplementation significantly decreases MDA and increases glutathione concentration, which is also supported by previous studies, just as in seminal plasma (Nairv 2005) [12]. Finally, in our study, hormone levels were analyzed and compared before and after selenium treatment.

Table 4: Changes in serum hormone levels as a result of treatment with selenium

Variable	Before selenium treatment	After selenium treatment	Increase (%)*
FSH mIU/mL	5.10 ± 0.79	7.95 ± 0.58	%55.88
LH mIU/mL	4.98 ± 0.82	7.65 ± 0.72	%53.61
Testosteron ng/mL	7.90 ± 1.02	10.49 ± 0.65	%32.78

$$* increase = \frac{(Last\ Measured - First\ Measured)\ x\ 100}{First\ Measured}$$

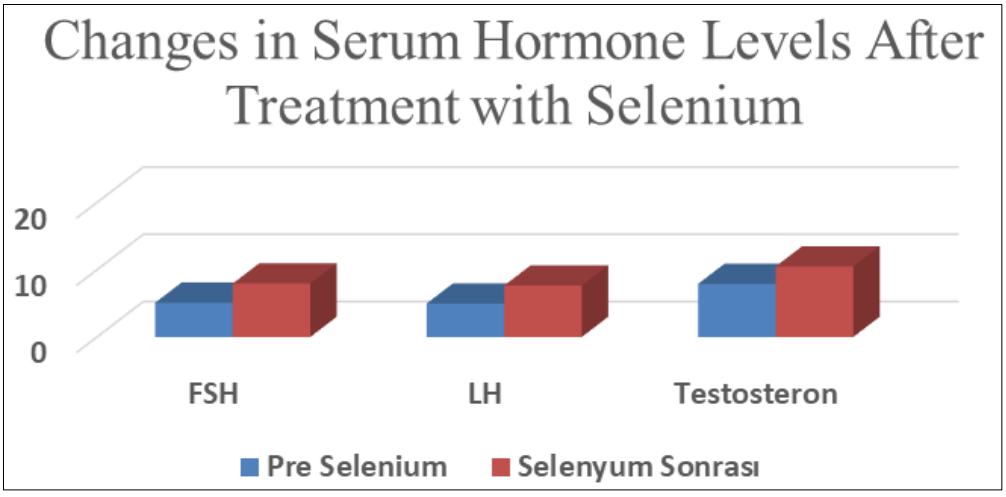

Fig 5: Changes in serum hormone levels after treatment with selenium

It has been observed that infertile patients treated with selenium supplementation have a significant increase in FSH, LH and testosterone levels. At this point, it would be appropriate to draw attention to the fact that the increase in testosterone level is directly proportional and related to the increase in zinc level.

Evaluation

While infertility can be related to genetic characteristics, it also varies depending on the person's daily life, activity level, preferred nutrition method, harmful habits such as smoking and alcohol, and even geographical region (Wiersemav 2006) [17]. In our study, patients living in the Tikar province of Iraq were discussed. While about 15% of married couples face infertility problem, about half of the infertility problems among total married couples are caused by male infertility. (Chaudhury *et al*. 2005) [4]. For the diagnosis of male infertility, experts suggest the condition that there is no pregnancy as a result of sexual intercourse that is protected for 12 months and the diagnosis is made accordingly (Graciav 2005, Philippovvd. 1998).

In the experiments carried out within the scope of our study, MDA, Glutathione, Zinc, Magnesium, Testosterone, LH, FSH values of infertile men, as well as sperm count, sperm motility, sperm viability, normal sperm morphology and ejaculation volume were analyzed. Diagnosis of infertility becomes easier with sperm count, sperm motility, normal sperm morphology and ejaculation analysis. Since magnesium, zinc, glutathione and MDA are elements found in semen plasma, they are considered to be indirectly related to infertility (Graciavd. 2005). LF, FSH and Testosterone measurements were also analyzed in relation to infertility.

MDA is a reactive strain. Naturally occurring MDA is considered as a marker of oxidative stress (Janero 1990). Glutathione plays an important role in protecting cells against oxidative and electrophilic stress caused by reactive oxygen species and radiation (Beckett 1993, Shanv 1990) [3]. It is known that zinc is very effective in sperm production and prostate secretions. In addition, the sperms take zinc with ejaculation, and therefore, the zinc content of the sperm increases with exposure to seminal fluid, thus affecting the maturation of spermatozoa (Stoltenberg *et al*. 1996) [14]. A diet deficient in zinc affects oxidative stress due to a decrease in glutathione and an increase in MDA in both semen and serum (Gavella and Lipovae 1998, Nairv 2005) [7, 12].

Selenium is a type of micronutrient required to protect male fertility (Maiorinovd. 1999). It is stated in various studies that approximately 20%-40% of male infertility is associated with insufficient sperm production associated with selenium deficiency (Griffin *et al*. 1994) [6]. Nevertheless, it is possible to talk about the availability of limited data on the effects of selenium on male reproduction. In a previous study, it was revealed that the seminal fluid of non-infertile men has a much higher amount of selenium than infertile men (Hurst 1999) [9]. Like all cells living in aerobic conditions, spermatozoa are constantly faced with the paradox of oxygen. Studies show that sperm damage mediated by Reactive Oxygen Species (ROS) contributes to a significant pathology in 30-80% of infertility cases (Nair *et al*. 2005) [12].

In our study, which is a long-term study, 100 infertile men were told to take a 50 mcg selenium supplement before going to bed, and this treatment was applied between the first and last measurements throughout our study. Medical history and clinical review were performed with semen analysis as well as serum and seminal plasma study for zinc, magnesium, Malondialdehyde (MDA) and glutathione. FSH, LH, and Testosterone measurements were also performed simultaneously with other measurements before and after selenium treatment. The results were evaluated by statistical analysis.

As a result-

- Significant increases in sperm count, sperm motility, sperm viability, normal sperm morphology and ejaculate volume have been found in infertile men after treatment with selenium..
- Serum Mg, Serum FSH, Serum LH and Serum testosterone and serum glutathione levels were found to be significantly increased.
- Serum MDA has been found to be significantly reduced after treatment with selenium in infertile men compared to pretreatment.
- These results are supported by many studies describing and concluding the effect of selenium on semen quality (Nair *et al*. 2005) [12]. It is evaluated that selenium can be used as a single agent in the treatment of male infertility.

Refernces

1. Abid S, Maitra A, Meherji P, Patel Z, Kadam S, Shah J, *et al*. Clinical and laboratory evaluation of idiopathic male infertility in a secondary referral center in India. J Clin Lab Anal. 2008;22:29-38.
2. Alizadeh F, Javadi M, Karami AA, Gholaminejad F, Kavianpour M, Haghighian HK. Curcumin nanomicelle improves semen parameters, oxidative stress, inflammatory biomarkers, and reproductive hormones in infertile men: A randomized clinical trial. Phytotherapy Research. 2018;32(3):514-521.
3. Beckett GJ, Hayes JD. Glutathione S-transferases: biomedical applications. Adv Clin Chem. 1993;30:281-380.
4. Chaudhury K, DAS T, Chakravarly B, Bhattacharyya A. Acrosin activity as a potential marker for sperm membrane characteristics in unexplained male infertility. Fertile Steril. 2005;83(1):104-109.
5. El Kissi Y, Romdhane AB, Hidar S, Bannour S, Idrissi KA, Khairi H. General psychopathology, anxiety, depression and self-esteem in couples undergoing infertility treatment: a comparative study between men and women. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2013;167(2):185-189.
6. Griffin E, Wilson D. Disorders of the testes. In: Isselbacher K, Braunwald E, Wilson J and Martin B (Eds.), Harrison’s Principles of Internal Med. (13 edn), New York, McGraw Hill, USA, 1994.

7. Gavella M, Lipovae V. In vitro effect of zinc on oxidative changes in human semen. Andrologia. 1998;30(6):317-323.
8. Gałecka E. Jacewicz R, Mrowicka M, Florkowski A, Gałecki P. Antioxidative enzymes-Structure, properties, functions. Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego. 2008;25:266-268.
9. Hurst R, Bao YP, Ridley S. Phospholipid hydroperoxide cysteine peroxidase activity of human serum albumin. Biochem J. 1999;338(pt 3):723-728.
10. Keskes-Ammar L, Feki-Chakroun N, Rebai T *et al*. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Archives of Andrology. 2003;49:83-94.
11. Janero DR. "Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and per oxidative tissue injury". Free Radic Biol Med. 1990;9(6):515-540.
12. Nair N, Bedwal S, Prasad S, Saini MR, Bedwal RS. Short-term zinc deficiency in diet induces increased oxidative stress in testes and epididymis of rats. Indian J Exp Biol Sep. 2005;43(9):786-794.
13. Nikoobakht MR, Aloosh M, Nikoobakht N, Mehrsay A, Biniaz F, Karjalian MA. The role of hypothyroidism in male infertility and erectile dysfunction. Urology Journal. 2012;9(1):405-409.
14. Stoltenberg M, Ernst E, Andreasen A. Histochemical localization of zinc ions in the epididymis of the rat. The Histochemical Journal. 1996;28(3):173-185.
15. Safarinejad MR. The effect of coenzyme Q 10 supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study. International Urology and Nephrology. 2012;44(3):689-700.
16. Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. Fertility and sterility. 2006;85(5):1409-1414.
17. Wiersema N, Drukker A, Dung M. Consequences of infertility indeveloping countries: results of a questionnaire and interview survey in the South of Vietnam. J Transl Med. 2006;4:54.
18. Zorn B, Osredkar J, Meden-Vrtovec H, Majdic G. Leptin levels in infertile male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. International Journal of Andrology. 2007;30(5):439-444.