Research Article

Gynecology & Reproductive Health

The Role of Micronutrients in the Treatment of Idiopathic Abnormal Sperm Parameters

Elmahaishi LM[1], Abbas AA[2] and Elmahaishi MS[2*]

[1]Faculty of pharmacy. Misurata university. Misurata, Libya.

[2]Lamis IVF center. Lamis clinic. Misurata, Libya.

[*]Correspondence:
Prof. Elmahaishi MS, Lamis clinic, P.O. Box 65, Misurata, Libya, Tel: 00218913945055; E-mail: Elmahaishi@Elmahaishi.com.

Received: 15 June 2017; Accepted: 13 July 2017

Citation: Elmahaishi LM, Abbas AA, Elmahaishi MS. The Role of Micronutrients in the Treatment of Idiopathic Abnormal Sperm Parameters. Gynecol Reprod Health. 2017; 1(2): 1-8.

ABSTRACT

In spite of the presence of large number of male infertility pharmaceuticals agents and strategies, there is a renewed interest in remedies from micronutrient sources because of their lesser side effects and low cost. In this clinical trial that included male subjects aged from 20 to 60 years with at least 1 year of infertility and abnormal semen analysis who received 3 -6 months' treatment with a Cyclofert men that contains (l-carnitine l-tartarate 1g, N – Asetyl - cysteine 600 mg, 40 mg zinc, Lycopene 20 mg, 60 mg vitamin E, 200 mg vitamin C, 80 mg glutathione, 200 µg selenium, 100 mg coenzyme Q10 and 400 µg folic acid. Theses micronutrients were given in a form of two tablets/ twice a day for 6 months). Sperm parameters (count, motility, and morphology) and pregnancy incidence were monitored before and after treatment. The collected data showed that there was an overall progressive motility after 3 and 6 months of treatment. Moreover, the sperm count increased by 64% (3 months), (6 months) 88% (α = 0.00, 1, at $P<0.05$) compared to the untreated group. Furthermore, treatment for three and six months significantly improved the sperm morphology ($p<0.05$). These positive changes ameliorated the women's ability to get pregnant in which 30 % of women got pregnant after 6 months of the treatment. However, all of these improvements are proportional with the duration of treatment.

Keywords
Male infertility, antioxidant, ROS, OS, semen parameters.

Introduction
According to the world health organization (WHO) infertility is the inability of a sexually active couple in absence of any contraceptive use to achieve spontaneous pregnancy in one year. This problem could affect both men and women. In the recent decades, fertility disorders affect steadily growing number of couples; approximately 15% of them are unable to conceive after one year of unprotected intercourse. In 50% of involuntarily childless couples, a male-infertility-associated factor is found together with abnormal semen parameters. Male fertility can be reduced as a result of different illnesses and disabilities including hormonal abnormalities, genetic causes, autoimmune factors, endocrine disturbances, environmental pollution, reactive oxygen species, and lifestyle factors which can impair semen quality such as heavy smoking, alcoholic abuse, use of anabolic steroids, and extreme sports [1-3]. In certain cases, the infertility is attributed to anatomical changes in which 40-90% of cases lie in insufficient sperm production of unknown etiology. Studies have shown a clear decrease of 58% in sperm count over the last 50 years which could be attributed to the lifestyle and the environmental factors. An example of these factors is the increasing environmental impact of estrogens, which are used in cattle breeding and milk production. Moreover, pesticides such as Dichlorodiphenyltrichloroethane (DDT) can have an estrogen-like effect and therefore negatively affect sperm production. Other factors include heavy metals in the environment, ongoing stress, alcohol, nicotine, and other narcotics as well as a nutrient deficiency [1].

In general, 25-30% of all infertility cases are due to male factor infertility [2,3], which falls into one of two categories either productive or obstructive. Many of these problems such as hypogonadism, varicocele, gonadotrophin deficiency, and genital tract infections and obstructions, or sperm autoimmunity could be treatable. On other cases, sub-fertility can be attributed to known irreversible conditions. However, no specific cause is found in about 50% of all infertile men with abnormal sperm parameters seeking treatment. These sperm disorders are the most common cause of male subfertility. Strong associations between sperm quality parameters and pregnancy have been established [4,5]. Thus, low sperm quality has increasingly raised therapeutic interest [2-6].

The Pathogenic mechanisms involved in this form of defective sperm production are unknown yet [7]. Thus, focusing on the development of effective treatments is not easy. Various agents have been used in the attempt to increase the fertility potential of men with decreased semen quality. Studies have rendered heterogeneous results, and the effect of gonadotrophins or antiestrogens on pregnancy rates remains controversial [2]. A recent review aimed at searching the rationale for the treatment of idiopathic male factor infertility over the past 20 years concluded that there is no evidence supporting androgen and gonadotrophin use for enhancing male fertility. Evidence regarding antiestrogen, aromatase inhibitor and antioxidant use were found clearly to be beneficial to idiopathic impaired male fertility in terms of natural conception [8].

However, the use of micronutrients is another approach that has been used recently. In this therapy, scientists use a combination of the most well-known antioxidants including carnitine, L-carnitine, L-arginine, zinc, vitamin E, glutathione, selenium, co-enzyme Q10 and folic acid. In this clinical trial, we used a cyclofert men food supplement tablets that contains nine of these essential compounds which are required for DNA synthesis and spermatogenesis and are derived from the diet. Therefore, concentration of required nutrients and other relevant factors may have substantial effects on sperm quality and reproduction [9,10]. In the same pattern, there are many studies that used anti-inflammatory drugs (NSARs such as e.g. Cinnoxicam) and antioxidant substances (vitamin E, glutathione, coenzyme Q10 etc.) in the treatment of varicocele and observed predominantly positive results [11].

In this study, we used the cyclofert men tablet which known to have a positive impact on improving the sperm parameters. L. carnitine, a naturally occurring vitamin-like substance, is one of these microelements that found in human tissues and cells and is important for the transport of long-chain fatty acids across the inner mitochondrial membrane where they are used for energy production via beta oxidation. Because L-carnitine appears in a high concentration in the human epididymis to provide an energetic substrate for spermatozoa, it contributes directly to sperm motility and may be involved in the successful maturation of sperm. In addition to carnitine, the presence of glutathione and N-acetyl cysteine in the used supplement has an important effect on sperm movement [12]. They also can improve sperm concentration and acrosome reaction [13]. Several studies have indicated a relationship between intracellular glutathione depletion and apoptosis in which intracellular glutathione modulated Fas-mediated apoptosis in malignant and normal human T lymphocytes (Fas is a death factor that controls apoptosis) [14-16]. Because of that the existence of glutathione protects cell membrane from lipid peroxidation and has a positive effect on sperm motility [17]. Moreover, DNA is usually protected by anti-oxidants in seminal fluid including selenium (Se) which is required for testicular development, spermatogenesis, Motility and function. It is known to protect against oxidative sperm DNA age. Combination treatment of Se with other antioxidant led to significant better sperm parameters than using it alone. There are some studies mentioned to the synergistic effect of selenium with Vitamin E as an anti peroxidant [18,19].

Almost the entire selenium content in the human testis is found in the seleno-protein phospholipid hydro-peroxide glutathione peroxidase (PGHPx). PGHPx, an active peroxidase in spermatozoa, is converted into an inactive oxidative protein in the matured sperm where it becomes a main component of the mitochondrial capsule in the midpiece of the spermatozoon. Male infertility, which develops due to impaired sperm motility via morphological changes in the sperm midpiece, is attributed to an insufficient concentration of PGHPx. Deficiencies of either substance can lead to instability of the mid-piece, resulting in defective motility [20]. Moreover, Zinc is a trace mineral essential for normal functioning of the male reproductive system. There are more than 200 enzymes in the body are zinc dependent. Zinc deficiency is associated with decreased testosterone levels and sperm count. Furthermore, the presence of vitamin C in seminal plasma directly reflects dietary intake, and lower levels of vitamin C may lead to infertility and increased damage to the sperm's genetic materials [9,10,12].

The other micronutrient that is found in the cyclofert formula is coenzyme Q10 (Co Q10) that is concentrated in the mitochondrial mid-piece, where it is involved in energy production. It also functions as an antioxidant, preventing lipid peroxidation of sperm membranes. Folic acid also is another compound that presents in a wide variety of foods, such as green-leafy vegetable, liver, bread, yeast and fruits. Folic acid is important for DNA synthesis as well as for protein biosynthesis while zinc is an essential component of the metalloenzymes involved in DNA transcription and expression, sperm quality and as a further result on male fertility [20,21]. It has also been reported that folic acid effectively scavenges oxidizing free radicals, and as such can be regarded as an antioxidant [22]. Folate plays a vital role in synthesis of DNA, transfer RNA and the amino acids cysteine and methionine. DNA synthesis plays an important role in germ cell development, and therefore, it is obvious that folate is important for reproduction. Despite its water-soluble character, folic acid inhibits lipid peroxidation (LPO). Therefore, folic acid can protect bio-constituents such as cellular membranes or DNA from free radical damage [19,22]. The last compound in this combination is lycopene which has been demonstrated to be the most potent antioxidant in comparison to other carotenoids. It has the ability to quench singlet oxygen and prevent oxidative damage to other molecules. This is because of its unique structure of 11 conjugated double bonds and no cyclic groups [17,21].

Aim of the study

For all of these benefits and because since to date, there is still no specific therapy to improve the abnormal semen parameters in cases of male infertility, we aimed to look at the possible effects of micronutrients on semen parameters including sperm count, motility, and morphology to improve male infertility.

Material and Method

Study design and subjects: This study started from the 1st of March 2016 until 30th of November 2016 (6 six months was the period of taking the treatment) while the duration time of the study including the follow up and the examination period was 9 nine months. A total number of six hundred (600) male patients aged from 20-60 years old who were sub-fertile were included in this study under the following criteria:

- Has primary infertility one year or more or secondary infertility two years or more.
- Both couple were not using any form of contraception.
- They had no disease affecting their fertility.
- Azozoospermic obstructive or non-obstructive were excluded.
- They had no disease which affecting sperm parameters.
- They had sperm count <20 million/ml, forward sperm progressive motility <20%, normal sperm morphology <10% and there were no signs of seminal infections.
- All patients had normal semen liquefaction (<5 minutes).
- These inclusion criteria can be all together present or one of them present.

Those patients with the above criteria were included in the study and the required micronutrients were prescribed to every patient as a dose of two tablets, twice a day (12 hours in between the two doses) for 3-6 months. The control group which contains one hundred patients had the same inclusion criteria and they request the treatment by intra-cytoplasmic sperm injection (ICSI). The semen analyses were performed after three and six months of the first semen analysis and again six months later.

Samples collection and analysis

The semen samples were received from the patient and carried to the lab directly. The analysis was performed before treatment, after three and 6 months from starting the first dose. All semen analysis was done under a controlled condition in one lab (Lamis clinic) by the same technician.

The abnormality can be in sperm concentration, motility and or morphology together or isolated. The diagnosis of these abnormal parameters was taken on two semen analysis one week in between in both groups; the therapeutic and the control group. The diagnosis of any of previous abnormal sperm parameters in one or more sample of two sperm analysis collected in (1-4 weeks) a part according to WHO.

The active compounds in the tablet: In this study, we are seeking to find out the effect of micronutrient supplement on the quality of sperm parameters since the previous multiple studies showed improvement in the sperm parameters. We used a tablet that contains anti-oxidants to improve sperm parameters in male infertility which is very accepted nowadays because they are safe with a low cost. On those bases, we are using the coming micronutrients together on daily bases divided in two doses during the 24 hours and for 6 months. The combination form of the used micronutrients composed of:

1	L – Carnitine L – tartarate	1 gram
2	N – Asetyl – cysteine	600 mg
3	Vitamin E	60 mg
4.	Selenium	200 µg
5	Folic acid	400 µg
6	Lycopene	20 mg
7	Co-enjyme Q10	100 mg
8	Zinc	40 mg
9	Vitamin C	200 mg

These 9 micronutrients were giving to the patient in a form of two tablets together twice a day for a period of six months. We used cyclofert male which contain the mentioned microelements (Surveal laboratories, Belgium. Figure 1 showed the type of drug that has been used in this study.

Figure 1: The drug used in the study.

Statistical analysis

The data obtained were expressed as means (±SDM) and analyzed using repeated measures of variance. The differences between the means were analyzed statistically with Chi-Square Tests using PSPP program (Linux operating system). Values of $p<0.05$ were taken to imply statistical significance.

Results and Discussion

Male infertility in Libya is responsible on more than 50% of the infertile couples. Infertility due to male factor can be as a result of obstructive azozoospermia, non-obstructive azozoospermia, non-identifiable causes, and infections or in a small group due to sexual dysfunctions plus general diseases. 25% of male cases have no identifiable causes [23]. To illustrate the effect of micronutrients including carnitine, vitamins, zinc, etc, the patients in this clinical trial received a micronutrient treatment (cyclofert) of 2 tab/ twice a day for 3-6 months. The age distribution of the total number of patients (600; 500 therapeutic group and 100 control group) is prescribed in table 1 and figure 1 in which most of the patients are aged from 30-34 (210) and 35-39 (160). This is the main reproductive age in Libya.

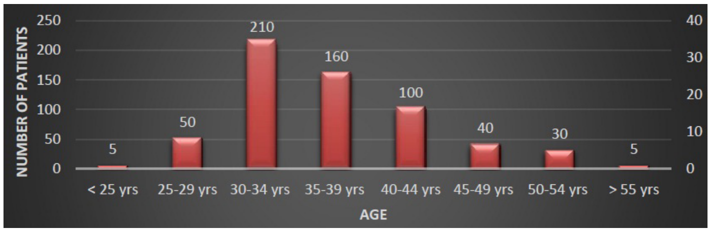

Figure 2: Distribution of Age in the control and experimental groups.

The changes in the semen parameters after three and six months of the treatment:

The effect of micronutrients on the sperm count: All the patients were requested to perform a semen analysis to detect the changes of sperm count after 3 months of treatment. It is well known that the normal semen supposed to have an over than 20 million sperm per milliliter. After using cyclofert for three months, the sperm count has been increased by 64% ($\alpha = 0.00$, 1, at $P<0.05$) compared to the untreated group which showed a 92% of unchanged count. The results are shown in table 2, figure 3. These results are constant with Netter A, who showed that the total number of sperm increased after the combined administration of folic acid and zinc in both sub fertile and fertile men [24].

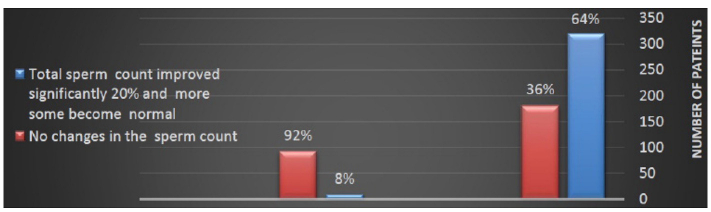

Figure 3: The changes of sperm count after 3 months of treatment.

The continuance treatment for six months significantly increased the number of sperm in the semen in which 48% of the patient had a normal value while 40% of them showed an improvement in the sperm count compared with the untreated group (Table 3, Figure 4). The collected data indicates that there is a noteworthy association between cyclofert treated group and the improvement in the sperm count where ($\alpha = 0.00$, 2at $P<0.05$).

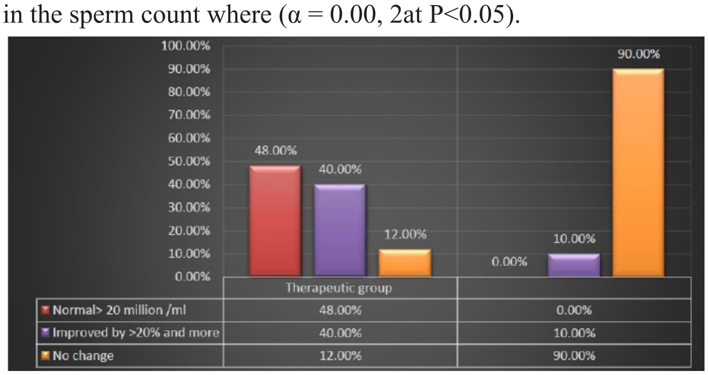

Figure 4: The changes of sperm count after 6 months of treatment.

The effect of micronutrients on the sperm motility:

In addition to the sperm count, we evaluated the changes in the forward progressive motility over a period of 3 and 6 months of treatment. Results showed that by the end of the three months' course of treatment, there were a 50% increase in the motility rate compared to the untreated group ($\alpha = 0.00$, 1 at $P<0.05$). (2%) (Table 4, Figure 5). Moreover, the long-term use (6 months) of micronutrients ameliorates the sperm's ability to move by 71% in which 16.9 % of them had a normal number of motile sperms as in the healthy people while 54.2 % showed an improvement by 10% or more (Table 5, Figure 6) ($\alpha = 0.00$, 2 at $P<0.05$). This progression could be contributed to the use of a combination of multi micronuternts. In other studies, the presence of a combination of L-carnitine and L-acetylcarnitine in spermatozoa during the passage through the epididymis leads to an improvement in sperm motility [25].

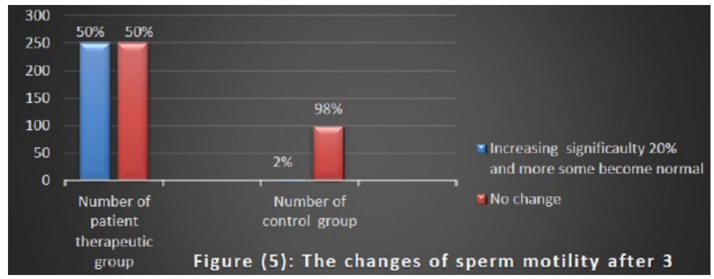

Figure 5: The changes of sperm motility after 3 months of treatment.

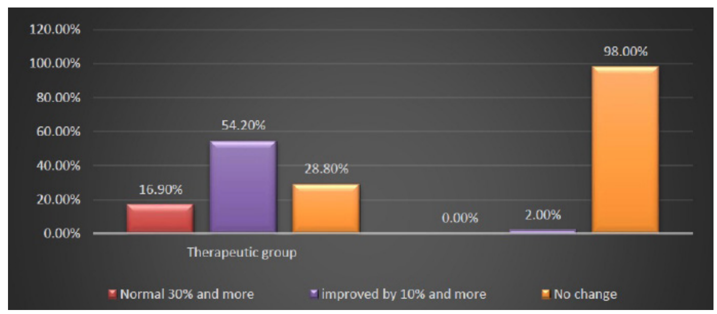

Figure 6: The changes of sperm motility after 6 months treatment.

These results are consistent with Ross and his lab mates (2010) who showed that oral supplement of carnitine improved the motility and concentration of sperm [26-28]. These changes could be attributed to the presence of micronutrients such as carnitine which is derived mostly from human diet and plays a role in sperm energy metabolism and provide the fuel for sperm motility. Spermatozoa exhibits increased L-carnitine and L-acetyl carnitine content during epididymal passage and acquisition of motility [29]. It enhances the cell energy in the mitochondria by facilitate the entry and utilization of free fatty acid and decreasing fatty acid oxidation [30-32]. Carnitines protect sperm DNA and cell membranes from reactive oxygen species (ROS) induced damage and apoptosis [33-36]. However, most of the used micronutrients in this therapy exhibited a positive effect on sperm motility.

The effect of micronutrients on the sperm morphology

The shape of a sperm is an important determinant of its fertilizing ability. A normal sperm has an oval head about 5-6 micrometers long and 2.5-3.5 micrometers wide, and a single long tail. In order to pass through the egg's protein coat, the sperm must be vigorously motile and the sperm head must be a symmetrical, oval shape of the appropriate size. Nevertheless, there are many problems related to sperm shape including Hypokinisis, sever oligozoospermia, sever asthenozoopermia, and sever teratozoospermia are considered as causes of male infertility. These morphological abnormalities are shown in figure 7.

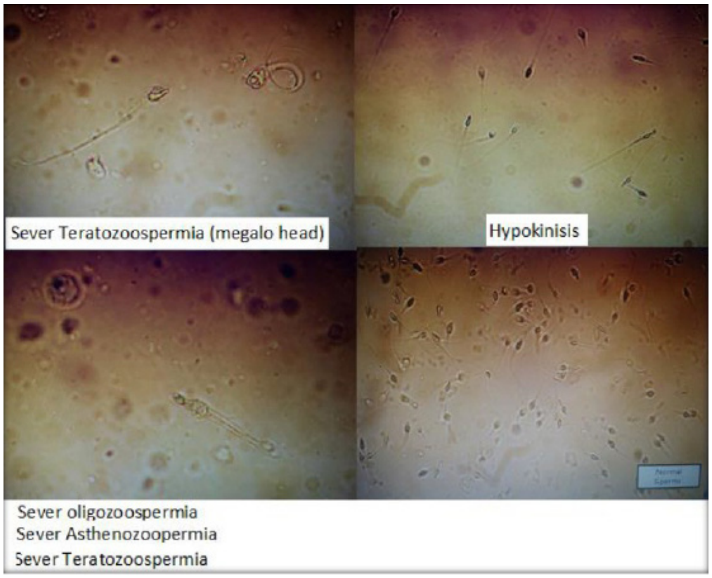

Figure 7: The abnormal and normal shape of the sperm.

The microscopic examination for our semen samples revealed that the use of cyclofert for three months has a notable effect on sperm shape in which 38.8% (205) of the patient showed significant changes in the sperm shape and some of them became normal compared to the untreated group ($p<0.05$). Results are showed in table 6 and figure 8.

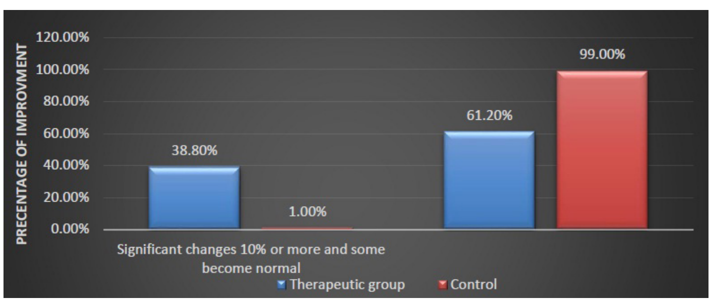

Figure 8: The changes of sperm motility after 3 months of treatment.

On the same way, the micronutrients use for six months improved the sperm morphology in which 16% (80) of the patients showed an ideal sperm shape while 40% (200) showed an improvement in the morphological appearance compared to the untreated group (2% improvement only (Figure 9). These findings are consistent with Menkveld and his colleagues who showed that there was a significant increase in the normal sperms shape after the use of supplementary treatment [37]. Our results were significant in which ($P <0.05$). These results could be attributed to the regulatory effect of L-carnitine, zinc, folic acid, etc. on the ROS. Scientists have been concluded that the production of ROS by spermatozoa is a normal physiological process, but the amount of ROS produced has to be carefully controlled because an imbalance between the generation and scavenging of ROS may lead to damage of DNA or other important structures. Spermatozoa are particularly susceptible to peroxidative damage because most of their cytoplasm is removed during the final stages of spermatogenesis. Therefore, the spermatozoa have almost no cytoplasmic defensive enzymes, such as catalase, glutathione peroxidase or GST, which are involved in the protection of most cell types from peroxidative damage induced by ROS. Furthermore, the plasma membranes of spermatozoa contain large amounts of unsaturated fatty acids, which are particularly susceptible to free radical attack [38]. Therefore, high concentrations of ROS are harmful to sperm. The oxidative stress (OS) arises when excess free radicals overwhelm the antioxidant defense of the male reproductive tract which causes a cell damage, cell death or sperm DNA fragmentation [39].

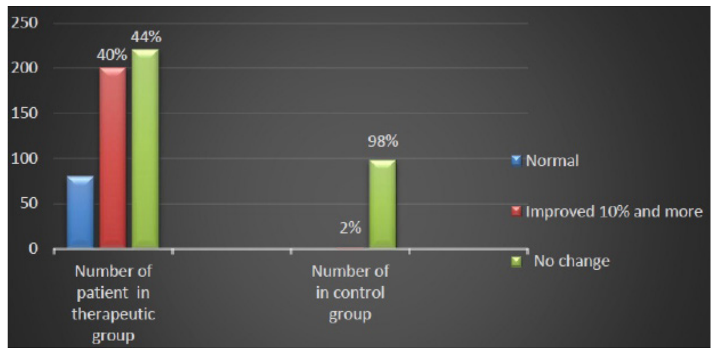

Figure 9: The changes of sperm morphology after 6 months of treatment.

The effect of micronutrients on getting pregnancy: As a result of all the significant changes on sperm count, motility and shape, there was some detectable changes on the women ability to get pregnant in which there were 90 (18%) of women got pregnant after 3 months of the treatment. furthermore, after a course of six months the number of pregnancy cases increased in which (150) 30% of the spouses became pregnant. These findings could be attributed to L-carnitine effects in which previous studies showed that the concentration of L-carnitine in the sperm was significantly less in infertile men than in fertile men. It can be assumed that Lcarnitine would make a good marker for estimating sperm quality. L-carnitine leads to an effective improvement in the proportion of sperm in the semen with linear progressive movement as well as sperm with normal morphology [5,7]. Folic acid also is an important for DNA synthesis as well as for protein biosynthesis while zinc is an essential component of the metalloenzymes involved in DNA transcription and expression, sperm quality and as a further result on male fertility [40]. It has also been reported that folic acid effectively scavenges oxidizing free radicals, and as such can be regarded as an antioxidant [41].

All the previous significant changes could be attributed to the ability of micronutrients to eliminate the reactive oxygen species (ROS) that present in seminal fluid and may disrupt cell functions and lead to affect cell's life [42]. Several clinical trials have examined the potential of anti-oxidant supplementation to treat oxidative stress induced male factor infertility. In more details, male germ cells at various stages of differentiation can generate ROS which needed in low physiological levels to regulate sperm capacitation, acrosome reaction and sperm –oocyte fusion [43,44]. Therefore, to keep a normal cell function, ROS should be continuously accompanied by seminal plasma antioxidants [45]. In healthy men balance between physiological ROS and anti-oxidants exists in the male reproductive tract. The oxidative stress (OS) arises when free radicals increase and over whelm the antioxidants defense of the male reproductive tract [46,47]. The seminal (OS) correlates negatively with sperm parameters and function – adversely affecting fusion events required for fertilization [48-51]. Patients with a high seminal fluid OS were found to have DNA disruptions [52]. Moreover, DNA damage during sperm transport through the epididymis is increasingly being recognized as one of the main mechanisms of DNA damage of human spermatozoa and is mainly related to ROS [53,54].

Even though there are a lot of studies that support our finding, some other studies were unable to show the significant effect of anti-oxidant on sperm parameters and pregnancy outcome, this can be due to short duration, small sample size, small doses of micronutrients or other factors. In addition to that, many investigations in the field of (sub) fertility reviewed here were performed on laboratory animals or cell cultures, which is a comprehensive first research design. However, it is very important to verify the results in human subjects. Also, most investigations actually performed in human beings involve couples participating in ART procedures. This is the only way to obtain research material in human. The disadvantage is that all such couples experience (sub) fertility, and because (sub) fertility is known to be a combination of female and male factors, no information on truly fertile couples is available. It would be a great advantage to investigate the same biochemical parameters in fertile couples [55,56].

Conclusion

The idiopathic male infertility is due to the abnormal semen function or low sperm production which caused by different factors such illnesses, injuries, chronic health problems, lifestyle choices and other factors. In the absence of these illnesses and known causes, these abnormalities caused by the lack of some important micronutrients that appeared to have a positive impact on sperm quantity and quality include carnitine, vitamins, and antioxidant compounds.

Compared with the model group, the micronutrient treated group with a dose of 2 tablets twice a day exhibited not only significantly increased sperm counts and motility but also significantly decreased sperm deformity rates (P<0.01). Moreover, the pregnancy incidence has been increased compared to the untreated group. This treatment improved also the pregnancy status in which it caused an elevation in the number of pregnant spouses compared with untreated ones. This improvement in the sperm parameters is proportional to the duration of treatment in which the 6 months' course gave more significant results than the three months' period.

Recommendations

Even though the recent study concluded the positive effect of sperm parameter, a need for further investigation with randomized controlled studies to confirm the efficacy and safety of antioxidant supplementation on the idiopathic male infertility as well as the need to determine the ideal dose of each compound to improve semen parameters, fertilization rates and pregnancy outcomes.

Moreover, many studies are supposed to be carried out to compare the short-term effects of a combination of micronutrients vs. a mono-substance (l-carnitine alone, Zinc alone, etc) on sperm parameters.

References

1. Sinclair S. Male infertility: nutritional and environmental considerations. Altern Med Rev. 2000; 5: 28-38.
2. Templeton A. Infertility-epidemiology, aetiology and effective management. Health Bull (Edinb). 1995; 53: 294-298.
3. Isidori AM, Pozza C, Gianfrilli D, et al. Medical treatment to improve sperm quality. Reprod Biomed Online. 2006; 12: 704-714.
4. Bonde JPE, Ernst E, Jensen TK, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998; 352:1172-7.
5. Zinaman MJ, Brown CC, Selevan SG, et al. Semen quality and human fertility: a prospective study with healthy couples. J Androl. 2000; 21: 145-153.
6. McLachlan RI, Baker HW, Clarke GN, et al. Semen analysis: its place in modern reproductive medical practice. Pathology. 2003; 35: 25-33.
7. Baker HW. Management of male infertility. Baillieres Best Pract Res Clin Endocrinol Metab. 2000; 14: 409-422.
8. Kumar R, Gautam G, Gupta NP. Drug therapy for idiopathic male infertility: rationale versus evidence. J Urol. 2006; 176: 1307-1312.
9. Wong WY, Thomas CM, Merkus JM, et al. Male factor subfertility: possible causes and the impact of nutritional factors. Fertil Steril. 2000; 73: 435-442.
10. Ebisch IM, Thomas CM, Peters WH, et al. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update. 2007; 13: 163-174.
11. Schauer I, Jost R, lmhof M. Micronutrients as an alternative to fertility treatment in men with subclinical varicocele. Bratislava EAU, 2010; 23.
12. Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr. 1990; 10: 357-382.
13. Lenzi A, Culasso F, Gandini L, et al. Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. Human Reproduction. 1993; 8: 1657-1662.
14. Chiba T, Takahashi S, Sato N, et al. Fas-mediated apoptosis is modulated by intracellular glutathione in human T cells. Eur J Immunol. 1996; 26: 1164-1169.
15. Beaver JP, Waring P A decrease in intracellular glutathione concentration precedes the onset of apoptosis in murine thymocytes. Eur J Cell Biol. 1995; 68: 47-54.
16. Lenzi A, Culasso F, Gandini L, et al. Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male

infertility. Hum Reprod. 1993; 8: 1657-1662.
17. Twigg JP, Irvine DS, Aitken RJ. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. Human Reproduction. 1989; 13: 1864-1871.
18. Maiorion M, Coassin M, Roveri A, et al. Microsomal lipid peroxidation: effect of vitamin E and its functional interaction with phospholipid hydroperoxide glutathione peroxidase. Lipids. 1989; 24: 721-726.
19. Foresta C, Flohe L, Garolla A, et al. Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. Biol Reprod. 2002; 67: 967-971.
20. Wong WY, Merkus HM, Thomas CM, et al. Effects of folic acid and zinc sulphate on male factor subfertility: a double-blind, randomized, placebo controlled trial. Fertil Steril. 2002; 77: 491-498.
21. Joshi R, Adhikari S, Patro BS, et al. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radic Biol Med. 2001; 30: 1390-1399.
22. Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. Fertility and Sterility. 2002; 77: 873-882.
23. Netter A, Hartoma R, Nahoul K. Effect of zinc administration on plasma testosterone, dihydrotestosterone, and sperm count. Arch Androl. 1981; 7: 69-73.
24. Matalliotakis I, Koumantaki Y, Evageliou A, et al. L-carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. Int J Fertil Womens Med. 2000; 45: 236-240.
25. Menchini Fabris GF, Canal D, Izzo PL, et al. (1984). Free L-carnitine in human semen: its variability in different andrologic pathologies. Fertility and Sterility. 1984; 42: 263-267.
26. Bornman MS, du Toit D, Otto B, et al. Seminal carnitine, epididvmal function and spermatozoal motility. South African Medical journal. 1989; 75: 20-21.
27. Ross C, Morriss A, Khairy M, et al. Asystematic review of the effect of oral antioxidants on male infertility . Reproductive biomedicine Online. 2010; 20: 711-723.
28. Jeulin C, Lewin LM. Role of free L-carnitine and acetyl-L-carnitine in post gonadal maturation of mammalian spermatozoa. Human Reproduction Update. 1996; 2: 87-102.
29. Gattuccio F, De Rose AF, Letteri MA. Varicocele. Palermo, Italy: Cofese Editore. 2000.
30. Lenzi A, Lombardo F, Sgrò P, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil Steril. 2003; 79: 292-300.
31. Vicari E, Calogero AE. Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. Human Reproduction. 2001; 16: 2338-2342.
32. Arduini A. Carnitine and its acyl esters as secondary antioxidants? Am Heart J. 1992; 123: 1726-1727.
33. Lenzi A, Sgro P, Salacone P, et al. Aplacebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetyl-carnitine treatment in men with asthenozoospermia . Fertility and Sterility. 2004; 81: 1578-1584.
34. Baker HW, Brindle J, Irvine DS, et al. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes. Fertility and Sterility. 1996; 65: 411-419.
35. Giovenco P, Amodei M, Barbieri C, et al. Effects of kallikrein on the male reproductive system and its use in the treatment of idiopathic oligozoospermia with impaired motility. Andrologia. 1987; 19: 238-241.
36. Moilanen J, Hovatta O. Excretion of alpha-tocopherol into human seminal plasma after oral administration. Andrologia. 1995; 27: 133-136.
37. Menkveld R, Stander FS, Kotze TJ, et al. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod. 1990; 5: 586-592.
38. Rolf C, Cooper TG, Yeung CH, et al. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double blind study. Human Reproduction. 1999; 14: 1028-1033.
39. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Human Reproduction Update. 2003; 9: 331-345.
40. Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. Urologic Clinics of North America. 2002; 29: 817-827.
41. Agarwal A, Said TM. Carnitines and male infertility. Reprod Biomed Online. 2004; 8: 376-384.
42. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology. 1996; 48: 835-850.
43. Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertility and Sterility. 2000; 74: 1200-1207.
44. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001; 8: 851-862.
45. Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. Journal of Reproduction and Fertility. 1987; 81: 459-469.
46. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod. 1989; 41: 183-197.
47. Comhaire FH, Christophe AB, Zalata AA, et al. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukotrienes and Essential Fatty Acids. 2000; 6: 159-165.
48. Alvarez JG, Storey BT. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. Gamete Res. 1989; 23: 77-90.
49. Alvares JG, Touchstone JC, Blasco L, et al. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl. 1987; 8: 338-348.
50. Jones R, Mann T. Lipid peroxidation in spermatozoa. Proc R Soc Lond B Biol Sci. 1973; 184: 103-107.

51. Sakkas D, Mariethoz E, St John JC. Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway. Exp Cell Res. 1999; 251: 350-355.
52. Hikim AP, Wang C, Lue Y, et al. Spontaneous germ cell apoptosis in humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. J Clin Endocrinol Metab. 1998; 83: 152-156.
53. Barroso G, Morshedi M, Oehninger S. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. Hum Reprod. 2000; 15: 1338-1344.
54. Hughes CM, Lewis SE, Mckelvey-Martin VJ, et al. A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Mol Hum Reprod. 1996; 2: 613-619.
55. Kodama H, Yamaguchi R, Fukuda J, et al. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril. 1997; 68: 519-524.
56. Comhaire FH, Christophe AB, Zalata AA, et al. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukotrienes and Essential Fatty Acids. 2000; 6: 159-165.