

Micronutrients in management of male infertility

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ABSTRACT

Male infertility is a common problem, which is gradually increasing in prevalence. The exact etiology can not be pinpointed in the majority of the cases, but seems to be due to increased oxidative stress.

This review summarizes the work done on micronutrients in management of male infertility, touching upon their efficacy and mechanism of action. [IJEM 2008;12(8):21-26]

Key words: Male infertility, reactive oxygen species, coenzyme Q, lycopene, zinc, l-carnitine, Vitamin C, Vitamin B12.

Defining the problem

Infertility is a common problem affecting one in six couples. WHO defines infertility as ‘Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year’(1).

The global incidence of infertility is about 13–18%, to which male infertility contributes approximately 50%. In India, nearly 10% of all married couples go childless despite unprotected intercourse. As in other countries, 50% of these shortcomings are in the male partner(2-4).

There is a growing body of scientific evidence supporting the idea that sperm counts have declined considerably over the last 50 years. Carlsen *et al* analyzed a total of 61 studies including 14,947 men from the years 1938 to 1991, for mean sperm density and mean seminal volume. Their results show a significant decline in mean sperm density from 113 million/ml in 1940 to 66 million/ml in 1990 ($p < 0.0001$). Seminal volume decreased from an average of 3.40 ml to 2.75 ml ($p = 0.027$)(5,6). This demonstrates a 20-percent drop in volume and a substantial 58-percent decline in sperm production in the last 50 years. Three other recent reports also found semen quality has declined among donors over the last 20 years(7-9). Because the decline in sperm production is relatively recent, one must suspect a combination of environmental, lifestyle, and dietary factors

might be interfering with spermatogenesis.

The management of male infertility starts with the efficient identification of the causative factors for male infertility. The etiology of male infertility includes environmental factors; acquired or developmental defects of the testis, prostate and sperm; systemic diseases; and hormonal and idiopathic causes. Infertility burden based on etiology is depicted in Table 1(10).

Table 1: Incidence of infertility based on cause

Etiology	Distribution
Sexual factors	1.7
Urogenital infection	6.6
Congenital anomalies	2.1
Acquired factors	2.6
Varicocele	12.3
Endocrine disturbances	0.6
Immunological factors	3.1
Other abnormalities	3.0
Idiopathic abnormal semen or no demonstrable cause	75.1

This table reveals that obvious factors cannot be pinpointed in the vast majority patients with infertility.

Known causes of male infertility are given in Table 2(11).

The World Health Organization (WHO) guidelines specify that the minimum sperm volume should be 2 mL, with a concentration of 20 million sperm cells/mL. At least

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50% of sperms should be motile and 30% should have normal morphology. However, there is no exact threshold under which sperm values can be considered abnormal. It has also been observed that a large fluctuation of sperm values is related to the duration of abstinence, the conditions of sperm collection, the season and possibly the time of the day(12). Ejaculate analysis has been standardized by the WHO and propagated by continuing work and publications in the WHO Laboratory Manual for Human Semen and Sperm-Cervical Mucus Interaction (4th edition)(1). The consensus is that modern spermatology has to follow these guidelines without exception.

Table 2: Known causes of male infertility

Sperm production problems	<ul style="list-style-type: none"> • Chromosomal or genetic causes • Undescended testis (failure of the testis to descend at birth) • Infections • Torsion (twisting of the testis in scrotum) • Heat • Varicocele • Drugs and chemicals • Unknown cause
Blockage of sperm transport	<ul style="list-style-type: none"> • Infections • Prostate-related problems • Absence of vas deferens • Vasectomy
Sperm antibodies	<ul style="list-style-type: none"> • Vasectomy • Injury of infection in the epididymis • Unknown cause
Sexual problems (erection and ejaculation problems)	<ul style="list-style-type: none"> • Retrograde and premature ejaculation • Failure of ejaculation • Infrequent intercourse • Spinal cord injury • Prostate surgery • Damage to nerves • Some medicines
Hormonal problems	<ul style="list-style-type: none"> • Pituitary tumors • Congenital lack of LH/FSH (pituitary problem from birth) • Anabolic (androgenic) steroid abuse.

Role of ROS in Male Infertility

Oxidative stress and the damage that results from it has been implicated in a wide number of disease processes. Reactive oxygen species (ROS) are ubiquitous and occur naturally in all aerobic species, coming from both exogenous and endogenous sources. Reactive oxygen species are quite reactive and readily damage biological molecules, including DNA(13).

Table 3: Overview of standard values for semen analysis according to the 1999 WHO criteria(1)

Volume	> 2.0 mL
pH	7.0-8.0
Sperm concentration	> 20 million/mL
Total no. of spermatozoa	> 40 million/ejaculate
Motility	> 50% with progressive motility or 25% with rapid motility within 60 min after ejaculation
Morphology	> 14% of normal shape and form*
Viability	> 50% of spermatozoa
Leukocytes	< 1 million/mL
Immunobead test (IBT)	< 50% spermatozoa with adherent particles
MAR test**	< 50% spermatozoa with adherent particles

* Assessment according to Kruger and Menkfeld criteria.

** MAR = Mixed antiglobulin reaction.

It has been observed that the human sperm cell exhibits the capacity to generate ROS and initiate peroxidation of the unsaturated fatty acids in the sperm plasma membrane. The limited diffusion of these molecules is consistent with their physiologic role in key biological events such as acrosome reaction and hyperactivation. The peroxidative damage induced by ROS, particularly H₂O₂ and the superoxide anion, has been proposed as a major cause of defective sperm function in cases of male infertility(13).

Reactive oxygen species can have both beneficial and detrimental effects on the spermatozoa and the balancing between the amounts of ROS produced and the amounts scavenged at any moment will determine whether a given sperm function will be promoted or jeopardized. The seminal plasma confers some protection against ROS damage as it contains enzymes such as catalase and superoxide dismutase that scavenge ROS(13).

Oxidative Stress and Sperm Motility

Excessive seminal ROS levels have significant toxic effects on both sperm quality and function. Decreased sperm motility, defective acrosome reaction and loss of fertility are found to be associated with increased seminal ROS. Production, nature, amount and duration of exposure to ROS determines the sperm cell dysfunction. In addition, the extent of ROS damage is also dependent upon surrounding environmental factors such as oxygen tension and temperature as well as the concentrations of molecular components such as ions, proteins, and ROS scavengers(14). It is found that low hydrogen peroxide concentrations suppress human sperm competence during oocyte fusion.

Decreased motility is a result of cascade of events including lipid peroxidation (LPO) of sperm plasma membrane that ultimately affect protein phosphorylation and sperm immobilization. Thus, reducing oxidative stress is important in management of infertility(14).

Oxidative stress and sperm chromatin damage

It has been shown that human spermatozoa express aryl hydrocarbon (dioxin) receptors, providing a mechanism by which environmental dioxins, polycyclic aromatic

hydrocarbons and polyhalogenated biphenyls could directly influence sperm function(15). In addition to damaging effect on all components of sperm, ROS have potential adverse effects on sperm nuclear DNA. Oxidative stress related damage to nonfunctional sperm is highly relevant.

Intracytoplasmic sperm injection (ICSI) technique, an effective therapy for severe male factor infertility, bypasses the majority of reproductive tract deficiencies but oxidative stress can cause defect in DNA of sperm. Thus, it is important to identify and treat DNA damage with antioxidants before planning ICSI. Sperm DNA damage is detrimental to reproductive outcomes.

Moreover, infertile men are suggested to carry more DNA damage than do the spermatozoa from fertile men. This DNA damage may likely increase the transmission of genetic diseases during the assisted reproductive procedures. Thus, evaluation of seminal ROS levels and extent of sperm DNA damage especially in an infertile male may help develop new therapeutic strategies and improve success of Assisted Reproductive Techniques (ART).

Micronutrients in management of infertility

Lycopene

Lycopene is a naturally synthesized carotenoid found in fruits and vegetables. It is also a component of the human redox mechanism that scavenges free radicals including ROS. It is found in high concentrations in seminal plasma and decreased levels have been demonstrated in men suffering from infertility(16).

Effective in Idiopathic Infertility

Oral lycopene therapy has a role in the management of idiopathic male infertility. Maximum improvement occurs in the sperm concentration with lycopene therapy(16). Cells exposed to lycopene secrete lycopene-enriched exosomes. Packaging into exosomes for export result in reduced degradation of antioxidant, and therefore maximize the effectiveness of delivery to the sites of action.

Thus, these organelles act as the transport vehicles for and have a role in the chemoprevention of male infertility. 15A clinical trial demonstrated improvement in sperm concentration in 66% patients, improved motility in 53% and 46% showed improvement in sperm morphology with lycopene therapy. In cases showing an improvement, the median change in concentration was 22 million/mL, motility 25% and morphology 10%. The improvement in concentration and motility were statistically significant. Higher baseline concentrations were associated with significant improvement(16).

Matos *et al.* investigated the effect of lycopene on lipid peroxidation and on the formation of 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodGuo) in an *in vitro* study. Cells supplemented with lycopene showed a reduction of 86% in lipid peroxidation. In addition, lycopene supplementation decreased in 77% the 8-oxodGuo levels.

These results indicate that lycopene can protect against

membrane and DNA damage and possibly play a protective role against oxidative damage(17).

Coenzyme Q10

Coenzyme Q10 is an important antioxidant which has good efficacy in improving sperm motility. Coenzyme Q10 is present in oxidized form and reduced form. Oxidized form is helpful in energy production and reduced form is helpful in preventing the sperm damage.

It is a liposoluble antioxidant that exists in mitochondria and has a role in causing oxidation resistance in male. It improves the sperm quality and reproductive ability of infertile patients as well as exerts the effects of an adjunctive therapy on male infertility. Coenzyme Q10 is significantly involved in mitochondrial bioenergetics and its antioxidant properties are at the basis of its role in seminal fluid. The increased concentration of Coenzyme Q10 in seminal plasma and sperm cells is associated with the improvement of semen kinetic features(18).

Increases Sperm Motility

Coenzyme Q10 is responsible for energy for movement and all other energy-dependent processes in the sperm cell. It prevents lipid peroxidation in sperm membranes. The enzyme is essential for maintenance of normal sperm motility. A study conducted by Lewin *et al.* also confirmed the effect of coenzyme Q10 in increasing sperm motility. Coenzyme Q10 showed a significant increase in sperm motility, with a motility rate of $35.7 \pm 19.5\%$, as compared to $19.1 \pm 9.3\%$ in the controls. Treatment with oral Coenzyme Q10, 60 mg/day, for a mean of 103 days caused significant improvement in fertilization rates (from $10.3 \pm 10.5\%$ in their previous cycles, to $26.3 \pm 22.8\%$ after coenzyme Q10) (Fig. 1)(19).

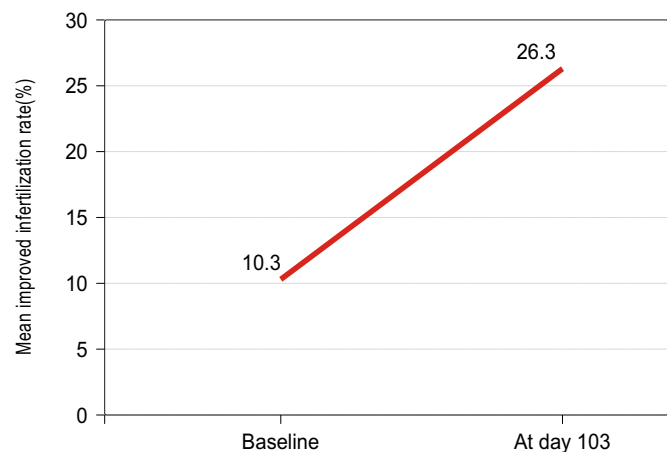


Figure 1: Improvement in fertilization rates with coenzyme Q10 treatment.

Role in asthenozoospermia

Reduction in levels of coenzyme Q10 is observed in sperm cells and seminal plasma of idiopathic and varicocele-

associated asthenozoospermic patients. It is suggested that ubiquinol (QH2)/ubiquinone (Q10) ratio might be an index of oxidative stress and its reduction. It is observed that sperm cells, characterized by low motility and abnormal morphology, have low levels of coenzyme Q10(20).

Presence of lower levels of coenzyme Q10 in idiopathic asthenozoospermic patients suggests the role of coenzyme Q10 in pathogenesis of asthenozoospermia(18). Thus, it can be concluded that coenzyme Q10 is effective and essential in maintaining normal sperm motility and morphology of sperm cells. A study conducted by Mohanty et al supported this in a well-structured clinical trial. Administration of coenzyme Q10 increased the pregnancy rate by 36% and with improvement of sperm count and functional sperm concentration in 70% and 60% individuals, respectively. In addition, there was significant improvement in sperm motility. Sperm motility and sperm motility index improved in 54% and 46% of individuals respectively; while 38% showed improvement in sperm morphology. There were no side-effects with good patient compliance (Fig.2)(21)

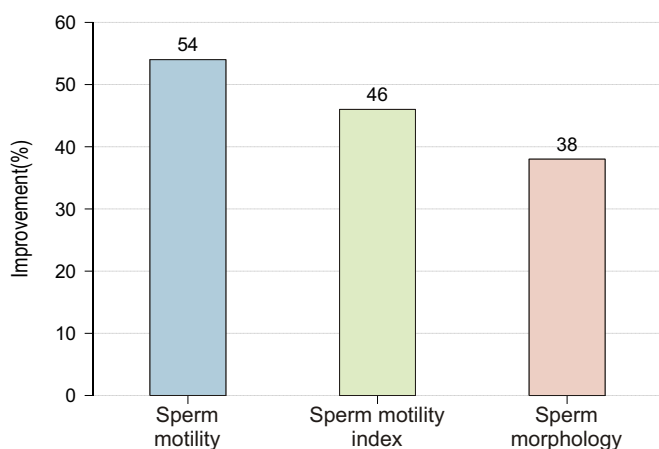


Figure 2: Improvement in sperm motility, sperm motility index and sperm morphology.

Carnitine

Infertility L-carnitine is a naturally occurring enzymatic antioxidant responsible for utilization of long chain fatty acids to produce energy. Excess of ROS in sperm cells and semen causes adenosine triphosphate (ATP) depletion leading to insufficient axonemal phosphorylation, lipid peroxidation and loss of motility and viability of sperm cells.

L-Carnitine has an important role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and the spermatogenesis process. Antioxidant therapy with carnitines may represent a new non-hormonal option within a broader therapeutic strategy in men with ROS-mediated infertility(22).

Improves semen quality

L-Carnitine is essential to maintain proper semen quality. Significantly high levels of free L-carnitine are observed in the seminal plasma of infertile men. The level of free L-carnitine in the semen has positive correlation with sperm concentration, sperm motility and vitality of sperm cells(23).

Improves sperm motility

L-Carnitine improves forward motility and viability of sperms. L-carnitine is effective in improving asthenozoospermia. It improves the spermatozoal motility, both quantitatively and qualitatively. In addition, oral administration of L-carnitine improves the spermatozoal output and might improve sperm quality at least in patients with idiopathic asthenozoospermia(24). In addition, it causes significant reduction in ROS production in sperms and helps in improving fertility. L-Carnitine is the only compound which is effective in infertility patients suffering from a bacterial prostatic-vesiculourethritis(25).

A study conducted by Shang et al reported that L-carnitine is helpful in asthenospermia patients with epididymal knob. In this study, 3-month therapy with L-carnitine, resulted in significant improvement in sperm concentration, forward sperm motility, total sperm motility, straight line velocity, curvilinear velocity and average path velocity in 78.13% of patients. It was concluded that oral L-carnitine therapy is efficacious for asthenospermia with epididymal knob(26).

Intraspermatic L-Carnitine and survival of sperm motility

Significant correlation between L-carnitine content and sperm motility survival in cervical mucus is reported in various studies. In cervical mucus, lipids are the energy source for sperm and to metabolize these lipids, intrasperm L-carnitine is essential. Thus, L-carnitine is an indicator of sperm motility life-span in cervical mucus. L-carnitine system modulates the reserves of free CoA, essential to the tricarboxylic acid cycle function. The intrasperm L-carnitine deficit could be due to following reasons(23).

- Alterations in the L-carnitine uptake mechanisms in the epididymis due to inflammatory processes.
- Lack of testosterone (L-carnitine uptake is androgen-dependent).

Thus, supplementation of L-carnitine has therapeutic value in male infertility. The supplementation is more important in presence of hypomotility due to intrasperm L-carnitine deficit(27).

Zinc

Zinc is a trace metal that constitutes a part of superoxide dismutase - a key enzymatic antioxidant. It is important to consume adequate amounts of zinc, since it is necessary to maintain the optimal functioning level of these enzymes(28).

Zinc is a potent antioxidant which helps in protecting

sperm against free radical. Each ejaculate contains 5mg of zinc, which is one third of the recommended daily nutrient intake. This suggests that zinc plays major role in sperm health. Apart from antioxidant function of zinc, it has following functions(29).

The genetic material (DNA chromatin) in the sperm nucleus is tightly wound with special proteins to form an insoluble, stable complex. This condensed structure is important for successful fertilization. Zinc is important for this structure and protects it from breaking down.

Zinc deficiency can cause early discharge acrosome reaction and sperm fails to penetrate the ovum resulting in infertility. High concentrations of zinc in semen reduce the acrosome reaction in a reversible way. Acrosome reaction reoccurs once zinc concentrations become diluted within the female genital tract. Deficiency of zinc causes inflammation of prostate gland. This slows down the speed of sperm traveling from testes.

Necessary for spermatogenesis and hormonogenesis

Significantly low serum and seminal plasma zinc levels are observed in oligospermic and azospermic infertile males with significantly low androgen. This suggests potential role in spermatogenesis and steroidogenesis. Thus, zinc concentration in seminal plasma is one of the factors responsible for decreased testicular function in infertile male subjects.

A study conducted by Ali *et al* confirmed the lower levels of zinc in infertile male. Results revealed significantly lower serum and seminal plasma zinc levels in oligospermic, and azospermic subjects when compared with normospermic control groups(30).

Effective in asthenozoospermia

Zinc has effect on cellular and humoral immunity by reducing the levels of antisperm antibodies and TNF-alpha and increasing IL-4. Zinc therapy causes higher T-helper cytokine, interleukin-4 level. Zinc causes significant improvement in the sperm quality; sperm count, progressive motility, fertilizing capacity and a reduction in the incidence of antisperm antibodies(23).

Oxidative stress with higher seminal malondialdehyde, TNF-, low total antioxidant capacity and glutathione peroxidase is observed in asthenozoospermia. Zinc as a monotherapy or in combination with other antioxidants, comparably improves sperm parameters with less oxidative stress, sperm apoptosis and sperm DNA fragmentation index (DFI)(25).

Vitamin C

Studies have shown the concentration of ascorbic acid 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 material. Fraga *et al* demonstrated this by reducing ascorbic acid intake in healthy men from 250 mg to 5 mg per day. Seminal plasma levels of

vitamin C decreased by 50 percent, with a concomitant 91-percent increase in sperm with DNA damage(31).

Cigarette smoking has been documented as having deleterious effects on sperm quality. In a University of Texas study on vitamin C and sperm quality in heavy smokers, 75 men were divided into three supplementation groups; one was given placebo, the other groups received 200 mg or 1000 mg ascorbic acid. While the placebo group showed no improvement, the ascorbic acid groups showed significant improvement in sperm quality, with the greatest improvement occurring in the 1000 mg group(32).

In one of the studies on vitamin C and male infertility, 30 infertile but otherwise healthy men were given a placebo, 200 mg, or 1000 mg vitamin C daily. After one week, the group receiving 1000 mg/day had a 140-percent increase in sperm count, while there was no change in the placebo group. The 200mg/day group had a 112-percent increase in sperm count, while both groups demonstrated significant reductions in the number of agglutinated sperm. Most importantly, by the end of the 60-day study every participant in the vitamin C group had impregnated their partner, while no pregnancies occurred in the placebo group(33).

Vitamin E

Vitamin E is a well-documented antioxidant and has been shown to inhibit free-radical- induced damage to sensitive cell membranes. 34 In one study, lipid peroxidation in the seminal plasma and spermatozoa was estimated by malondialdehyde (MDA) concentrations. Oral supplementation with vitamin E significantly decreased MDA concentration and improved sperm motility, resulting in a 21-percent pregnancy occurrence during the study(35).

In one randomized, cross-over, controlled trial, 600 mg/day vitamin E improved sperm function in the zona binding assay, therefore enhancing the ability of the sperm to penetrate the egg in vitro(36).

Nine men with low sperm count and alterations in sperm motility were given vitamin E with the antioxidant trace mineral selenium for six months. Compared to the baseline pre-supplementation period of four months, the combination of vitamin E and selenium significantly increased sperm motility and the overall percentage of normal spermatozoa(37).

Vitamin B12

Vitamin B12, in its various forms, has been studied for its effect on male infertility. Vitamin B12 is important in cellular replication, especially for the synthesis of RNA and DNA, and deficiency states have been associated with decreased sperm count and motility.

Methylcobalamin (1,500 mcg/day) was given to a group of infertile men for a period of 8-60 weeks. They were evaluated periodically by semen analysis, and standard sperm parameters were increased by 60 percent. 38 In another methylcobalamin study, 1,500 mcg/ day was given to 26 infertile men for a period of 4-24 weeks. Sperm analysis was

conducted eight weeks into the study. Sperm concentration increased in 38.4 percent of the cases and total sperm count increased in 53.8 percent of the men. Sperm motility increased in 50 percent of the participants. Serum LH, FSH, and testosterone levels were unchanged.³⁹ When 6000 mcg/day was given to men with low sperm count, it resulted in a 57-percent improvement⁽⁴⁰⁾. Vitamin B-12 (1000 mcg/day) was administered to men with a sperm count less than 20 million/ml. By the end of the study, 27 percent of the men had a sperm count over 100 million/ml⁽⁴¹⁾.

CONCLUSION

Male infertility is a multifactorial disease process with a number of potential contributing causes. Considering the majority of male infertility cases are due to deficient sperm production of unknown origin, environmental and nutritional factors must be evaluated. Occupational risk factors, including exposure to heat, chemicals, and heavy metals needs to be examined. Lifestyle and dietary choices, pesticide residues, and xeno-estrogens all may adversely affect spermatogenesis.

Spermatogenesis is an energetically demanding process, which requires an optimal intake of antioxidants, minerals, and nutrients. Various nutritional strategies have been presented which have a beneficial impact on sperm count, motility, and ultimately, fertility.

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