# The management of male infertility: from nutraceuticals to diagnostics

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## INTRODUCTION

Natural fertility is changing worldwide due to the impact of environmental exposure, population density, ethnicity, nutritional style and social constructs. Subfertility is one of the most intriguing menace for reproductive medicine, often concerning undiagnosed issues of male factor[1]. For the evaluation of male fertility, a medical and reproductive history, a clinical examination by a specialist and at least two semen analyses every 6 months should be comprised. However, not always these data are sufficient for a complete diagnosis. Additional analyses might be required as post-ejaculatory urinalysis, endocrine examination, medical ultrasound and genetic screening and anyhow an important rate of male with fertility problems cannot find an answer to clarify the origin of these problems. This because the male subfertility is a multifactorial disorder to all effects. Factors such as life style, diets and oxidative stress (OS) might be the causes of sperm quality decline[2-5]. In addition, considering that approximately 30% of the male reproductive age population is challenged with fertility problems, the repercussions of this condition on the worldwide population should not be underestimated. As reported, is likely to be a critical and plausible cause of idiopathic male infertility. OS is caused by the imbalance between the production of reactive oxygen species (ROS) and their neutralization or removal by the antioxidant system. Consequently, the excessive presence of ROS in the sperm induces damage to the proteins and lipids in plasma and mitochondrial membranes[6]. Moreover, chemical and structural modifications to DNA of spermatozoa have also been implicated to the action of ROS, reducing their ability to fertilize the oocyte[7]. Evidence from the literature was collected and analyzed in a qualified meta-analysis showing a correlation between ROS levels and *in vitro* fertilization (IVF) rate[8]. High ROS levels in spermatozoa have shown to impair sperm fertilizing ability and reduce pregnancy rates following IVF as well as embryo development to the blastocyst

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stage[9]. As OS is a heterogeneous phenomenon, supplementations with combined antioxidant vitamins and trace elements might be more appropriate and recommendable than a single element. Indeed, their pharmacodynamics features ensemble might improve the management of sperm OS and basic sperm parameters[10]. It has also been recognized that the restriction of nutrient intake or deficiency of particular nutrients may impair spermatogenesis[11]. Antioxidants, by scavenging ROS, are considered an effective therapy to protect gonadal cells and mature spermatozoa from oxidative damage. Indeed, intake of selective micronutrients has a good impact on sperm health and fertilizing capacity. A number of molecules commonly used for male infertility have been investigated for endocrine and antioxidant activity. An interesting evidence on supplementation of oral antioxidants in subfertile men comes from the Cochrane analysis that showed a significant increase in live-birth rate (pooled odds ratio: 4.21; 95% CI, 2.08-8.51; $p < 0.0001$, $I^2 = 0\%$) when compared to placebo[12].

## NUTRITIONAL THERAPIES

The word "Nutraceutical" was coined in the 1989 from "nutritional" and "pharmaceutical" terms, and it is today used to indicate food, or part of the food, which provides important benefits for human health, as prevention and/or treatment of disease[13]. In recent years, especially in industrialized countries, this concept has received an important boost towards the development of new therapeutic opportunities that can improve patients' health, by the contemporary identification of unbalanced food regimens. Poor heterogeneous diets, caused by environmental factors such as air pollution, stress, chemicals and other toxic agents in foods, have been considered in some studies as possibly responsible of the decreased male semen quality observed in the last 50 years[14-16]. Recently, other studies[17,18] evaluated the effects of specific nutrients and nutritional supplements on male infertility. The aim of this article is to highlight the activities of specific nutrients able to improve the male fertility, and more generally, the latest news relatively to male infertility.

### Myo-inositol

It is the most important stereoisomer of inositol, a carbocyclic polyol found in cereals, nuts, fruits as well as in human cells. Myo-inositol is synthesized from glucose-6-phosphate[19] and in the male reproductive system is mainly produced by follicle-stimulating hormone (FSH)-responsive Sertoli cells. It plays a pivotal role in the regulation of calcium intracellular concentration and as a second messenger in the cellular signal transduction system[20] and mediator in the insulin cascade[21]. The seminiferous tubule fluid contains greater levels of myo-inositol compared to the seminal plasma. Myo-inositol is found in high concentrations in the epididymis and its depletion has been linked to reduced fertility[22]. It is involved in many processes such as capacitation, acrosome reaction and regulation of sperm motility[23]. Furthermore, it restores mitochondrial cristae morphology and improves the mitochondrial membrane potential[24,25]. Myo-inositol plays a crucial role during spermatozoa migration through the epididymis and in stabilizing tubuline, a fundamental protein for sperm motility. A prospective double-blind randomized placebo-controlled study showed how myo-inositol supplemented for three months to idiopathic infertile men significantly increased the sperm concentration, the percentage of acrosome-reacted spermatozoa, total count and progressive motility compared to placebo, also rebalancing serum luteinizing hormone (LH), FSH and inhibin B concentrations[26].

### L-carnitine

It is highly concentrated in the epididymis and seminal fluid. It is involved in the mitochondrial β-oxidation of long chain free fatty acids[27]. Low levels of L-carnitine reduce fatty acid within mitochondria, reducing energy production and sperm motility[28,29]. L-carnitine administration results in an improvement in seminal parameters, increasing sperm quality and motility[30-33]. A study[33] carried out in Italy showed that the combination of L-carnitine and L-acetyl-carnitine for 6 months-treatment improved sperm motility, especially in men with asthenozoospermia. Another similar study[34] one year later confirmed the same results in addition to an improvement of the total oxyradical scavenging capacity of the seminal fluid in men with idiopathic asthenozoospermia.

### L-arginine

It is one of the most metabolically versatile amino acids, that can be found also in high concentration in haploid cells nuclei and it reaches a 50% level in spermatozoa nuclei. Several studies[35] have shown that exogenous L-arginine intake has multiple beneficial pharmacological effects. It stimulates the production of nitric oxide, thus assuring an adequate oxygen supply to muscles, whose stimulation at the level of either the cavernous bodies of the penis or the anococcygeal muscles causes penile erections;

indeed, few studies report an improvement of erectile function by l-arginine supplementation. Clinical observations indicate that oral administration to infertile men considerably increases overall percentage of healthy spermatozoa and motility resulting in successful pregnancies[36].

## Selenium

It is an essential trace element mainly found in grains, fish, meat, poultry, eggs. It is a constituent of selenoproteins that serves as structural components of mature spermatozoa, and is involved in some reproductive functions such as testosterone metabolism. Sperm capsular selenoprotein has an important structural role in spermatozoa in the form of glutathione peroxidase, which protects against peroxidation[37]. Furthermore, deficiencies of selenium and/or glutathione can lead to instability of the mid-piece, resulting in defective motility[38]. Selenium supplementation improves sperm characteristics of subfertile men from the mean total sperm count, concentration, normal morphology percentage and motility[39]. Combination of selenium with vitamin E has also shown to improve semen quality of male diagnosed with asthenoteratospermia or asthenospermia[38]. The recommended daily intake of Se is 55 µg.

## Vitamin E

It is a well-known antioxidant, located inside cell membrane, thus inhibiting free radical-induced damage. Vitamin E acts as a biological deterrent to lipid peroxidation. Oral supplementation of vitamin E significantly reduces malondialdehyde levels in spermatozoa and improves sperm motility[40]. An interesting study has indicated that vitamin E works synergistically with selenium enhancing their efficacy and increasing significantly sperm motility and the sperm counts[41].

## N-acetyl-cysteine

N-acetyl cysteine is a precursor of glutathione and is an excellent scavenger of hydroxyl radicals. Many studies[42,43] have shown its action in improving semen parameters such as volume, motility, and viscosity. Its administration is enhanced by the simultaneous intake of selenium. Indeed, it has been shown that their combination improves serum testosterone levels as well as the parameters of sperm quality in infertile men with idiopathic oligo-asthenoteratospermia[39]. The effect of N-acetyl cysteine in improving semen quality was also shown in patients following varicocelectomy[44].

## Folic acid

It is one of the most important vitamins because it is involved in cell development and is essential for DNA, transfer RNA and protein synthesis. Alteration of folic acid metabolism has been linked to male subfertility. In a murine model it has been shown that a paternal insufficient folate intake alters the mouse sperm epigenome negatively impacting on pregnancy outcomes[45]. In oligospermia and asthenospermia a pathologic increase in homocysteine has been observed. Folic acid plays a fundamental role in the treatment of decreased reproductive function as well as in the improvement of spermatic quality. In a placebo-controlled study, folic acid and zinc had proven to increase significantly the concentration of spermatozoa improving the morphology of the same[46].

## Zinc

It is a trace mineral essential for normal functioning of the male reproductive system. Zinc deficiency has been linked with male sterility and subfertility, playing a crucial role in normal testicular development and spermatogenesis[47]. It affects the stability of sperm chromatin and in general biological membranes as influences the fluidity of lipids[48,49]. Exposure to stress, cigarette smoke, pollution, and alcohol can deplete zinc and its deficiency has been linked also to low sperm counts and testosterone levels[50]. A recent meta-analysis indicated that the seminal plasma zinc concentrations were significantly lower in infertile men compared to those from normal controls. These results were harmonized by a significant improvement of the sperm volume, motility and morphology following zinc supplementation in infertile males[51].

## Coenzyme Q10

It is a molecule acting as a liposoluble chain-breaking antioxidant[12]. It has a pivotal role in the energy metabolism for cell membranes and lipoproteins. A meta-analysis found a statistically significant increase of seminal concentration, sperm concentration and motility after supplementation of coenzyme Q10[52]. However, there are no available data regarding the live birth rate and any positive effect on pregnancy rates.

## Omega-3

Omega-3 fatty acids are polyunsaturated fatty acid (PUFA) acquired through the food consumption and the main ones are docosahexaenoic acid

(DHA), eicosapentaenoic acid (EPA) and a-linolenic acid (ALA). Normally, these molecules are fundamental for the cell membrane constitution for their role in the maintenance of lipid bilayer properties[53]. The importance of their role is even more clear for the spermatozoa membrane, where lipids guarantee fluidity and flexibility of spermatozoa, and successful fertilization[54]. At this regard, some studies evaluated the membrane composition of spermatozoa[55] from asthenozoospermic, oligozoospermic and normozoospermic men and most interesting data highlighted lower levels of DHA in the pathological conditions[55-57]. Moreover, higher omega-3 levels and ratios were found directly correlated with improved sperm motility, concentration, and structure[53,54]. Since omega-3 fatty acids are widely available and have an excellent safety profile, they can be used as nutraceuticals that improve semen quality. Nowadays, many different studies investigated the effect of a balanced diet on the semen quality, reporting that higher consumption of fruits, green vegetables, fish, chicken, whole grains, and low-fat dairy products associated with a reduced intake of meat, processed foods, sweets, and high-fat products is able to improve the semen quality. Generally, intake of high levels of saturated fatty acids as well as the presence of preservative agents or hormonal residues represents a threat for the male fertility[58-60]. Deleterious effects of dietary factors can impair spermatogenesis, reduce sperm concentration and motility, and increase sperm DNA damage, especially in infertile obese men with diabetes, dyslipidemia, or metabolic syndrome, who are at increased risk of OS in the testicular microenvironment or the excurrent ductal system[61]. For this reason, an increased intake of antioxidants can help to obtain a better sperm quality[58,60,62-66], referred as parameters defined in the WHO guidelines of 2010[67]. Table 1 illustrates the main nutrients, which are benefits for spermatozoa, further improving sperm quality by their synergistic action.

## LIFESTYLE AND SEMEN QUALITY

As just reported, the adoption of unbalanced diet and/or an incorrect lifestyle represents an alarm bell for the male fertility. In fact, male sedentarism/obesity negatively impacts fertility, reducing total testosterone and luteinizing hormone (LH) levels[68] with consequent changes in sperm quality. A number of studies have shown how exercise training may be useful for improving reproductive function in infertile patients[69,70]. Although, excessive physical exercise compromises testicular androgen secretion[71]. Over-trained men might have serious complications on the hypothalamic regulation of

**Table I.** Supplements.

| Supplement | Description | Metabolic Effects |
|---|---|---|
| Myo-inositol | Carbocyclic polyol found in cereals, nuts, fruits | Promotes spermatozoa maturation and motility; restores mitochondrial cristae morphology and the mitochondrial membrane potential |
| Vitamin E | Antioxidant | Reduces levels of MDA that cause toxic stress in cells and improves sperm motility |
| ↕ | | |
| Selenium | Essential trace element | Regulates spermiogenesis, promoting motility, increasing the number and quality of the spermatozoa. |
| ↕ | | |
| N-acetyl-cysteine | Aminoacid and antioxidant | Reduces sperm viscosity improving motility as well as sperm quality |
| L-carnitine | The biologically active form of carnitine, a small amino acid derivative | Improves sperm quality and increases mitochondrial fatty-acid oxidation reducing cell death in the testes |
| L-arginine | Conditionally essential amino acid | Increases sperm counts and motility |
| Coenzyme Q10 | Antioxidant | Increases sperm concentration and motility |
| Folic acid | Vitamin B complex | Improves sperm quality |
| ↕ | | |
| Zinc | Trace mineral essential | Improves sperm volume, motility and morphology |
| Omega-3 | Fundamental for the cell membrane constitution | Guarantees fluidity and flexibility of spermatozoa, and successful fertilization |

Supplements improving sperm quality. The synergistic action of nutrients is indicated by the arrow.

gonadal hormones, causing idiopathic hypogonadotropic hypogonadism[72]. Furthermore, the use of anabolic androgenic steroids suppresses spermatogenesis, reduces testicular volume, with a negative feedback on the hypothalamic-pituitary-gonad axis. Changes in the hormonal levels such as LH, follicle-stimulating hormone, testosterone or prolactin may adversely impact male fertility[73]. Smoking habit is associated with a reduction in sperm count and motility, induction of OS as well as an increase in atypical morphologies, aneuploidy and sperm DNA damage. It has also been found that the presence of pesticides (carbon tetrachloride, pentachlorophenol, diazinone etc.) causes sperm quality alterations in a dose-dependent way. As might be expected, the greatest reduction of seminal liquid parameters is recorded in the most industrialized and developed countries, and spermatozoa motility belongs to these parameters. The exposition to fine particles, lead and carbon monoxide, present in the air and in many types of food, determined over time an increase of the concentration of these same substances at the testicular level, leading to a drastic reduction not only of the number but also of spermatic quality, in addition to an increased risk of male mammary tumor, cryptorchidism and hypospadias[74].

## DIAGNOSTICS OF MALE INFERTILITY

Male infertility is best tested by determining the ability of sperm to achieve a successful pregnancy. Nowadays, the analysis of seminal fluid is evaluated according to the World Health Organization criteria including semen volume, sperm concentration, total number of spermatozoa, total motility, sperm morphology and viscosity[75]. However, the evaluation of these parameters indicates only partially the sperm quality and the fertilizing capacity. Myo-inositol has a role in the chemotaxis and human sperm thermotaxis, via the phospholipase C signaling pathways; the production of inositol 1,4,5-trisphosphate ($InsP_3$) modulates $Ca^{++}$ intracellular concentrations through the activation of its receptors, resulting in the calcium channels opening and the release of $Ca^{++}$ internal stores. This increases the $Ca^{++}$ intracellular concentrations in the flagellum[76]. This mechanism increases the cytosolic and mitochondrial $Ca^{++}$ triggering the oxidative metabolism and the ATP production. In spermatozoa, the key site of ATP production is the inner mitochondrial membrane, where a chemiosmotic proton gradient, usually translated into the mitochondrial membrane potential (MMP), is required[77]. MMP can be considered an important laboratory parameter as a predictor of male fertility. Few recent publications[25,78] have shown the effect of myo-inositol in significantly increasing the number of spermatozoa with high MMP and significantly reducing the number of those with low MMP in patients with oligozoospermia.

Andrositol test (Patent number 2764361, Lo.Li. Pharma, Rome, Italy), is a diagnostic test that measures the MMP categorizing the seminal fluid in three different groups: Low, Medium and High responders. A semen sample identified as medium or high responder contains an underperforming mitochondrion with a low MMP. In this condition, a nutritional therapy would be recommended to improve the sperm quality before trying to conceive. Therefore, this innovative test can be associated to the standard analyses as a non-invasive method to evaluate the sperm functionality and the potential to achieve a pregnancy.

## DNA FRAGMENTATION

Assessment of sperm DNA integrity has been evaluated for correlation with inability to conceive by intercourse, intrauterine insemination or intracytoplasmic sperm injection (ICSI). Elevated seminal ROS as well as DNA fragmentation present in idiopathic infertile men contribute to their impaired reproductive potential[79]. However, there are conflicting results about the correlation between sperm DNA fragmentation and the outcome of IVF or ICSI[80,81]. Antioxidants and micronutrients have the propriety to protect somatic and germ cells against age-associated genomic damage. Indeed, a study has shown how older men with a high intake of micronutrients and antioxidants had the same levels of sperm damage as younger men[82].

## CONCLUSIONS

Despite the knowledge collected in the last fifty years, an important rate of male with fertility problems cannot find an answer to clarify the origin of these problems. The recent innovations to help a patient affected by this rising problem is a multi-faceted treatment based on new diagnostics approaches, changes of lifestyle, dietary support of an expert nutritionist and an integrative therapy targeted. Nutritional strategies have been endorsed with a beneficial impact on sperm count, motility and fertility. The modern medicine is continuously evolving and should stay abreast of this pioneering concept of food and health benefits. At this regard, patients should be nutritionally educated and oriented to treat male infertility at the root of the problem. A healthy lifestyle should be the mainstay of management.

**Conflicts of interest:**
The Authors declare that there are no conflicts of interest.

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