Antioxidants and Male Infertility

43

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Key Points

- Oxidant radical excess has been associated with male infertility. In particular many authors have focused on the role of reactive oxygen species (ROS).
- Spermatozoa are very susceptible to oxygen effects because their membranes are rich in polyunsaturated fatty acids that assure fluidity and flexibility; this one makes spermatozoa more vulnerable to lipid peroxidation.
- The most important sources of ROS in semen are immature spermatozoa and leucocytes, and the protective antioxidant system in semen is divided into enzymatic factors, such as superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxins, and nonenzymatic factors.
- Carnitine, coenzyme Q10, vitamin E, vitamin C, zinc, and myo-inositol are the most important nonenzymatic systems for which a seminal parameter improvement was demonstrated.
- There are emerging evidences that herbal products can also improve male reproductive functions.

43.1 Introduction

Infertility is defined as the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. Infertility affects an estimated 15% of couples globally, especially in developed countries, with around 20–50% due to male factors [1].

Unfortunately, many causes of male infertility are unknown, and for this reason, numerous studies have been carried out, analyzing gene expression, epigenetics modifications, and mostly the role of reactive oxygen species (ROS) and antioxidants.

It is well recognized that oxidative stress is a cause of male infertility, but the use of antioxidants as a treatment is still debated, and it is considered as a supportive therapy, rather than etiological or physiopathological, on the real effect of oral supplementation. Many models have been introduced to explore the role of different antioxidants in vitro, and some differences can be discovered regarding the protective effects exerted by specific enzymatic or nonenzymatic molecules.

43.2 The Role of Reactive Oxygen Species

An oxidant radical excess has been associated with male infertility. In particular many authors have focused on the role of ROS [2, 3, 4].

In the last 20 years, an assay called "TOSC" (total oxyradical scavenging capacity) was developed to measure the total capacity of the biologic fluids or cellular antioxidants that neutralize oxygen radical toxicity. This assay has been used by our group in andrology, and it helped us to show a reduced antioxidant capacity in infertile men's seminal fluid and a correlation between the scavenging action versus oxygen radicals and sperm cell parameters, in particular the motility [5, 6, 7]. Another parameter of antioxidant evaluation is the determination of total antioxidant capacity (TAC), the method that we employed in our laboratory [8], which is

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a modification of the method of Rice-Evans and Miller [9]. It is a kinetic assay, based on the reaction of H_2O_2-metmyoglobin with a chromogen (ABTS), whose radical species is spectroscopically detected. The latency time before the appearance of radical ABTS (lag phase or LAG, measured in sec) is proportional to the amount of small-chain-breaking molecules, which interrupt the propagation of oxidative reactions, mainly urate, ascorbate, and glutathione, and moreover, the rapid increase of ABTS is followed by a more gradual increase, in which antioxidant properties of proteins are involved. The interest of these parameters is based on its modulation by hormonal milieu: in fact we have shown that plasma LAG is lower in hypogonadism [10] and hypoadrenalism [11] and is influenced by growth hormone [12], another hormone involved in the control of fertility [13]. Even in seminal plasma, TAC exhibits a modulation by endocrine factors, since it is inversely related to systemic thyroid hormone levels [14].

Oxidative stress is determined when there is an imbalance between the production of ROS and the neutralizing activity of antioxidant system. It leads to a pathological and often irremediable cell damage. In particular spermatozoa are very susceptible to oxygen effects because their membranes are rich in polyunsaturated fatty acids that assure fluidity and flexibility; this one makes spermatozoa more vulnerable to lipid peroxidation [15]; in addition their cytoplasm is poor in scavenging systems. On the other hand, ROS are important for some processes like the sperm chromatin condensation, capacitation, acrosome reaction, cell signaling, and sperm motility [16].

The most important sources of ROS in semen are immature spermatozoa [17] and leucocytes [18], and the protective antioxidant system in semen is divided into enzymatic factors, such as superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxins [19], and nonenzymatic factors.

43.2.1 Nonenzymatic Systems

The first line of studies about antioxidants and fertility concerns dietary influences; in fact it is known that fertility is related to lifestyle [20, 21] and is an index of general health of men [22]. We have shown that modification of the diet in patients with metabolic syndrome, with enrichment of antioxidant by seasonal vegetable and fruits, has a synergy with metformin in reducing insulin resistance [23]; preliminary data shows that, in patients with metabolic syndrome during treatment with metformin, such kind of diet can influence testosterone levels (Fig. 43.1). Even if fertility has not been investigated in this pilot study, hormonal effects could be obviously important [24]. Moreover, a recent review of the literature about the influence of diet in male fertility indicated that a healthy diet improves at least one measure of semen quality, while diets high in lipophilic foods, soy isoflavones, and sweets lower semen quality [25]. However, the more diffuse approach was the effect of exogenous administration of antioxidants. We focused our attention on the main natural antioxidants, the efficacy of which has been supported by clinical trials. In particular our group focused on the study of coenzyme Q_{10} and carnitine, but we will also review the other main antioxidants.

43.2.1.1 Carnitine

L-carnitine (LC) or 3-aminobutyric acid is involved in intermediary metabolism being a shuttle of the activated long-chain fatty acids (acyl-Coa) into the mitochondria. The high levels found in epididymal fluid (2000 times higher than blood concentration) suggest the central role in sperm cell metabolism. There are evidences that show a positive correlation between an increase of L-carnitine in the epididymal lume and L-acetyl-carnitine (LAC) in sperm cells and initial sperm movement [26, 27]. Our group performed a 6-month double-blind randomized placebo-controlled trial using LC or LAC or combined LC and LAC treatment in infertile males affected by idiopathic asthenozoospermia [28]. The evaluation of the effectiveness of these treatments in improving semen kinetic parameters and the variation of TOSC in semen after treatment was the end points of the study. Sixty patients (mean age, 30 years) affected by idiopathic asthenozoospermia have been enrolled in the study, and all subjects underwent medical screening, including history and clinical examination, and presented a clinical history of primary infertility >2 years. The selected patients were submitted to a double-blind therapy of LC (10 ml phials containing 3 g/day orally of Carnitene – Sigma Tau, Italy, n. 15 patients), LAC (tablets containing 3 g/day orally of Zibren – Sigma Tau, n. 15 patients), and a combination of LC (10 ml phials containing 2 g/day orally of Carnitene) and LAC (tablets containing 1 g/day orally of Zibren) (n. 15 patients) or a seemingly identical placebo (each 10 ml placebo phial contains malic acid, sodium benzoate, sodium saccharinate dihydrate, anhydrous sodium citrate, pineapple flavoring, and demineralized water; each placebo tablet contains a core with l-hydro lactose, magnesium stearate, polyvinylpyrrolidone, and cornstarch and a coating with cellulose acetophtalate, dimethicone, and ethyl phthalate, Sigma Tau, Pomezia, Rome, Italy). All patients assumed a total of one phial and two tablets three times a day. The study design was 1-month run-in, 6 months of therapy (45 patients) or placebo (15 patients), and further 3 months of follow-up (controls at months T − 1, T0, T + 3, T + 6, and T + 9). Our results demonstrated that patients treated with the combination of the two molecules improved significantly during the first 3-month period of the administration. A significant improvement in total sperm motility was found in patients to whom

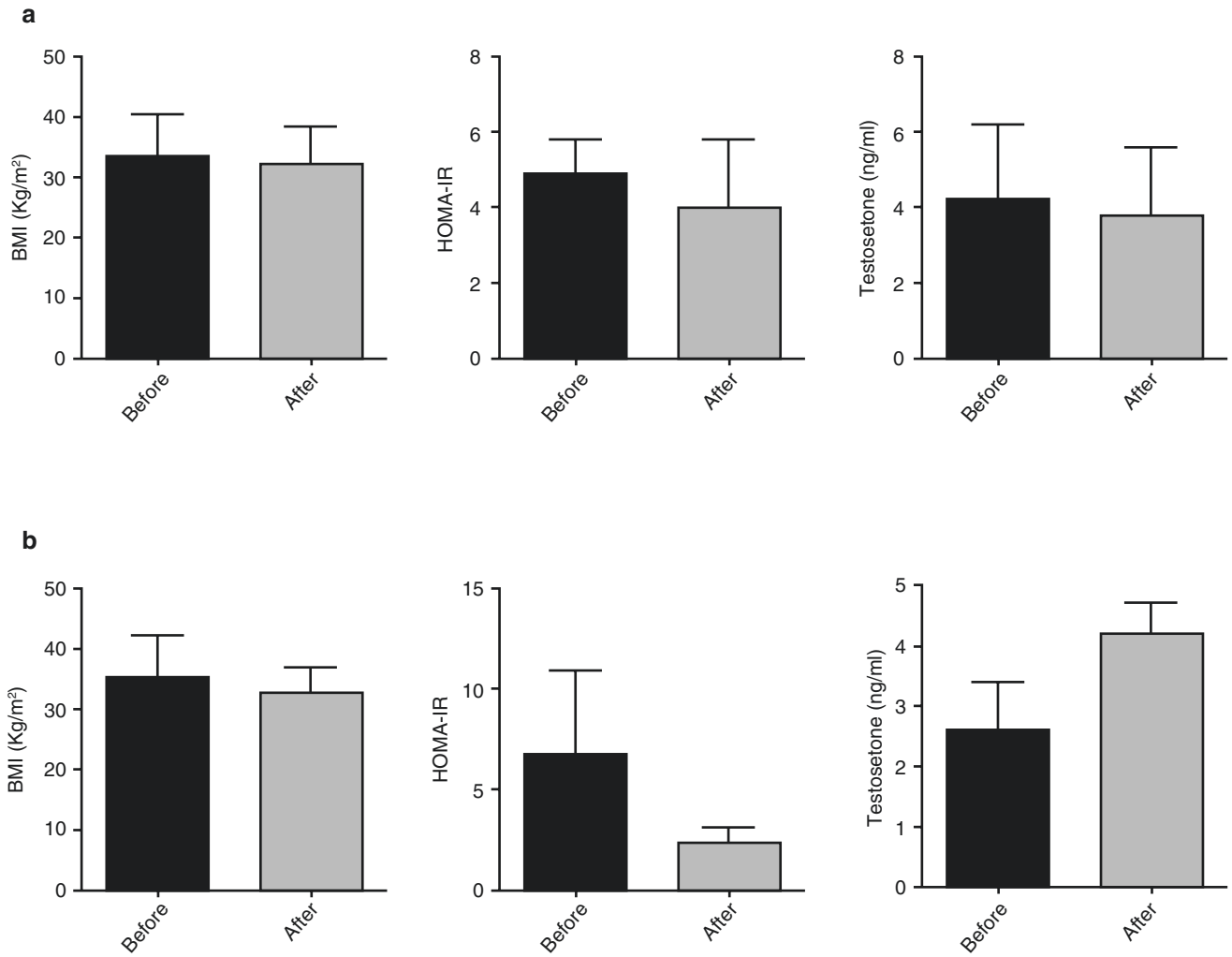

Fig. 43.1 Mean ± SEM levels of body mass index, HOMA-IR index, and testosterone levels in men with metabolic syndrome during metformin treatment: group A, hypocaloric diet; group B, hypocaloric diet enriched with natural antioxidants

LAC was administered, either alone or combined with LC. The analysis of forward sperm cell motility showed the same results. An improvement of forward motility was found when combined LC–LAC was compared with LC or LAC therapy alone, although the variations of kinetics sperm parameters were not significant. No significant modifications were found in placebo group. In all carnitine therapy groups, a significant dependence of the total and forward motility variations on the baseline values was found, and patients with lower baseline values of motility had a significantly higher probability to be responders to the treatment. Several controlled and uncontrolled studies support a potential positive effect of therapy with LC and its acyl derivatives in selected forms of oligo-astheno-teratozoospermia [29]. Lenzi et al. [30] demonstrated, in a controlled study, the efficacy of LC and LAC combined treatment in improving sperm motility, especially in patients with lower baseline levels. However, new evidence is needed to define the effective role and mechanisms of action of carnitine as an antioxidant.

43.2.1.2 Coenzyme Q10

Coenzyme Q10, also called ubiquinone, is an important component of the mitochondrial oxidative phosphorylation process because of its role of redox link between flavoproteins and cytochromes in the inner mitochondrial membrane [31]. CoQ10 also plays an import role as antioxidant; it contributes to membrane fluidity and can participate in many aspect of redox control of the cellular signaling origin and transmission; it has a probable involvement in cellular proliferation [32]. Clinically the significance of antioxidant action of ubiquinone has been clarified by many studies on lipoproteins. In fact LDL proteins are very susceptible to oxidative stress, and reduced CoQ10 present in LDL is oxidized before vitamin E, and the appearance of fatty acid hydroperoxides occurs after the oxidation of ubiquinol [33]. It can be suggested a possible

role in male infertility; in fact spermatozoa are rich in mitochondria, and they are strongly subject to oxidative stress. The previous coenzyme Q10 studies were performed with the administration of this antioxidant in cohort of unselected infertile patients, and the endogenous levels of CoQ10 were not measured [34, 35]. The first analytical data are represented by two of our works. The first one shows that the levels of CoQ10 in seminal plasma correlated with sperm count and motility except in the varicocele population. We also studied the varicocele patients after surgical repair, and the correlation between cellular CoQ10 and motility was no more detectable in postoperative VAR patients.

We conducted two different studies that showed an increase in spermatozoa motility in idiopathic asthenospermic patients undergoing CoQ10 therapy [36, 37].

We investigated the potential therapeutic role of CoQ10 by administering CoQ10 to a group of 22 idiopathic asthenozoospermic infertile patients [37], classified according to the WHO 1999 criteria [38] as having <50% forward motile forms at two distinct sperm analyses and normal sperm morphology >30%.

An increase of CoQ10 was found in seminal plasma after treatment, the mean value rising significantly from 42.0 ± 5.1 at baseline to 127.1 ± 1.9 ng/ml after 6 months of exogenous CoQ10 administration ($p < 0.005$). A significant increase of CoQ10 content was also detected in sperm cells (from 3.1 ± 0.4 to 6.5 ± 0.3 ng/106 cells; $p < 0.05$). Similarly, PC levels increased significantly both in seminal plasma and sperm cells after treatment (from 1.49 ± 0.50 to 5.84 ± 1.15 μM, $p < 0.05$, and from 6.83 ± 0.98 to 9.67 ± 1.23 nmoles/106 cells, $p < 0.05$, respectively).

Regarding semen, a significant difference was found in forward motility of sperm cells after 6 months of CoQ10 dietary implementation (from 9.13 ± 2.50 to 16.34 ± 3.43%, $p < 0.05$). The improvement of motility was also confirmed by means of computer-assisted determination of kinetic parameters. A significant increase of VCL (from 26.31 ± 1.50 to 46.43 ± 2.28 μm/s, $p < 0.05$) and VSL (from 15.20 ± 1.30 to 20.40 ± 2.17 μm/s, $p < 0.05$) was found after treatment. No significant differences were found in sperm cell concentration and morphology.

This study indicates a significant improvement of kinetic features of sperm cells after 6 months of administration of CoQ10, both on the basis of manual and computer-assisted evaluation. Moreover, these results constitute the first demonstration that exogenous administration of CoQ10 increases its levels in seminal plasma and in spermatozoa.

Similar increases of CoQ10 concentration (two to three times higher than baseline value) are commonly found in blood plasma after chronic administration of the quinine [39]. Statistical analysis did not reveal any significant functional relationship among the therapy-induced variations of CoQ10 and kinetic parameters of spermatozoa, probably due to the low number of samples. Nevertheless, the good degree of association among these variables, according to Cramer's V index of association, supports the hypothesis of a pathogenetic role of CoQ10 in asthenozoospermia, according to previously reported data [40]. Improvement of the spontaneous pregnancy rate also suggests that this therapeutic approach is beneficial.

These results were confirmed by a double-blind, placebo-controlled clinical trial, also from our group [36]. The selected patients underwent a double-blind therapy with CoQ10 (Q-Absorb softgels, Jarrow Formulas LA, USA), containing 100 mg of CoQ10, lecithin, and medium-chain glycerides. Placebo had the same composition but the softgels did not contain any CoQ10. All patients were given a total of two soft capsules in two separate daily administrations, with meals. The CoQ10 dose was similar to that used in our previous open trial on male infertility.

The study design was 1-month run-in, 6 months of therapy (30 patients) or placebo (30 patients), and further 3 months of follow-up (controls at months T − 1, T0, T + 3, T + 6, and T + 9).

CoQ10 levels increased in seminal plasma after treatment, and a significant increase of CoQ10 content was also detected in sperm cells (from 2.44 ± 0.97 to 4.57 ± 2.46 ng/106 cells, $p < 0.0001$). Similarly, QH2 levels increased significantly both in seminal plasma and sperm cells after treatment (from 31.54 ± 10.05 to 51.93 ± 16.44 ng/ml, $p < 0.0001$, and from 0.95 ± 0.46 to 1.84 ± 1.03 ng/106 cells, $p < 0.0001$, respectively). No statistically significant modifications were found in the placebo group.

A significant improvement of sperm cell total motility (from 33.14 ± 7.12 to 39.41 ± 6.80%, $p < 0.0001$) and forward motility (from 10.43 ± 3.52 to 15.11 ± 7.34%, $p = 0.0003$) was observed in the treated group after 6 months (T + 6) of CoQ10 administration. No statistically significant modifications in kinetic parameters were found in placebo group.

A significant inverse correlation between baseline (T0) and T + 6 relative variations of seminal plasma or intracellular CoQ10 or QH2 content and kinetic parameters was also found in treated group. In fact, patients with lower baseline value of motility and levels of CoQ10 had a statistically significant higher probability to be responders to the treatment.

After washout (T + 9), sperm cell kinetic features (total and forward motility, VSL) resulted to be significantly reduced in treatment groups when compared with month T + 6.

Also the group of Safarinejad confirmed a positive effect of CoQ10 treatment on sperm motility [41], in particular in improving sperm count, motility, and morphology. Our more recent work investigated the protective effects of coenzyme Q10 and aspartic acid on oxidative stress and DNA damage in subjects affected by idiopathic asthenozoospermia [42].

We found that only CoQ10 seemed to play a protective role against oxidative stress and DNA damage, thus contradicting some previous findings, which suggested these effects also for D-Asp.

43.2.2 Vitamin E

Vitamin E (alpha-tocopherol) is a fat-soluble antioxidant vitamin that can neutralize free radicals and protect cell membranes against ROS. Vitamin E can also protect against DNA damage, as demonstrated by Greco et al. in a placebo-controlled study of infertile men [43]. In this study vitamin E didn't improve seminal parameters (motility and concentration). Other studies, instead, reported an improvement in motility or morphology or both with a combination of vitamin E and selenium [44].

43.2.3 Vitamin C

Vitamin C, known as ascorbic acid, is a water-soluble vitamin that plays a role as cofactor in several hydroxylation and amidation processes, and it can be found in seminal plasma. There are several studies that demonstrated that a vitamin C oral integration can improve sperm motility and count in men with idiopathic oligozoospermia [45]. Cyrus et al. demonstrated, in a double-blind randomized controlled trial, that ascorbic acid improved sperm motility but not sperm count [46]. Other studies investigated the effect of administration of vitamin C with other antioxidants with good results in terms of sperm concentration and motility [47].

43.2.4 Zinc

It has been hypothesized that even a zinc deficiency may contribute to unexplained forms of infertility. In fact zinc plays an important role in testicular development as well as in sperm maturation [48, 49]. A recent review demonstrated that seminal zinc levels were lower in infertile men and that zinc supplementation increased semen volume, sperm morphology, and motility [50]. Some studies have demonstrated that oral supplementation of zinc can improve sperm motility in men with idiopathic oligozoospermia and/or asthenozoospermia [51]. Low seminal plasma zinc levels have been associated with a decrease in fertility, and in oligospermic patients, there are lower zinc levels than in normozoospermic men [52]. Also in this case, the data are contradictory as numerous studies have not shown a statistically significant association between the zinc levels and the seminal parameters [53, 54].

43.2.5 Myo-inositol

Inositol is a polyalcohol and in nature is present in nine isoforms, and myo-inositol (MYO) is the most abundant. Recently it was studied the important role of MYO on male reproduction. Its concentrations are higher in seminiferous tubules than in serum, and it is secreted in response to FSH.

It has been suggested that MYO could be involved in some processes such as the regulation of motility, capacitation, and acrosome reaction of spermatozoa [55, 56].

MYO probably has a role in the osmoregulation of seminal fluid contributing to reduce viscosity and improve the progressive motility and velocities [57, 58].

Few studies have investigated the role of MYO as a possible antioxidant agent for the treatment of male infertility and to improve the quality of sperm used to medically assisted reproductive procedures. Colone et al. [59] demonstrated that MYO reduces the presence of amorphous material. At a functional level, MYO seems to increase the membrane potential [60] and improve the synthesis of DNA, tRNA, and proteins [61].

43.2.6 Herbal Remedy

There are emerging evidences that herbal products can also improve male reproductive functions. Recently it was demonstrated that *Eurycoma longifolia* Jack extract has, in an in vivo study, androgenic and pro-fertility effect [62, 63].

Cardiospermum halicacabum has been found to increase testosterone levels and sperm count and motility [64]. Also the *Tribulus terrestris* has long been identified for treating male infertility in Asia and Europe [65].

"MARJORAM essential oil" has demonstrated faculty to increase spermatogenic and sperm cells in a study in which degenerative changes in seminiferous tubules were determined by fatty diet [65].

43.3 Perspectives

Although several specific genetic causes of male infertility have been acknowledged, the etiology of many forms of infertility remains unknown. It would be desirable to deepen the study of the exogenous antioxidants in order to hypothesize new therapeutic horizons, especially for those patients who do not benefit from conventional therapies, and we will hope to standardize the dosage to produce new evidences.

43.4 Conclusion

Oxidant radical excess has been associated with male infertility; in fact spermatozoa are very susceptible to oxygen effects. Also healthy diet and antioxidant agents (such as carnitine, coenzyme Q10, vitamin E, vitamin C, zinc, and myo-inositol) are the most important systems for which it was demonstrated a seminal parameter improvement. There are emerging evidences that herbal products can also improve male reproductive functions. It needs more studies to confirm and refresh data from literature and to standardize new therapy for infertility.

43.5 Review Criteria

We performed an extensive research of reviews examining the relationship between antioxidants and male infertility using PubMed and MEDLINE. We considered the reviews published in the last 5 years, including the articles researched by the following keywords: "nutraceutical," "coenzyme Q10," "nutrition," "reactive oxygen species," "diet," "male infertility," "semen parameters," and "reproduction." Articles published in languages other than English were not considered.

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