Chapter 22
Oxidative Stress and the Use of Antioxidants for Idiopathic OATs

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Abstract  Aim: To examine the effects of ROS and OS on male fertility and to evaluate the use of antioxidants as a means of treatment to improve fertilization rates in subfertile males suffering from idiopathic oligoasthenoteratozoospermia (iOAT).


Results: Current research notes ROS-associated male factor infertility to be the most common potential etiology of impaired sperm quality. The various effects of these oxidants may be neutralized by antioxidants. Although antioxidant therapy has shown to potentially treat iOAT by improving semen parameters, its success remains limited. Our review calls for a deeper look and understanding of the type(s), dosage, and duration of antioxidant treatment used in order to apply its use in a clinical setting.

Keywords  Male infertility • Oxidative stress • Antioxidant treatment • Idiopathic oligoasthenoteratozoospermia • Antioxidants • Anti-inflammatory drugs
22.1 Introduction

Infertility affects eighty million people worldwide [1]. Male factor subfertility is now thought to account for up to 50% of these cases [2]. However, the majority of the causes remain unknown [3]. Oxidative stress (OS) is implicated in pathogenesis of 30–80% of male factor subfertility cases [4]. This state arises when there is an imbalance between reactive oxygen species (ROS) generation and the semen’s natural antioxidant defense.

Although the pathophysiological role of ROS in human sperm dysfunction and male infertility has been extensively explored in recent years, the etiology of suboptimal sperm quality has yet to be completely understood. There is substantial evidence to suggest that low levels of ROS are necessary for spermatozoa to acquire fertilizing capabilities [5, 6]. However, in excessive amounts, ROS can disrupt sperm maturation pathways. Exogenous factors such as pollution, high temperature, and electromagnetic radiation, and endogenous factors including infections, chronic disease, and autoimmunity can act as major sources of oxidative stress [4, 7, 8].

Endogenous seminal antioxidant compounds act as free radical scavengers to neutralize ROS. Semen of subfertile men was found to have lowered antioxidant levels compared to those found in healthy, fertile controls [9]. Idiopathic oligoasthenoteratozoospermia (iOAT) is considered one of the most prevalent causes of male infertility. iOAT is a disorder related to defects in sperm concentration, motility, and morphology and has been attributed to free radical-induced OS.

Although many treatments have been developed to overcome male factor infertility, no reliable strategy has been established. Intracytoplasmic sperm injection (ICSI), a highly efficacious but costly assisted reproductive technique to circumvent deficits in sperm function, has displayed some success in fertilization. A new area of study has focused on pharmacotherapy targeting the oxidative stress in iOAT. Men with a high dietary intake of antioxidants were reported to demonstrate improved semen quality [10]. This chapter investigates the effects of OS on semen quality, as well as the use of antioxidants as a potential treatment strategy to improve semen parameters, such as concentration, motility, and morphology, of men with iOAT.

22.2 Oxidative Stress

While oxygen is essential to sustaining normal cell function, the breakdown of its products may generate free radicals that can act as beneficial cell-signaling molecules or induce irreversible cellular damage and death [11]. The chemically unstable ROS by-products from aerobic cellular metabolism react almost instantaneously with neighboring species within their vicinity. The interaction of these stability-seeking agents causes them to propagate a cascade of reactions, which may ultimately disrupt and damage living cells and tissues. A few ROS include superoxide,
hydrogen peroxide, and the hydroxyl radical, as well as the common ROS subclass—reactive nitrogen species (RNS)—such as nitric oxide (NO).

Generally, free radical production is counterbalanced by several mechanisms that include both enzymatic and nonenzymatic antioxidants. However, in a period of imbalance between ROS and the body’s antioxidants OS ensues. OS may be a consequence of excess ROS production and/or reduced antioxidant capacity. The inability of the human biological system to detoxify/reduce oxidants or to repair detrimental damage disrupts physiological homeostasis. OS has been implicated in the pathogenesis of many other human diseases including cancer, diabetes, AIDS and Parkinson disease; however, only recently have ROS been considered toxic to human spermatozoa [12].

22.3 Effects on Sperm

Numerous studies continue to explore the role of ROS in male infertility. Virtually, every human ejaculate is thought to be contaminated with potential sources of ROS [13]. Physiologically, ROS are vital for modulating gene and protein activities for sperm proliferation, differentiation, and fertilization [6]. The exact levels and duration of normal exposure as well as the pathways that induce the pathological and physiological effects on sperm remain unclear.

ROS-induced sperm damage has been suggested to contribute to at least 40% of all male factor infertility cases [14, 15]. Excessive levels of nitric oxide have been shown to inhibit both motility and sperm competence for zona binding [12]. OS may impair spermatogenesis, impede sperm migration, and even cause abnormal sperm morphology and improper sperm function. Therefore, controlled regulation of ROS levels is essential for proper reproductive function.

22.4 Idiopathic Oligoasthenoteratozoospermia

iOAT is a complex medical condition that affects approximately 30% of all infertile men and is the most common cause of male infertility [16, 17]. As a three-part disorder, low sperm concentration and motility and morphologically abnormal sperm characterize iOAT. Three classifications have been put into place to evaluate the severity of the disease: (1) isolated astheno ± teratozoospermia (no alteration in sperm concentration); (2) moderate (sperm concentrations between <20×10⁶/mL and >5×10⁶/mL); and (3) severe (sperm concentrations <5×10⁶/mL) [18].

Although iOAT is often thought to be related to defects in spermatogenesis, its actual cause remains unknown and cannot be diagnosed using common laboratory techniques. In addition, a majority of the infertile patients suffering from iOAT have normal physical examinations, normal hormonal profiles, and no discernable cause for their subfertile status. The age of patients may have an adverse effect on sperm
motility and volume, which have been reported to continuously decrease from the age of 22–80 [19]. The lower metabolic rate frequently observed in older age is thought to contribute to this decrease in sperm motility.

Additional etiologies for iOAT have been suggested. An increase of ROS in the tubules and seminal plasma may cause apoptosis, consequently affecting sperm concentration, motility, and morphology [16]. ROS are also able to penetrate through the sperm membrane, thereby disrupting sperm motility. Leukocytospermia has been reported to have similar adverse effects; however, much controversy remains behind the exact mechanistic pathways and reasoning of the noted positive correlation [20].

Infection and the subsequent release of cytokines, including IL-6, IL-8, and tumor necrosis factor-alpha, have been associated with decreased sperm quality. Inflammation-mediated cytokines have been shown to induce ROS production, causing lipid peroxidation (LPO) of the sperm cell membrane [20]. A recent study conducted by Fraczek et al. revealed that cytokines released during the oxidative burst from inflammation intensify the degree of OS associated with leukocytes [21]. Furthermore, it has been reported ROS levels in whole ejaculates to be negatively correlated with sperm concentration ($r = -0.52, p = 0.0003$), motility ($r = -0.41, p = 0.006$), and morphology ($r = -0.34, p = 0.02$), while also correlating positively to seminal leukocyte concentration ($r = 0.65, p < 0.0001$) [22]. Another study that compared 167 infertile patients with 19 controls found that sperm concentration, motility, and morphology were significantly reduced, while the mean ROS levels were significantly higher in infertile subjects than controls [23]. The positive correlation between ROS levels in whole ejaculates and seminal leukocyte concentrations provides a rationale for treatment of OS-positive infertile patients with antioxidant supplementation and other strategies to control excessive leukocyte infiltration.

Cytoplasmic droplets have also been implicated in the pathogenesis of iOAT. These residues have been found to develop from defects in spermatogenesis. They contain large amounts of glucose-6-phosphate dehydrogenase (G6PD), which is known to generate NADPH. NADPH activation generates ROS production in spermatozoa via the action of NADPH oxidase, thereby promoting OS in the sperm membrane [24]. Interestingly, male infertility seems to have a familial relation among brothers and maternal uncles [25]. A genetic etiology is currently considered the most accepted theory for iOAT. Bujan et al. suggests an autosomal recessive mode of inheritance [26]. Thus, it is essential for future genomic and proteomic studies to focus on identifying possible genetic defects or mutations that could give rise to male infertility.

Oligozoospermia. Multiple mutations in mitochondrial DNA (mtDNA) have been correlated with oligozoospermic patient [27]. An in vitro study using a mouse model revealed that different proportions of mutated mtDNA had respiratory chain defects from meiotic arrest during spermatogenesis [28]. This indicated that respiration-deficient spermatocytes were incapable of completing meiosis and eliminated by apoptosis. The study confirmed that the mutated mtDNA that affected mitochondrial respiration function also resulted in not only oligozoospermia, but also asthenozoospermia and teratozoospermia. Moreover, since mtDNA is more susceptible than the
nuclear genome, any impairment of the electron transport chain consequently enhances ROS generation in mitochondria from incomplete reduction of oxygen [29]. Other reports indicate that mitochondrial dysfunction is associated with decreased membrane potential and increased production of superoxide anions, hydroxide radicals, and hydrogen peroxide [30, 31]. An experimental study suggests that genetic alterations found in sperm mtDNA may have occurred during cell differentiation, spermatogenesis, or from random segregation of mtDNA during cell division [32].

Asthenozoospermia. Impairment in sperm motility has been attributed to increased ROS generation [33]. Impaired motility is thought to result from a cascade of events, involving lowered axonemal protein phosphorylation and sperm immobilization, both of which are associated with reduced membrane fluidity that is essential for sperm–oocyte fusion [34]. It has also been hypothesized that membrane-soluble hydrogen peroxide may potentially diffuse across membranes into cells and become oxidized even further, thereby inhibiting the activity of enzymes, particularly G_{6}PD. This cytosolic enzyme controls the rate of glucose flux through the hexose monophosphate shunt, regulating the intracellular availability of NADPH. Spermatozoa use this as a source of electrons to stimulate the generation of ROS by NADPH oxidase [35]. Any impairment or inhibition of G_{6}PD leads to a decrease in the availability of NADPH and a concomitant accumulation of oxidized glutathione (GSH). Subsequently, this can lower antioxidant defense of spermatozoa and result in LPO [36]. Since energy production is critical in order to facilitate movement of sperm, any impairment in the process of ATP production may have a detrimental effect on sperm motility.

Teratozoospermia. Morphologically abnormal spermatozoa have been established as a predominant source of high ROS generation in human ejaculate [37]. The irregular structure is thought to result from impairments during spermatogenesis, whereby cytoplasmic extrusion pathways become impaired. As a result, spermatozoa released from the germinal epithelium during spermiation carry excess residual cytoplasm, and under such conditions, are believed to be immature and functionally defective [38]. Studies have shown that levels of ROS production in semen were negatively correlated with the percentage of normal sperm forms [39, 40].

22.5 Antioxidants

Antioxidants are naturally occurring or synthetic biomolecules that prevent free radical-induced damage by averting the formation of radicals, scavenging them, or promoting their decomposition in the body. Their neutralizing capabilities reside in their ability to donate an electron to ward off the deleterious effects of the highly reactive radicals or by converting ROS into different, less harmful molecules.

There are three main routes of defense antioxidants utilized in combating detrimental ROS levels: prevention, interception, and repair. The seminal plasma transition metals are capable of preventing ROS from initiating chain reaction. These chelating metals are vital in controlling LPO damage to sperm. However,
as these chelators become loosely bound to reduced oxygen products, they can generate secondary or even more reactive oxidants.

Certain antioxidants can intercept and impede free radical-induced chain reactions, forming nonradical end products. α-Tocopherol is a form of vitamin E antioxidant with chain-breaking/intercepting abilities \[41\]. It is capable of inhibiting LPO in membranes by scavenging ROS, such as the peroxyl and alkoxyl radicals. However, this system is highly regulated and α-tocopherol’s ability to maintain a steady rate of radical reduction in the plasma membrane is dependent on its recycling by external reducing agents. Therefore, α-tocopherol is able to function even at low concentrations.

The last mechanistic process of defense employed by antioxidants is to simply repair any damage caused. In cases of minimal oxidative damage, normal physiological function may be restored. However, if OS-induced damage subsists, spermatozoa are incapable of repairing this level of injury. This is often due to the lack of an intricate cytoplasmic enzyme/antioxidant system in mature sperm that is required to treat this type of damage, which demonstrates the high susceptibility of spermatozoa to oxidative insults \[42\].

Antioxidants come in a variety of forms, ranging from those generated endogenously by the body to those acquired exogenously through the consumption of certain foods or dietary supplements. When the natural balance between oxidants and antioxidants within the body is perturbed via antioxidant deficiency or overwhelming ROS production, the subsequent adverse effects have been found to be diminished, and sometimes resolved, through bodily antioxidant defense and supplementation \[43\]. Since human spermatozoa membranes are rich in unsaturated fatty acids, they are sensitive to oxygen-induced damage mediated by LPO.

Normally, the seminal plasma is well endowed with an array of antioxidant defense mechanisms to quench ROS and protect against any possible damage to spermatozoa. The variety of pathways compensate for the deficiency in cytoplasmic enzymes in sperm. Potential anti-ROS enzymes and other low molecular weight nonenzymatic substances make up the total antioxidant capacity (TAC) of the seminal plasma. Fertile men were reported to have a higher, more effective TAC in comparison to infertile male subjects \[44\]. If the male reproductive system becomes compromised by infection or any impairment in spermatogenesis that elevates ROS levels, antioxidant defense mechanisms may be overwhelmed and depleted, and thus result in OS. Given that various studies implicate OS in poor sperm samples, medical treatments that reduce or protect against OS may be an appropriate strategy to alleviate infertility in men with iOAT.

### 22.6 Antioxidants: A Practical Approach for Treating iOAT

As a multifactor disorder, iOAT presents a clinical challenge in the diagnosis and medical treatment of infertile men. ICSI has enabled iOAT to be circumvented mechanically; however, it fails to address the fundamental factors contributing to male factor infertility. Oral supplementation of hormones and antioxidants has been
the primary area of focus for pharmacotherapy treatment of iOAT. Significant improvements in sperm concentration, motility, and morphology have been reported [45]. Therefore, if it were possible to achieve a particular regimen of antioxidant(s) with the proper dosage and duration of intervention necessary to fully repair each aspect of the disorder, these men would have a greater probability of gaining normal sperm function.

In order to consider a drug as active, it must improve sperm parameters and pregnancy rates in at least one blind, prospective, placebo-controlled trial, as well as additional trials from independent groups [46]. The aim of the following sections is to evaluate evidence gathered from experimental trials with oral antioxidants that may serve in potentially treating impaired sperm quality. The mode of action of each antioxidant will be discussed and evaluated for their clinical efficacy. Table 22.1 is provided to summarize collected data, along with critical commentary for each antioxidant.

### 22.7 Carnitine

Carnitine is a water-soluble antioxidant derived mostly from the human diet. Some sources include fish, meat, poultry, and dairy products. It is formed from the biosynthesis of lysine and methionine. Carnitine is found in nearly all cells of the body and is essential in sperm energy production by transporting long-chain fatty acids from the cytosol into the mitochondria. In addition, carnitine is involved in the removal of cytotoxic compounds from the cell. Normally, the body produces a sufficient amount of carnitine to meet daily needs; however, in some cases individuals are incapable of producing enough. For instance, vegetarians were found to be significantly deficient in carnitine as opposed to an omnivorous control group [47].

Carnitine is also important with respect to many properties specific to sperm maturation. In a meta-analysis by Ross et al., oral supplementation with carnitine improved spermatozoa motility in three out of four randomized controlled studies [48]. Intra- and extracellular carnitine may be essential in sperm energy metabolism, and thereby provide the fuel for posttesticular processes involved in sperm motility. Motile spermatozoa have been found to have a greater degree of carnitine acetylation than immotile spermatozoa [49]. Moreover, free-L-carnitine is acetylated only in mature spermatozoa [50]. Studies have suggested that the initiation of acquiring sperm motility occurs parallel to an increase in carnitine concentration in the epididymal lumen and acetyl-L-carnitine in spermatozoa [49, 50]. Carnitine that accumulates in the epididymis, in both free and acetylated forms, is utilized by spermatozoa for mitochondrial β-oxidation of long-chain fatty acids, as well as for enhancing the cellular energetic in mitochondria. This is done by facilitating the entry and utilization of fatty acids and restoring the phospholipid composition of its membrane by decreasing fatty acid oxidation. The resulting increase in metabolic rate allows for a greater production of energy and increased sperm motility. Table 22.1 summarizes the most recent studies on carnitine supplementation for improving sperm quality.
<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
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<tbody>
<tr>
<td>Costa et al. [51]</td>
<td>Cross-sectional</td>
<td>100</td>
<td>LC (3 g/day)</td>
<td>4m</td>
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<td>↑ Motility (p≤0.001)</td>
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<td>✅ Rapid linear progression (p≤0.001)</td>
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<td>Vitali et al. [52]</td>
<td>Case–control</td>
<td>47</td>
<td>LC (3 g/day)</td>
<td>3m</td>
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<td>↑ Motility (80%)</td>
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<tr>
<td>Lenzi et al. [53]</td>
<td>Double-blind, placebo-controlled, crossover</td>
<td>86</td>
<td>LC (2 g/day)^a; Placebo^b</td>
<td>2-m wash-out + 2-m therapy/placebo + 2-m wash-out + 2-m placebo/therapy + 2-m wash-out</td>
<td>20–40</td>
<td>↑ Total motility^A,B (p=0.04)</td>
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<td>↑ Forward motility^A,B (p=0.05)</td>
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<td>↑ Concentration^A,B (p=0.01)</td>
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<td>↑ Linearity^A,B (p=0.03)</td>
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<td>Cavallini et al. [54]</td>
<td>Double-blind, randomized controlled</td>
<td>195 (39^A; 44^B; 47^C)</td>
<td>LC (2 g/day) + LAC (1 g/day)^a; LC (2 g/day) + LAC (1 g/day) + Cinnoxicam (30 mg/4 day)^b; Placebo^c</td>
<td>6-m intervention + 3-m follow-up</td>
<td>27–40</td>
<td>↑ Motility^A,B (p&lt;0.05)</td>
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<td>✅ Concentration^A,B (p&lt;0.05)</td>
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<td>✅ Morphology^A,B (p&lt;0.05)</td>
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<td>✅ Pregnancy rates^A,B,a</td>
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<tr>
<td>Lenzi et al.</td>
<td>Double-blind, randomized controlled</td>
<td>56 (26&lt;sup&gt;A&lt;/sup&gt;; 30&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>LC (2 g/day) + LAC (1 g/day)&lt;sup&gt;B&lt;/sup&gt;; Placebo&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2-m wash-out + 6-m intervention + 2-m follow-up</td>
<td>↑ Total motility&lt;sup&gt;A&lt;/sup&gt; (p&lt;0.05)</td>
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<td>↑ Forward motility&lt;sup&gt;A&lt;/sup&gt; (p&lt;0.05)</td>
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<td>↑ Concentration&lt;sup&gt;A,B&lt;/sup&gt; (p&gt;0.05)</td>
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<td>↑ Morphology&lt;sup&gt;A,B&lt;/sup&gt; (p&gt;0.05)</td>
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<td>Balercia et al.</td>
<td>Double-blind, randomized controlled</td>
<td>59 (15&lt;sup&gt;A&lt;/sup&gt;; 15&lt;sup&gt;B&lt;/sup&gt;; 14&lt;sup&gt;C&lt;/sup&gt;; 15&lt;sup&gt;D&lt;/sup&gt;)</td>
<td>LC (3 g/day)&lt;sup&gt;A&lt;/sup&gt;; LAC (3 g/day)&lt;sup&gt;B&lt;/sup&gt;; LC (2 g/day)&lt;sup&gt;B&lt;/sup&gt; + LAC (1 g/day)&lt;sup&gt;C&lt;/sup&gt;; Placebo&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1-m wash-out + 6-m intervention + 3-m follow-up</td>
<td>↑ Total motility&lt;sup&gt;A,B,C&lt;/sup&gt; (p&lt;0.05)</td>
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<td>↑ Forward motility&lt;sup&gt;A,B,C&lt;/sup&gt; (p&lt;0.05)</td>
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<td>↑ TAC&lt;sup&gt;A,B,C&lt;/sup&gt; (p&lt;0.05)</td>
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<td>Sigman et al.</td>
<td>Double-blind, randomized controlled</td>
<td>21 (12&lt;sup&gt;A&lt;/sup&gt;; 9&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>LC (2 g/day) + LAC (1 g/day)&lt;sup&gt;A&lt;/sup&gt;; Placebo&lt;sup&gt;B&lt;/sup&gt;</td>
<td>24-w intervention</td>
<td>↓ Motility&lt;sup&gt;A,B&lt;/sup&gt; (p&gt;0.05)</td>
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<td>↑ Total motility&lt;sup&gt;A,B&lt;/sup&gt; (p&gt;0.05)</td>
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*LC l-carnitine; LAC l-acetyl-carnitine; m month; w week; TAC total antioxidant capacity

<sup>A</sup>Group A had significantly higher pregnancy rate compared with group C (p<0.01), and group B had a significantly increased pregnancy rate compared with group A (p<0.05)
22.7.1 Critical Commentary

The findings of preliminary, uncontrolled studies suggest that oral carnitine supplemen-
tation improves sperm motility both in a quantitative and qualitative manner [51, 52]. This is in agreement with recent literature which reported that both L-carnitine (LC) and L-acetyl-carnitine (LAC) improved sperm motility parameters, especially in groups with lower baseline motility [54, 55]. A metabolic alteration has been postulated. Defective function in one of the intracellular carnitine-dependent systems may lead to a reduction in fatty-acid oxidation, thereby impairing energy-dependent sperm functions such as motility. Carnitine supplementation provides additional substrate for sperm energy metabolism and motility.

Carnitine’s action to improve sperm parameters may differ according to baseline semen characteristics. Lenzi et al. were unable to demonstrate a change in seminal plasma levels in the carnitine arm [53], whereby baseline levels may have possibly not allowed, although unproven, detection of small but physiologically important increases in carnitine levels. Hence, the lack of improvement in semen parameters may in fact be due to no increase in seminal plasma or sperm carnitine levels.

LC is thought to work in a dose-dependent manner, as 2 g/day was reported to elevate sperm concentration, while a 3 g/day yielded no improvement. The lack of significant variation in sperm concentration following therapy excludes a direct positive effect on the spermatogenic process. The effects of carnitine may be post-testicular, as many reports do not account for any improvement in morphology [53, 56]. Carnitine supplementation in combination therapy with the antioxidant cinnamicam was seen to improve sperm concentration, motility, and morphology in patients with iOAT [54].

Importantly, no side effects were reported in any subjects receiving LC/LAC treatment. In some cases, sperm parameters failed to improve. However, many of the studies reported elevated pregnancy rates as a result of the intervention. Therefore, the mechanism by which long-term combined carnitine treatment may improve fertilization capacity may not be directly evident from microscopic analysis [55]. Large-scale randomized controlled and dose-finding trials are necessary to confirm carnitine’s effects on sperm characteristics. In vitro studies may help to further elucidate the compound’s mechanism of action.

22.8 Lycopene

Lycopene is a naturally synthesized carotenoid-class molecule commonly found in fruits and vegetables. It is particularly useful in the human redox defense mechanisms against free radicals. Lycopene has been reported to have the highest quenching rate constant with singlet oxygen and to have a higher plasma level than that of beta-carotene [58]. Normally, it is found in high concentrations in the testes and seminal vesicles; however, decreased levels have been demonstrated in men suffering from infertility. Table 22.2 summarizes recent studies pertaining to lycopene intervention for improving sperm parameters.
<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
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<td>Mohanty et al. [59]</td>
<td>Case–control</td>
<td>50</td>
<td>Lycopene (8 mg/day)</td>
<td>3-month intervention + 12-month</td>
<td>21–50</td>
<td>† Count (70%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;† Concentration (60%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;† Motility (54%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;† SMI (46%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;↑ Pregnancy (36%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;↑ Morphology (38%)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Gupta et al. [58]</td>
<td>Case–control</td>
<td>30</td>
<td>Lycopene (4 mg/day)</td>
<td>3-month intervention + follow-up</td>
<td>23–45</td>
<td>† Concentration (67%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;† Motility (53%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;↑ Morphology (40%)&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;↑ Pregnancy (20%)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mendiola et al. [60]</td>
<td>Case–control</td>
<td>61 (30&lt;sup&gt;A&lt;/sup&gt;; 31&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>Dietary habits and nutrient consumption recorded&lt;sup&gt;AB&lt;/sup&gt;; higher intake of lycopene&lt;sup&gt;B&lt;/sup&gt; (p&lt;0.05)</td>
<td>–</td>
<td>29–38</td>
<td>Low intake of lycopene&lt;sup&gt;A&lt;/sup&gt; is associated with ↓ Concentration ↓ Motility ↓ Morphology</td>
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<td>Oborna et al. [61]</td>
<td>Double-blind, placebo-controlled, crossover</td>
<td>44 (18&lt;sup&gt;A&lt;/sup&gt;; 26&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>Lycopene (20 mg/day); Placebo Lycopene (20 mg/day) followed by placebo&lt;sup&gt;A&lt;/sup&gt;; Placebo followed by lycopene (20 mg/day)&lt;sup&gt;B&lt;/sup&gt;</td>
<td>27–38</td>
<td>‡ sRAGE levels in seminal plasma (p=0.012)&lt;sup&gt;A&lt;/sup&gt;, (p=0.008)&lt;sup&gt;B&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

<sup>SMI</sup> sperm motility index; <sup>sRAGE</sup> soluble isoforms of receptor for advanced glycation end products
<sup>a</sup>Percent of patients
22.8.1 Critical Commentary

Lycopene is one of the most potent, highly lipophilic antioxidants. Low lycopene intake has been associated with a decrease in sperm concentration, motility, and morphology levels [60]. While its main action is antioxidative, it also has antiproliferative, immunomodulatory, and anti-inflammatory effects and influences on cell differentiation, communication, and signaling. The receptor for advanced glycation end products (RAGE) is a cell surface multiligand receptor that is activated by advanced glycation end products (AGEs). Activation of RAGE induces cellular response and results in OS [62]. Since OS is recognized as an important contributing factor to male infertility [63], the release of sRAGE may aid in the removal/neutralization of proinflammatory ligands. Lycopene has been shown to reduce seminal sRAGE levels [61], illustrating a potential role in treating ROS-associated male infertility. Lycopene’s effects are most likely an antioxidative course of action or from an increased clearance of AGE ligand/sRAGE complexes.

Preliminary, controlled studies indicate a promising role for lycopene in treating ROS-associated male infertility. Lycopene supplementation has been shown to improve sperm count, concentration, motility, morphology, and pregnancy rates [59, 64]. Moreover, there were no reported side effects or complications from treatment. Future studies using larger sample sizes must be conducted to further investigate the role of lycopene supplementation in the treatment of iOAT.

22.9 N-Acetyl-Cysteine

N-acetyl-cysteine (NAC) is a metabolite of L-cysteine produced within the human body. It helps to synthesize GSH, one of the body’s most important and powerful natural antioxidant and detoxification mechanisms. NAC increases GSH levels, subsequently alleviating OS by neutralizing free radicals. GSH is also known to aid in the transport of nutrients to lymphocytes and phagocytes, as well as protect cell membranes. NAC plays a vital role in germ cell survival in human seminiferous tubules. Several studies have indicated an optimistic role of NAC in improving semen parameters (Table 22.3).

22.9.1 Critical Commentary

NAC is an N-acetyl derivative of the naturally occurring amino acid L-cysteine [67]. Due to the antioxidant nature of NAC, it is thought that NAC supplementation may improve redox status in idiopathic male infertility and semen parameters.
### Table 22.3 Quality assessment of oral N-acetyl-cysteine supplementation

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
</table>
| Paradiso Galatioto et al. [65] | Randomized controlled             | 42 (20\(^a\); 22\(^b\)) | NAC 600 mg/d + vitamin-minerals\(^a\); Placebo\(^b\) | 90-d intervention + 12-m follow-up | 23–36 | \(\uparrow\) Count \(^a\)(\(p=0.009\)), \(b(p=0.1)\)
|                                |                                   |                        |                                     |                                 |      | \(\uparrow\) Motile number \(^a\)(\(p=0.752\)), \(b(p=0.976)\)        |
|                                |                                   |                        |                                     |                                 |      | \(\uparrow\) Morphology \(^a\)(\(p=0.926\)), \(b(p=0.833)\)          |
| Safarinejad and Safarinejad [45]| Double-blind, randomized controlled | 468 (116\(^a\); 118\(^b\); 116\(^c\); 118\(^d\)) | Se (200 µg/d)\(^a\); NAC (600 µg/d)\(^b\); Se (200 µg/d) + NAC (600 µg/d)\(^c\); Placebo\(^d\) | 26-week intervention + 30-w treatment-free | 25–48 | \(\uparrow\) Count \(^a\)(\(p=0.02\)), \(b(p=0.04)\), \(c(p=0.01)\), \(d(p=0.08)\) |
|                                |                                   |                        |                                     |                                 |      | \(\uparrow\) Concentration \(^a\)(\(p=0.03)\), \(b(p=0.04)\), \(c(p=0.01)\), \(d(p=0.1)\) |
|                                |                                   |                        |                                     |                                 |      | \(\uparrow\) Motility \(^a\)(\(p=0.03\)), \(b(p=0.07)\), \(c(p=0.02)\), \(d(p=0.1)\) |
|                                |                                   |                        |                                     |                                 |      | \(\uparrow\) Morphology \(^a\)(\(p=0.03\)), \(b(p=0.03)\), \(c(p=0.03)\), \(d(p=0.1)\) |

(continued)
<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciftci et al. [66]</td>
<td>Randomized controlled</td>
<td>120 (60&lt;sup&gt;a&lt;/sup&gt;; 60&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>NAC 600 mg/d&lt;sup&gt;a&lt;/sup&gt;; Placebo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3-m intervention</td>
<td>33</td>
<td>↓ Concentration&lt;sup&gt;a&lt;/sup&gt;(NS)</td>
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<td></td>
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<td></td>
<td></td>
<td>↑ Motility&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.05)</td>
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<td></td>
<td>↑ Morphology&lt;sup&gt;a&lt;/sup&gt;(NS)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>↑ Volume&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.05)</td>
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<td></td>
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<td></td>
<td>↓ Viscosity&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.001)</td>
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<td>↑ TAC&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.001)</td>
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<td></td>
<td></td>
<td></td>
<td>↓ TP&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.001)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ OSI&lt;sup&gt;a&lt;/sup&gt;(p&lt;0.001)</td>
</tr>
</tbody>
</table>

*NAC* N-acetyl-cysteine; *Se* selenium; *TAC* total antioxidant capacity; *TP* total peroxide; *OSI* oxidative stress index; *NS* not significant; *d* day; *m* month
Oxidative stress is thought to cause inflammation and fibrosis leading to the entanglement of spermatozoa. This is thought to prevent proper migration of sperm through to cervical tract fluids and the site of fertilization [68]. NAC’s reducing nature is thought to lower these effects of ROS activity in semen and decrease semen viscosity. Human semen samples incubated with NAC (1.0 mg/mL) at room temperature exhibited improved total sperm motility and significantly reduced ROS [69]. Ciftci et al. demonstrated that sperm volume, viscosity, liquefaction time, and motility, as well as oxidative status in the plasma were significantly improved in the NAC-treated group compared to a control group [66]. Another study confirmed the effect of NAC administration to decrease seminal oxidative stress. However, no improvement in sperm motility was seen [70].

The safe and effective dosage of NAC has yet to be determined. An overdose of NAC has been to cause a minimal allergic reaction. No contraindications other than hypersensitivity have been demonstrated. In a study by Ciftci et al., no side effects were reported in any patients following 3 months of NAC (600 mg/day) treatment [66]. Taken together, the evidence presented suggests that NAC can act as an antioxidant to protect sperm from ROS damage by reducing the production of oxygen radicals. Nevertheless, its efficacy and clinical usefulness remain controversial. To fully understand its mechanism of action, additional studies should be performed on a molecular level. Randomized controlled trials to evaluate the safety and efficacy of NAC-combination therapy are essential to confirm its benefits and standardize therapeutic values.

### 22.10 Vitamins C and E

Vitamin C, also known as ascorbic acid (AA), is a water-soluble antioxidant necessary for normal tissue growth and repair in the body. Since AA is not manufactured endogenously, it must be incorporated in one’s daily diet. AA is commonly found in a variety of fruits and vegetables. It is an essential antioxidant for blocking some of the oxidative damage inflicted by free radicals and is found at concentrations higher in seminal plasma than in serum [71, 72]. A positive correlation was noted between seminal plasma AA concentration and the percentage of morphologically normal spermatozoa [73]. Additionally, poor semen samples associated with OS have been found to contain significantly lowered AA concentrations [74]. These studies demonstrate the importance of AA in warding off the adverse effects of ROS.

On the other hand, vitamin E is a lipid-soluble vitamin. Its antioxidant properties are involved in protecting vitamin A and essential fatty acids from oxidation and preventing breakdown of tissues. Similarly to vitamin C, the body cannot generate vitamin E. Hence, it must be supplemented into a daily diet from corn, lentils, wheat, rice, or nuts. Vitamin E has family of eight isomers; α-tocopherol is the only form that is actively maintained in the human body, and thus, found in the
largest quantities in the blood and tissues, specifically in cell membranes. It is believed to display defensive mechanisms by acting as a reducing agent to inhibit LPO, as well as elevating the activity of other antioxidants to aid in the scavenging of free radicals [75, 76].

Combination therapy of vitamins C and E has been hypothesized to be potentially effective in the treatment of idiopathic male infertility. Administration of hydrophilic and lipophilic antioxidants concurrently may allow for a synergistic effect in reducing the amount of peroxidative attack on spermatozoa. Recent studies assessing vitamins C and E on sperm parameters are summarized in Table 22.4.

22.10.1 Critical Commentary

Vitamin E is an essential antioxidant, located mainly in the cell membrane. It is thought to have a chief role in interrupting free radical cascade reactions, as well as acting to scavenge free radicals during univalent reduction of molecular oxygen during electron transport between complexes in the mitochondria. Sperm motility oxidation of the fatty acid bilayer by ROS will damage the mitochondrial sheath of spermatozoa, impairing their motility.

Studies show an increase in sperm motility and in vitro fertilizing potential in response to Vitamin E treatment [76, 77]. Although combined vitamin C and E therapy failed to improve conventional semen parameters during the treatment stage, prolonged abstinence time was found to significantly increase ejaculate volume, sperm count, sperm concentration, and the total number of motile spermatozoa [78]. These substantial improvements following a period of prolonged abstinence suggests that Vitamin C and E may have a synergistic effect, which enhances long-term reproductive success.

While in vitro studies have demonstrated that vitamin E has a protective effect on the sperm motility [79, 80], no significant improvement was seen in conventional semen analysis parameters in vivo [77]. Moreover, vitamin E supplementation had no effect on ROS levels in semen [77]. Conflicting findings among studies may be attributed to the fact that some authors used a chemiluminescent assay that measures both intracellular and extracellular ROS. Since vitamin E is more likely to be chain-breaking rather than a scavenging antioxidant, it would be expected to protect the membrane components without influencing ROS production. Despite the fact that no significant improvements in sperm parameters have been confirmed, Kessopoulou et al. believe the in vivo administration of vitamin E has the potential to act as a successful treatment in treating male infertility and warrants further evaluation [77]. Additional randomized controlled trials using patients with identified sperm abnormalities as well as a deeper understanding of the synergistic mode of action of vitamin C and E are essential to confirm these reports and ascertain a standardized intervention for effective treatment.
<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kessopoulou et al. [77]</td>
<td>Double-blind randomized crossover</td>
<td>30 (15\textsuperscript{A}; 15\textsuperscript{B})</td>
<td>Vitamin E (600 mg/d) followed by placebo\textsuperscript{A}; Placebo followed by vitamin E (600 mg/d)\textsuperscript{B}</td>
<td>3-m intervention + 1-m wash-out + crossed-over to other treatment</td>
<td>32</td>
<td>↑ Zona binding test (p&lt;0.004)\textsuperscript{A,B}</td>
</tr>
<tr>
<td>Suleiman et al. [76]</td>
<td>Double-blind randomized controlled</td>
<td>87 (52\textsuperscript{A}; 35\textsuperscript{B})</td>
<td>Vitamin E (300 mg/d)\textsuperscript{A}; Placebo\textsuperscript{B}</td>
<td>6-m intervention</td>
<td>–</td>
<td>(\uparrow) Motility (\uparrow p&lt;0.001), (\downarrow LPO \downarrow \uparrow p&lt;0.001), (\downarrow p&lt;0.001)</td>
</tr>
<tr>
<td>Rolf et al. [78]</td>
<td>Double-blind randomized controlled</td>
<td>31 (15\textsuperscript{A}; 16\textsuperscript{B})</td>
<td>Vitamin C (1,000 mg/d) + vitamin E (800 mg/d)\textsuperscript{A}; Placebo\textsuperscript{B}</td>
<td>56-d intervention</td>
<td>–</td>
<td>(\uparrow) Total count (\uparrow \downarrow p&lt;0.05) (\uparrow) Concentration\textsuperscript{A,B}((p&lt;0.05)) (\uparrow) Total motile sperm\textsuperscript{A,B}((p&lt;0.05)) (\uparrow) Ejaculate volume\textsuperscript{A,B}((p&lt;0.05))</td>
</tr>
</tbody>
</table>

\(mg\) milligram; \(m\) month; \(d\) day; \(h\) hour; \(LPO\) lipid peroxidation

\(^a\)While significant \((p<0.05)\), results only appeared upon prolonged abstinence; treatment did not improve conventional semen parameters nor 24-h sperm survival rate.
22.11 Selenium

Selenium (Se) is a trace mineral found in plants, as well as in some meat and seafood. Se is incorporated into proteins to form selenoproteins that are essential antioxidant enzymes. These antioxidant properties aid in preventing and diminishing cellular damage from free radicals. Studies have noted at least 25 selenoproteins in the human body to help maintain normal sperm structure integrity [46].

Se has been suggested to be vital for proper testicular development, spermatogenesis, and spermatozoa motility and function [81]. Deficiency of Se has been linked to a loss of sperm motility, instability of the mitochondrial midpiece, and morphological abnormalities [46, 81]. The loss of motility may be a result of depletion in energy supply from mitochondrial instability.

Much controversy still remains concerning the exact mode by which Se eliminates oxidative damage to improve semen quality. It has been suggested that Se may be mediated by selenoenzymes, such as phospholipid hydroperoxide GSH-peroxidase (GSH-Px) or the sperm capsular selenoprotein GSH-Px, which are related to the production of functional spermatozoa [82]. The most recent studies are summarized in Table 22.5.

22.11.1 Critical Commentary

Selenium (Se) is an essential element that has a demonstrated role in normal testicular development, spermatogenesis, and spermatozoa function [81]. The incorporation of selenium into proteins has allowed them to work in maintaining membrane integrity. Sperm capsular selenoproteins play a structural role in spermatozoa in the form of GSH-Px [86, 87], which is an effective hydroperoxide scavenger in the prevention of oxidative damage to spermatozoa [88, 89]. Iwanier et al. reported elevated GSH-Px activity in the plasma and red cells of selenium-supplemented patients [83]. However, the exact mechanism by which Se exerts its antioxidant effect on semen is unknown.

Several reports have indicated correlations between semen Se concentration and sperm parameters. Bleau et al. reported maximal sperm motility in semen samples with Se levels ranging between 50 and 69 ng/mL, while concentrations below and above this range resulted in a high incidence of asthenozoospermia [90]. Additionally, significantly lowered Se concentrations were found in tetrazoospermic than in normozoospermic men [91]. Hence, Se supplementation appears to be dose-dependent such that antioxidant GSH-Px activity would increase upon Se intake until the dose–response relationship achieved a plateau. The dosage required to achieving optimal Se levels to maximize antioxidant enzyme activity needs to be determined in order to use this treatment to reduce ROS levels and improve sperm quality and fertilization rates.
### Table 22.5 Quality assessment of oral selenium supplementation

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iwanier and Zachara [83]</td>
<td>Double-blind randomized controlled</td>
<td>33 (16(^a); 17(^b))</td>
<td>Se-rich-yeast (200 μg/d)(^a); sodium selenite (200 μg/d) mixed with</td>
<td>12w</td>
<td>19–38</td>
<td>† Whole blood, plasma and seminal fluid [Se] (^A) ((p&lt;0.001))</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>baker’s yeast(^b)</td>
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<tr>
<td>Scott et al. [84]</td>
<td>Double-blind randomized controlled</td>
<td>64 (16(^a); 30(^b); 18(^c))</td>
<td>Se (100 μg/d)(^a); Se(100 μg/d) + vitamins A (1 mg/d), C (10 mg/d),</td>
<td>6m</td>
<td>33</td>
<td>† Count (^A) (^B) (^C) ((p&lt;0.243))</td>
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<td></td>
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<td></td>
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<td></td>
<td>† Motility (^A) (^B) (^C) ((p&lt;0.068))</td>
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<td>† Plasma Se (^A) (^B) (^C) ((p&lt;0.001))</td>
</tr>
<tr>
<td>Hawkes and Turek [85]</td>
<td>Double-blind randomized controlled</td>
<td>12 (6(^a); 6(^b))</td>
<td>Se (47 μg/d) for 21 days + Se (13 μg/d) (^A) or Se (297 μg/d) (^B) for 99 days</td>
<td>120d</td>
<td>–</td>
<td>† Plasma [Se] (^A) (50%)</td>
</tr>
<tr>
<td>Safarinejad and Safarinejad [45]</td>
<td>Double-blind randomized controlled</td>
<td>468 (116(^a); 118(^b); 116(^c); 118(^d))</td>
<td>Se (200 μg/d)(^a); NAC (600 μg/d)(^b); Se (200 μg/d) + NAC (600 μg/d)(^c); Placebo(^d)</td>
<td>26-w intervention + 30-w treatment-free</td>
<td>25–48</td>
<td>† Count (^A) (^B) (^C) ((p≤0.05))</td>
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<td></td>
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<td></td>
<td></td>
<td>† Motility (^A) (^B) (^C) ((p≤0.05))</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>† Morphology (^A) (^B) ((p≤0.05), (^p=0.07))</td>
</tr>
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</table>

Se selenium; NAC N-acetyl cysteine; \(w\) week; \(m\) month; \(d\) day
The mechanism by which Se induced its effects on sperm is still poorly understood and its effects on male fertility and semen quality in humans remain controversial. Some studies have reported no effect of Se supplementation at all [92, 93]. While supplementation was found to cause incremental increases in seminal fluid Se levels, spermatozoal quality characteristics showed no improvement [83]. However, combination therapy with Se, Vitamin E, and NAC has shown promising results [45, 84]. These improvements were most likely supplement-dependent as all parameters were seen to return to baseline values during the posttreatment period. Conflicting study results may be explained by differences in the baseline fertility status of control subjects, andrological history, methodological variations in study design, and semen analysis, as well as demographic characteristics. There is a need for larger studies to fully assess the potential side effects of Se supplementation. Additional studies are necessary to evaluate the route and proper state by which Se directs its neutralizing effects, its side effects, as well as to confirm the findings of improving sperm motility and fertility rates.

22.12 Nonsteroidal Anti-Inflammatory Drugs

Although most commonly known as treatment for pain relief and inflammation, nonsteroidal anti-inflammatory drugs (NSAIDs) have exhibited antioxidant effects in improving sperm quality and fertility in rabbits [94]. They function mainly through the reversible inhibition of cyclooxygenase, thereby inhibiting the production of prostaglandins and thromboxanes. Elevated prostaglandin levels have been found in men suffering from OAT [95]. Combination therapy of NSAIDs and carnitine, which are thought to both undergo similar mechanistic pathways, may facilitate the beneficial results of NSAIDs therapy by suppressing excess prostaglandin production. Table 22.6 reviews recent studies conducted with NSAIDs on sperm.

22.12.1 Critical Commentary

Although there is a paucity of information in the literature, preliminary reports suggest a promising role for NSAIDs in improving sperm quality. They are thought to stabilize lysosomal membranes, thereby partially preventing apoptosis [94, 97, 98]. Some evidence has shown that an increase in prostaglandin concentration in seminal plasma can inhibit spermatogenic function [98]. One study found success in treating men with iOAT and prostaglandin-F2 in seminal plasma by treating them with a NSAID called flubiprofen [97]. Cinnoxicam is a lipophilic NSAID that has been shown to enhance sperm concentration, motility, and morphology [96] and even lead to higher pregnancy rates when combined with LC and LAC [54]. Its fat-soluble nature facilitates lymphatic and prostatic absorption [97, 99]. No specific biochemical study has found a mechanism by which cinnoxicam elicits its actions, although
### Table 22.6 Quality assessment of oral supplementation of nonsteroidal anti-inflammatory drugs (NSAIDs)

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
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<tbody>
<tr>
<td>Cavallini et al. [96]</td>
<td>Double-blind randomized controlled</td>
<td>156 (41&lt;sup&gt;A&lt;/sup&gt;; 61&lt;sup&gt;B&lt;/sup&gt;; 54&lt;sup&gt;C&lt;/sup&gt;)</td>
<td>Surgery&lt;sup&gt;A&lt;/sup&gt;; Cinnoxicam (30 mg/4d)&lt;sup&gt;B&lt;/sup&gt;; Placebo (glycerine suppository 1×/4d)&lt;sup&gt;C&lt;/sup&gt;</td>
<td>12-m intervention</td>
<td>34–37</td>
<td>↑ Concentration&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>↑ Motility&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>↑ Morphology&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Cavallini et al. [54]</td>
<td>Double-blind randomized controlled</td>
<td>130 (47&lt;sup&gt;A&lt;/sup&gt;; 39&lt;sup&gt;B&lt;/sup&gt;; 44&lt;sup&gt;C&lt;/sup&gt;)</td>
<td>Placebo (starch tablet 2×500 mg/d) + (glycerine suppository 1×/4d)&lt;sup&gt;A&lt;/sup&gt;; LC (1×2 g/d) + LAC (500×2 mg/d) + glycerine suppository (1×/4d)&lt;sup&gt;B&lt;/sup&gt;; LC (1×2 g/d) + LAC (500×2 mg/d) + cinnoxicam suppository (1×30 mg/d)&lt;sup&gt;C&lt;/sup&gt;</td>
<td>6-m intervention + 3-m/6-m follow-up</td>
<td>28–40</td>
<td>↑ Concentration&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;p&lt;0.05&lt;/sub&gt;&lt;/sup&gt;</td>
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<td>↑ Motility&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;p&lt;0.05&lt;/sub&gt;&lt;/sup&gt;</td>
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<td>↑ Morphology&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;p&lt;0.05&lt;/sub&gt;&lt;/sup&gt;</td>
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<td>↑ Pregnancy&lt;sup&gt;C&lt;/sup&gt;&lt;sup&gt;&lt;sub&gt;p&lt;0.01&lt;/sub&gt;&lt;/sup&gt;</td>
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</table>

<sup>LC</sup> l-carnitine; <sup>LAC</sup> l-acetyl-carnitine; <sup>d</sup> day; <sup>mg</sup> milligram; <sup>m</sup> months

<sup>a</sup>Significant differences in sperm concentrations (<sup>p</sup> < 0.001), but not with replicates

<sup>b</sup>Significant increases with oligoasthenospermia associated with a grade III (<sup>p</sup> < 0.05), but not in those with grade IV or V varicocele
it is known that NSAIDs inhibit ROS and prostaglandin synthesis. Preliminary results appear to be best following 4 months of intervention, while returning to baseline levels following therapy suspension [54, 96]. A balance is critical in regulating ROS concentration for proper sperm function; too high of concentrations inhibit sperm motility and modify sperm morphology; however, too low of concentrations downregulate sperm capacitation and acrosomal reactions [100]. For instance, one study revealed chronic treatment with NSAIDs at low doses to improve sperm quality and fertility [99], while in vitro model showed that high cinnoxicam concentrations in seminal plasma inhibit sperm motility by most likely lowering the ROS in seminal plasma to excessive low levels [97]. Additional studies are required to establish the proper dosage of cinnoxicam treatment.

Only a few minor side effects were reported—mild euphoria, mild epigastralgia, and nausea—but never resulted in therapy suspension [54]. Future randomized controlled trials should test the utility of other common NSAIDs such as aspirin and ibuprofen in the treatment of male infertility. LAC/LC+cinnoxicam suppositories reveal much potential as a reliable treatment. However, there is still not enough data to support the use of NSAIDs in the treatment of iOAT.

22.13 Pentoxifylline

Pentoxifylline is an oral supplement commonly used to improve blood flow in patients with circulation problems by decreasing the viscosity of blood. As a xanthine derivative, pentoxifylline acts as a competitive nonselective phosphodiesterase inhibitor, thereby raising intracellular cAMP. It has also shown an anti-inflammatory effect in neutralizing ROS by controlling the release of superoxide anions [101, 102]. This presumably reduces the amount of OS by downregulating the body’s ability to initiate an inflammatory response. Table 22.7 summarizes the most recent studies conducted with pentoxifylline on improving sperm parameters.

22.13.1 Critical Commentary

Pentoxifylline is an oral supplement commonly used to improve blood flow in patients with circulation problems by decreasing the viscosity of blood. As a xanthine derivative, pentoxifylline acts as a competitive nonselective phosphodiesterase inhibitor, thereby raising intracellular cAMP. It has also shown an anti-inflammatory effect in neutralizing ROS by controlling the release of superoxide anions [101, 102]. This presumably reduces the amount of OS by downregulating the body’s ability to initiate an inflammatory response.

Pentoxifylline’s antioxidant nature has been studied for its role in improving sperm quality [101, 102]. It has been demonstrated to be effective in preserving sperm motility in vitro [103] and in improving semen parameters in vivo [105].
**Table 22.7** Quality assessment of oral pentoxifylline supplementation

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pang et al. [103]</td>
<td>Prospective</td>
<td>–</td>
<td>Pentoxifylline (3.6 mM)(^a); 1-h incubation + 24-h incubation</td>
<td>–</td>
<td>↑ VCL (^a)(p &lt; 0.05)</td>
<td>↑ ALH (^a)(p &lt; 0.05)</td>
</tr>
<tr>
<td>Okada et al. [104]</td>
<td>Case–control</td>
<td>71 (15(^a); 35(^b))</td>
<td>Pentoxifylline (300 mg/day); Pentoxifylline (1,200 mg/day)</td>
<td>4-m intervention + 4-m intervention (follow-up every 4w)</td>
<td>–</td>
<td>↑ Motility (^a,*(^b)(p &lt; 0.05)</td>
</tr>
</tbody>
</table>

\(^m\) month; \(^w\) week; \(VCL\) curve linear velocity; \(ALH\) amplitude of lateral sperm head displacement

\(^a\) Sperm preparations generated detected ROS levels at steady state

\(^b\) 18/35 with asthenospermia whose sperm preparations failed to generate detectable ROS levels at steady state
In a study by Okada et al., asthenozoospermic patients with detectable steady state levels of ROS were seen to have lowered ROS production levels and preserved sperm motion parameters following pentoxifylline treatment [104]. A low dosage (300 mg/day) treatment was found to be relatively ineffective in comparison to a higher dosage (1,200 mg/day), which increased sperm motility and motion parameters. However, no improvement in pregnancy rate was seen to result from either treatment regimen. Another report found that pentoxifylline increased the curvilinear velocity, path velocity, and straight-line velocity in both normozoospermic and asthenozoospermic specimens, but did not modify the percentage of motile spermatozoa [106].

These studies may suggest that pentoxifylline may serve to enhance sperm motility, making it a potential therapeutic approach for treatment of idiopathic male infertility. Nevertheless, data remain inconclusive. Studies focusing on the pharmacokinetics of pentoxifylline are essential in understanding its mechanism of action. In addition, prospective, double-blind clinical trials with fecundity as the main outcome measure are necessary to validate the full effects of pentoxifylline.

22.14 Zinc

Zinc (Zn) is a ubiquitous trace element that protects proteins and enzymes against free radical attack. The Zn molecule in Zn-containing enzymes is thought to shield specific regions of the enzyme from being oxidized, and thus, preserving its stability and activity. Additionally, since Zn does not readily undergo redox reactions, it functions to prevent free radical formation by other highly reactive metals, such as copper and iron. Therefore, Zn therapy may be an effective treatment for ROS-associated iOAT. It may act to neutralize the effects of ROS, thereby protecting sperm function. Interestingly, fertile and subfertile men have shown significant differences in their seminal plasma Zn concentrations and sperm motility [107]. Other reports indicate similar results, as it was hypothesized for Zn to work through various mechanisms in preventing OS by virtue of its stabilizing effects as an antioxidant [108, 109]. The basis of these findings may suggest that Zn may contribute to fertility through its positive effect on spermatogenesis. Recent studies conducted with Zn sulfate and its efficacy in combinational treatment are summarized in Table 22.8.

22.14.1 Critical Commentary

Zn is a ubiquitous trace element that protects proteins and enzymes from free radical attack. Since Zn does not readily undergo oxidation-reduction reactions, it can function to prevent free radical formation by other highly reactive metals such as copper and iron. The Zn molecule in Zn-containing enzymes shields specific
### Table 22.8  Quality assessment of oral zinc supplementation

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Cases</th>
<th>Dosage</th>
<th>Duration</th>
<th>Ages</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong et al. [110]</td>
<td>Double-blind randomized controlled</td>
<td>94 (22(^{A}); 23(^{B}); 24(^{C}); 25(^{D}))</td>
<td>Folic acid (5 mg/day)(^{A}); Zn sulfate (66 mg/day)(^{B}); Zn sulfate (66 mg/day) + Folic acid (5 mg/day)(^{C}); Placebo(^{D})</td>
<td>26-week intervention</td>
<td>–</td>
<td>↑ Concentration (^{C}(p&lt;0.05))</td>
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<td></td>
<td>↑ Morphology (^{A,B}(p&lt;0.05))</td>
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<td></td>
<td>↑ Total normal count (^{C}(p&lt;0.05))</td>
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<tr>
<td>Omu et al. [107]</td>
<td>Case–control</td>
<td>45 (11(^{A}); 12(^{B}); 14(^{C}); 8(^{D}))</td>
<td>Zn sulfate (400 mg/day)(^{A}); Zn sulfate (400 mg/day) + vitamin E (20 mg/day)(^{B}); Zn sulfate (400 mg/day) + vitamin E (20 mg/day) + vitamin C (10 mg/day)(^{C}); nontherapy control(^{D})</td>
<td>3-month intervention</td>
<td>35 ± 6(^{A})</td>
<td>↑ Motility (^{A,B,C}(p&lt;0.001))</td>
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<td>35 ± 1(^{B})</td>
<td>↑ Fertilizing capacity (^{A,B,C}(p&lt;0.05))</td>
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<td>34 ± 9(^{C})</td>
<td>↑ Total antioxidant capacity (^{A,B,C}(p&lt;0.001))</td>
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<td></td>
<td></td>
<td></td>
<td>↓ Seminal Bcl-2 (^{A,B,C}(p&lt;0.05))</td>
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<td></td>
<td>↓ Bax (^{A,B,C}(p&lt;0.01))</td>
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<td></td>
<td></td>
<td>↓ MDA (^{A,B,C}(p&lt;0.01))</td>
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<td></td>
<td></td>
<td>↓ TNF-α (^{A,B,C}(p&lt;0.001))</td>
</tr>
</tbody>
</table>

\(\text{Zn zinc; MDA malone dialdehyde}\)
regions of the enzyme from being oxidized, thereby preserving enzymatic stability and activity.

For such reasons, Zn therapy is thought to be an effective treatment for ROS-associated male infertility. Interestingly enough, fertile and subfertile men have shown significant differences in their seminal plasma Zn concentrations and sperm motility [107, 111]. Zn is hypothesized to exert its effects via numerous pathways to prevent OS formation, apoptosis, and sperm DNA fragmentation by virtue of its stabilizing nature as an antioxidant. Chia et al. noted seminal plasma zinc concentration to be significantly correlated with sperm density, motility, and viability [111]. These findings suggest that Zn may promote male fertility via its positive effect on critical steps in spermatogenesis.

Wong et al. reported an increase in total normal sperm count in both fertile and subfertile men following combination treatment with Zn sulfate (66 mg/day) and folic acid (5 mg/day) [110]. Hence, Zn and folic acid may work in a synergistic manner to protect spermatozoa. There is a need for additional randomized, placebo-controlled trials with larger sample sizes as well as various dosages and intervention periods to confirm the efficacy and safety of Zn and folic acid combination therapy. Establishing the beneficial effects of Zn treatment on fertility may aid in a therapeutic approach for treating iOAT.

### 22.15 Conclusion

Regulated levels of ROS are essential in physiologically regulating normal sperm function. However, in an environment with uncontrolled, elevated ROS levels, OS ensues and sperm function and viability are endangered. OS resulting from excessive production of ROS, impaired antioxidant defense mechanisms, or both precipitates in a wide range of pathologies that are currently believed to adversely affect sperm quality. Despite the established role of OS in the pathogenesis of male infertility, there is a lack of consensus as to the clinical utility of seminal OS testing in an infertility clinic. A major reason for this disconnect is related to the weakly defined standard protocol for assessing seminal OS.

Nevertheless, antioxidant therapies have illustrated promising results in improving the semen parameters of subfertile men suffering from iOAT. They have become the most widespread utilized and studied novel therapy for treating male factor infertility. However, the proper dosage, type, and duration of antioxidant treatment for clinicians to administer have yet to be confirmed and standardized. Many studies point to improvements in just one or two of the three parameters of iOAT, failing to fully address the disease as a whole. Additionally, safety becomes the primary concern, as high dosages of antioxidant therapy are capable of resulting in adverse effects. It has not been established whether antioxidant therapy is the proper management in cases of elevated ROS production, because intracellular sperm antioxidant status, abstinence time, sperm count, as well as other confounding factors must be considered. Since there are no reliable, predictive, and inexpensive methods in
determining the extent of ROS exposure and antioxidant capacity in patients, further advances in this area may prove valuable for assigning ROS values to serve as potential indicators of the correct antioxidant therapy to prescribe. Moreover, if ROS exposure values are established, this may help to establish levels in which antioxidant treatment may be administered. Since the liberation of transition-metal ions from metalloproteins during a prooxidative state is thought to serve as catalysts for free radical damage, especially in the reduced state, antioxidant treatment may even enhance oxidation damage. The body faces an “antioxidant paradox” in which the administration of a potent antioxidant to reduce a prooxidative state can worsen conditions. Therefore, establishing “cut-off” values of ROS in which an antioxidant can be used will help to control ROS damage and determine therapeutic values necessary for treating iOAT.

Men with iOAT are often administered a number of various therapies based on experimental studies, yet their supporting evidence in controlled human studies is sparse. Thus, in the absence of approved and effective treatment, medications prescribed are based solely on rationale. Assessment of OS status may also help in selecting the patient population that would most benefit from antioxidant supplementation. Further studies in this area are essential. Antioxidant treatment holds for a promising future as a conservative, inexpensive remedy in treating infertility worldwide.

References


