

# Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: Is it justified?

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## ABSTRACT

Oxidative stress contributes to defective spermatogenesis and the poor quality of sperm associated with idiopathic male factor infertility. The aim of this study was to review the current literature on the effects of various types of antioxidant supplements in patients to improve fertilization and pregnancy rates in subfertile males with idiopathic oligoasthenoteratozoospermia (iOAT). Review of recent publications through PubMed and the Cochrane database. Oxidative stress is implicated in impaired spermatogenesis leading to the poor semen parameters and increased DNA damage and apoptosis in iOAT. Strategies to modulate the level of oxidative stress within the male reproductive tract include the use of oral antioxidant compounds to reinforce the body's defence against oxidative damage. In our evaluation, carnitines were considered the most established pharmacotherapeutic agent to treat iOAT, as evidence and data concerning carnitine supplementation have been shown to be most consistent and relevant to the population of interest. Other therapies, such as combined vitamin E and C therapy, are still considered controversial as vitamin C can act as a pro-oxidant in certain instances and the results of randomized controlled trials have failed to show significant benefit to sperm parameters and pregnancy rates. There is a need for further investigation with randomized controlled studies to confirm the efficacy and safety of antioxidant supplementation in the medical treatment of idiopathic male infertility as well as the need to determine the dosage required to improve semen parameters, fertilization rates and pregnancy outcomes in iOAT.

**Key words:** Antioxidants, idiopathic oligoasthenoteratozoospermia, male infertility, oxidative stress

## INTRODUCTION

Infertility is defined as the failure to achieve a pregnancy within one year of regular (at least three times per month) unprotected intercourse. It affects approximately 15% of sexually active couples; a causative male factor is present in approximately 40% of cases.<sup>[1]</sup> Infertility is regarded as 'male factor' when an alteration in sperm concentration and/or motility and/or morphology is present in at least one sample of two sperm analyses, which comply with

the World Health Organization (WHO) 1999 guidelines, collected between one to four weeks apart.<sup>[2]</sup> Male infertility may be characterized by oligoasthenoteratozoospermia-low sperm count with a high percentage of slow-moving and abnormal semen. Approximately 30% of cases are considered idiopathic, meaning that no cause can be found with common clinical, instrumental or laboratory methods. Treating idiopathic oligoasthenoteratozoospermia (iOAT) can be problematic--although a variety of drugs and dietary supplements are available, many are prescribed without any rationale and without any evidence supporting their efficacy. In addition, although assisted reproductive techniques have been proposed as a possible solution for male factor infertility in general, they are expensive, not universally available, and have limited success.

This review article will discuss the causes of iOAT, the role played by oxidative stress in impairing spermatogenesis and the current knowledge regarding pharmacotherapy in the medical management of this condition.

## IDIOPATHIC OLIGOASTHENOTERATOZOOSPERMIA

Idiopathic oligoasthenoteratozoospermia is related to

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defective spermatogenesis, the origin of which is unknown and often regarded as undetectable by common laboratory methods. This condition is characterized by necrosis and apoptosis of gametes (oligoteratozoospermia), reduction in the percentage of normal sperm forms, and impairment of sperm motility (asthenospermia).<sup>[3]</sup> In addition to sperm analysis findings, there may be a histological picture of mixed atrophy of the testicles with normal serum levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). iOAT can be classified as: isolated astheno ± teratospermia (no alteration in semen concentration); moderate (sperm concentration  $<20 \times 10^6/\text{mL}$  and  $>5 \times 10^6/\text{mL}$ ); or severe (sperm concentration  $<5 \times 10^6/\text{mL}$ ).<sup>[3]</sup>

A large number of iOAT infertile patients have a normal physical examination, normal hormonal profile and no discernable cause of their subfertile status.<sup>[4,5]</sup> Although hormonal abnormalities are not always evident, iOAT is sometimes associated with lower serum testosterone and inhibin levels and higher serum estradiol, LH and FSH levels.<sup>[6,7]</sup> These changes may also be age-related as semen volume and sperm motility are seen to continuously decrease from 22 to 80 years of age, while sperm concentration is not affected.<sup>[8]</sup> Non-inflammatory functional alterations in post-testicular organs such as the epididymis may be implicated in iOAT by altering the DNA methylation of gametes.<sup>[9]</sup> Methylation mediates transcriptional repression by recruiting histone deacetylase. Genes must undergo demethylation in order for gene transcription to occur.<sup>[10]</sup> Asymptomatic infections without significant leukospermia, due to herpes virus, adeno-associated virus and Chlamydia trachomatis (CT), have been linked to iOAT.<sup>[11,12]</sup> The prevalence of asymptomatic CT has been estimated at 20% in men with iOAT.<sup>[13,14]</sup> As this percentage is higher than in the control population, asymptomatic CT infection has been regarded by some as a cause of iOAT. However, other researchers have found the CT prevalence (of approximately 5%) to be similar among iOAT and fertile subjects.<sup>[15]</sup> In general, male infertility appears to have a familial occurrence, especially among brothers and maternal uncles, who often have normal blood levels of FSH and LH.<sup>[16]</sup> An autosomal recessive mode of inheritance has been suggested.<sup>[17]</sup> A genetic etiology is currently considered the most acceptable theory.

### THE ROLE OF OXIDATIVE STRESS IN IDIOPATHIC OLIGOASTHENOTERATOZOOSPERMIA

Reactive oxygen species (ROS) are molecules that have at least one unpaired electron, rendering them highly unstable and highly reactive in the presence of lipids, amino acids and nucleic acids.<sup>[18]</sup> At physiologic levels, ROS are essential for normal reproductive function, acting as metabolic intermediates in the metabolism of prostanoid, in the regulation of vascular tone, in gene regulation, and

in facilitated sperm capacitation and acrosome reaction. However, at higher concentrations, they exert negative effects.<sup>[18]</sup> The main source of ROS production in seminal plasma is leukocytes and immature spermatozoa. Spermatids and mature spermatozoa are deemed highly sensitive to ROS because their membranes are particularly rich in polyunsaturated lipids.<sup>[19]</sup> It is not known at which point the peroxidative damage to spermatozoa occurs, whether within semen (during the time required for liquefaction), in the epididymis (where spermatozoa are stored before ejaculation), or in the testis. By altering membrane integrity, ROS may impair sperm motility and morphology and can lead to sperm cell death.<sup>[20]</sup>

The seminal plasma has a high concentration of antioxidants, which protect gametes from ROS. The total antioxidant capacity of seminal plasma represents the sum of the potential anti-ROS enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px)), low molecular weight substances (a-tocopherol, b-carotene, ascorbate, urate) and transition metal chelators (transferrin, lactoferrin, ceruloplasmin).<sup>[21]</sup> Impaired spermatogenesis leads to abnormal spermatozoa and yields excess ROS, which can overwhelm and deplete the antioxidant defenses and results in oxidative stress [Figure 1].

Oxidative stress occurs when there is an excess of ROS, a decrease in antioxidant levels, or both. ROS has been found in the seminiferous tubules and seminal plasma of most patients with iOAT.<sup>[22-25]</sup> The concentration of malondialdehyde (MDA), a marker of lipid peroxidation, was found to be almost twice as high in sperm pellet suspensions of asthenospermic and oligoasthenospermic patients compared to normospermic males.<sup>[24]</sup> Kobayashi demonstrated that the *in vitro* addition of exogenous SOD to semen samples led to improved sperm motility and a decrease in MDA concentration.<sup>[26]</sup> Zinc deficiency was seen to confer vulnerability to oxidative stress *in vitro*, leading to increased sperm DNA fragmentation and apoptosis.<sup>[27]</sup> Intracellular zinc modulates the levels of pro-apoptotic p53, p21 and bcl 2 family gene expression and caspase-3 activity. Bcl-2 has a role in regulating programmed cell death by functioning as an antioxidant at the outer membrane of the mitochondria.<sup>[28,29]</sup> In a study by Omu *et al.*, the antiapoptotic protein Bcl-2 was more highly expressed in normozoospermia than in oligozoospermia and asthenozoospermia men whereas the pro-apoptotic Bax was greatly expressed in men with oligozoospermia and asthenozoospermia.<sup>[27]</sup> Apoptosis and markers of programmed cell death are inversely correlated with sperm motility,<sup>[30]</sup> morphology, vitality and concentration.<sup>[30,31]</sup> Decreased expression of survivin, a programmed cell death inhibitor, has been correlated with increased severity of iOAT. However, it should be noted that increased apoptosis is implicated in several types of OAT, including that associated with hormonal infertilities,<sup>[32]</sup> anti-sperm antibody-associated infertility,

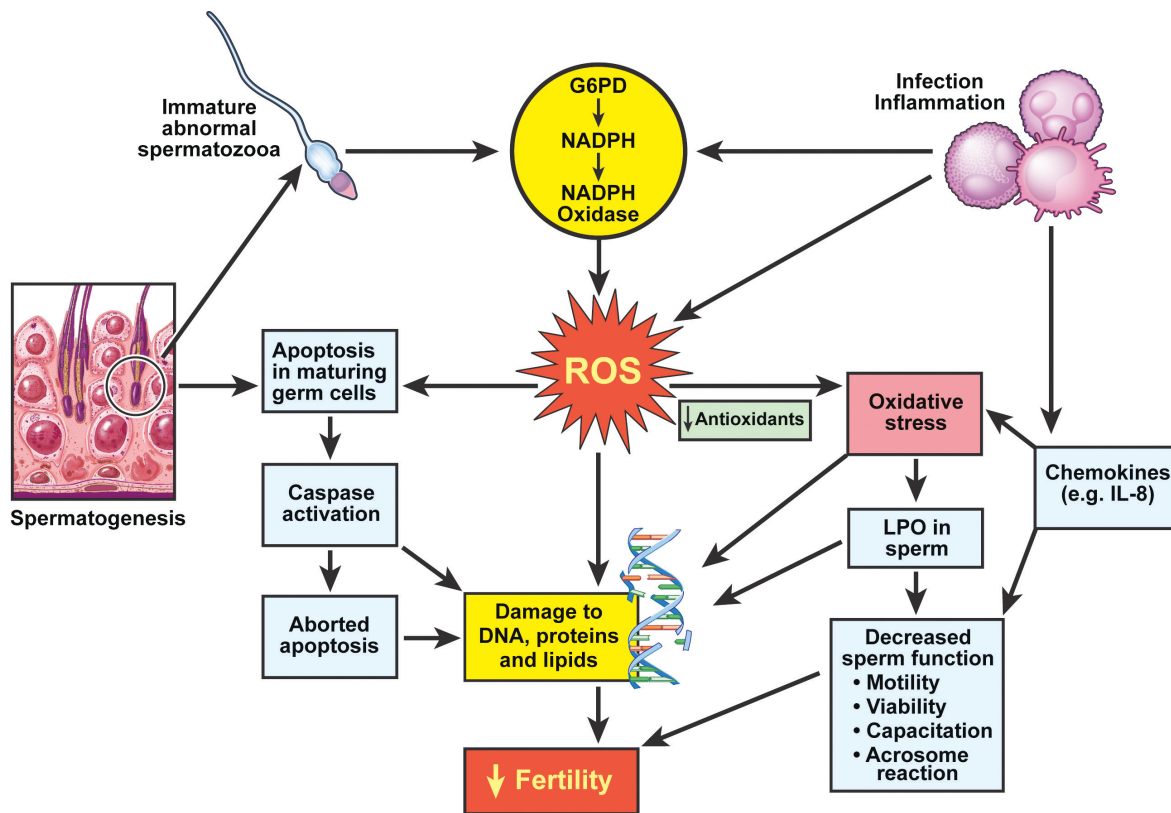


Figure 1: Mechanism of oxidative stress in human semen

varicocele, testicular torsion<sup>[33]</sup> and inflammation.<sup>[34]</sup> Therefore, accentuated programmed cell death is not pathognomonic for iOAT. Several studies have shown that, in addition to an increased susceptibility to apoptosis and DNA fragmentation, spermatozoa from OAT patients often exhibit chromosomal aneuploidy and mitochondrial dysfunction.<sup>[35]</sup> Mitochondrial DNA oxidative damage has been observed in asthenospermic infertile men<sup>[36]</sup> and confirmed in experimental models.<sup>[37]</sup> These alterations are very similar to the mitochondrial modifications seen in apoptotic sperm cells.<sup>[38-41]</sup>

### ANTIOXIDANT TREATMENT OF IOAT

Medical treatment for male infertility can often be a frustrating problem because it is a multifactorial disorder for which an identifiable cause cannot be found in the vast majority.<sup>[3]</sup> Unlike with genetic factors, nutritional factors can be changed by altering the patient's diet. Pharmacotherapy for iOAT consists of oral supplementation with hormones and antioxidants with the aim to improve and maintain semen parameters. Increased sperm concentration in men with OAT has been associated with a disproportionately higher fecundability.<sup>[42]</sup> Treatments that improve sperm concentration will result in stronger probability of conception when the initial sperm count is low, rather than high.<sup>[43]</sup> Therefore, selecting patients with iOAT who

may benefit from a particular therapy will depend on initial sperm parameters.

Treatment with antioxidants is a widely used therapy for several medical indications including male factor infertility, although its efficacy has yet to be well established. Safety is a concern as well because high doses of certain antioxidants, including vitamin A, may have embryotoxic and teratogenic effects.<sup>[44]</sup> It is unknown whether ROS production can be used as a criterion to select men for antioxidant therapy, since intracellular sperm antioxidant status, sperm count, abstinence time and other confounding factors must also be considered. In addition, there are no reliable, predictive and inexpensive tests that can determine the extent of ROS exposure or the antioxidant capacity of patients.<sup>[44]</sup> Seminal fluid oxidative stress levels can be quantified either by direct methods such as chemiluminescence assays, cytochrome-c and nitroblue tetrazolium reduction, flow cytometry, electron spin resonance spectroscopy, and xylenol orange-based assay, or by indirect methods which measure the levels of biomarkers of oxidative stress such as thiobarbituric acid-reactive substances, isoprostane, DNA damage and total antioxidant capacity. The measurement of ROS may help to identify those patients who could benefit from antioxidant supplementation.<sup>[45]</sup>

Men with iOAT are likely to be prescribed a number of

**Table 1: Scoring and evaluation of the quality of evidence regarding antioxidant therapy in idiopathic oligoasthenoteratozoospermia**

Antioxidant	GRADE score	Quality of evidence
Tamoxifen	2	Low
FSH	2	Low
Selenium	2	Low
Vitamin E	2	Low
Vitamin E and C	3	Moderate
Carnitine	4	High
Zinc	3	Moderate
Tranilast	2	Low

GRADE: Grading of recommendations, assessment, development and evaluations

empirical therapies. However, the scientifically acceptable evidence showing their benefit in controlled human studies is sparse. In the absence of approved and effective medical treatment for iOAT, certain medications continue to be prescribed based purely on their rationale and the fact that they lack significant side-effects. To define a drug as active, it should improve sperm parameters and pregnancy rates in at least one blind, prospective, placebo-controlled trial; more of these trials from independent groups are needed in order to define a drug as unquestionably active.<sup>[46]</sup> The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) working group has developed a systematic and explicit approach to making judgments regarding the quality of evidence and strength of recommendations.<sup>[47]</sup> A scoring system based on this framework<sup>[48]</sup> was used by us to evaluate each pharmacotherapeutic agent based on the current available evidence regarding its effectiveness in the treatment of iOAT. Evidence was evaluated across studies for specific clinical outcomes, taking into account the methodological flaws within the component studies, the consistency of results across different studies, how generalizable the research results are to the wider patient base, and how effective the treatments have been shown to be. All treatment comparisons were given one of four GRADE scores reflecting the quality of the evidence: high, moderate, low or very low [Table 1]. We believe this approach may give clinicians a clear view of the evidence relating to key treatment interventions.

## HORMONE PHARMACOTHERAPY

The reproductive tract microenvironment of men with iOAT has been established as androgen-deficient. Androgen-dependent epididymal and accessory gland function<sup>[49]</sup> may be boosted by androgen stimulation.<sup>[50]</sup> Tamoxifen was first introduced as an empirical treatment<sup>[51]</sup> as it acts on hypothalamic receptor sites to stimulate gonadotropin release.<sup>[52]</sup> Furthermore, it affects Leydig cell function to increase tubular and epididymal 5 $\alpha$ -dihydrotestosterone. Willis *et al.*, conducted a single-blind placebo-controlled

trial in which 10 mg of daily tamoxifen given for six months failed to significantly improve sperm count, although significant hormonal changes were seen.<sup>[53]</sup> A more recent study by Vanderkerckove *et al.*, further confirmed the lack of efficacy of tamoxifen in improving semen parameters in patients with iOAT.<sup>[54]</sup> Interestingly, Comhaire *et al.*, found that tamoxifen led to the greatest increase in pregnancy rates in the groups where the pre-treatment pregnancy rate was the lowest.<sup>[43]</sup> Combining tamoxifen with testosterone (T) undecanoate may improve any beneficial effects each of these treatments might exert independently on sperm quantity and quality. Previous studies have shown that short and long-term administration of tamoxifen citrate and Testosterone undecanoate promote pituitary and Leydig cell activity in men with idiopathic oligozoospermia.<sup>[55]</sup> Testosterone undecanoate administration exerts its actions mainly on epididymal function, improving sperm motility, morphology and fertilization capacity.<sup>[55-57]</sup> Androgens are given as a safeguard against possible disturbances of synthesis, metabolism and binding of these steroids, which is often implicated in the infertility associated with iOAT.<sup>[58-60]</sup> Adamopoulos *et al.* conducted a randomized control trial and found that this combined regimen produced a satisfactory improvement in total sperm number, motility and functional sperm fraction after three and six months.<sup>[50]</sup> Follicle-stimulating hormone (FSH) has been shown to stimulate spermatogenesis in an animal model. Administration of a high dose of FSH was seen to be particularly promising for improving disturbed sperm structure.<sup>[61,62]</sup> Based on this, various attempts have been made to increase sperm production in patients with OAT by administering FSH, but the results are still controversial. The subjects of an uncontrolled study by Ben-Rafael *et al.*, demonstrated both an increased pregnancy rate and improved classical semen parameters,<sup>[63]</sup> whereas other uncontrolled studies have shown only improved semen parameters.<sup>[64-66]</sup> Randomized controlled trials by Kamische *et al.*, and Foresta *et al.*, failed to demonstrate improvement in sperm parameters or pregnancy rates. However, testicular volume and DNA condensation increased, which suggests a potential role for FSH in the treatment of iOAT. Another randomized controlled trial by Baccetti *et al.*, reported that FSH therapy led to a significant improvement in sperm ultrastructure and increased probability of embryo implantation, as evidenced by higher pregnancy rates in the partners of the treated patients.<sup>[67]</sup> However, as patients with iOAT are a highly heterogeneous group, not all patients are likely to respond to hormone therapy.

## CARNITINES

Carnitine is a water-soluble antioxidant mostly derived from the human diet; approximately 25% is synthesized from lysine and methionine. Extracellular and intracellular carnitine may play a role in sperm energy metabolism, providing the primary fuel for sperm motility via post-



testicular effects. Spermatozoa exhibit increased L-carnitine and L-acetyl carnitine content during epididymal passage; this is concurrent with the acquisition of motility.<sup>[68]</sup> Carnitines accumulate in the epididymis in both free and acetylated forms and are used by spermatozoa for mitochondrial  $\beta$ -oxidation of long chain fatty acids, this being the principal shuttle and transfer system of the acyl to the mitochondrial CoA.<sup>[69,70]</sup> Carnitines enhance the cellular energetics in mitochondria by facilitating the entry and utilization of free fatty acids within the mitochondria and also restore the phospholipid composition of mitochondrial membranes by decreasing fatty acid oxidation.<sup>[71-73]</sup> Carnitine protects sperm DNA and cell membranes from free radical-induced damage and apoptosis<sup>[72,74,75,76]</sup> and has been correlated with sperm parameters such as concentration and motility, which relate to higher fecundity.<sup>[77,78]</sup> The initiation of sperm motility is thought to occur in parallel to an increase in carnitine concentration in the epididymal lumen and L-acetyl-carnitine in spermatozoa.<sup>[68,79,80]</sup> The degree of acetylation of carnitine was shown to be greater in motile than in immotile spermatozoa.<sup>[81]</sup> This is signified by the fact that patients with defective sperm motion parameters were shown to have a reduced L-acetyl-carnitine/L-carnitine ratio.<sup>[82]</sup>

The results of preliminary, uncontrolled studies<sup>[83-85]</sup> suggest that oral carnitine supplementation has a favorable effect on sperm motion characteristics of men with iOAT. A daily carnitine dose of 3 g given for four months<sup>[83]</sup> or for three months<sup>[84]</sup> was seen to significantly improve patients' sperm motility from baseline (pretreatment) levels. A higher dose of 4 g per day over a shorter treatment duration (two months) increased progressive sperm motility in 15 of 20 patients. This effect was more pronounced in seven patients whose partners achieved pregnancy during treatment and follow-up. The utility of carnitine to improve sperm motility of patients with iOAT is supported by more recent, randomized controlled trials in which 2 g carnitine was administered daily.<sup>[72,74,75]</sup> The most significant improvement in motility was seen in the groups with lower baseline motility.<sup>[72,74]</sup> Therefore, carnitine's action may differ depending on the pretreatment semen characteristics. Studies conducted by Lenzi *et al.*, failed to demonstrate any improvement in morphology.<sup>[72,74]</sup> which suggests that carnitine's effects are post-testicular. Conversely, Cavallini *et al.*, reported improved morphology at three and six months in the course of therapy.<sup>[75]</sup> In all of the studies, there was no further improvement in sperm parameters at three and six months, demonstrating that the treatment effects were stable.

### NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

The tubuloseminal plasma of OAT patients has been shown to exhibit elevated levels of prostaglandin.<sup>[86,87]</sup> Treatment with low-dose NSAIDs was shown to improve sperm quality and fertility in an animal model.<sup>[88]</sup> Interestingly, carnitine administration was reported to promote the accumulation

of prostaglandin E2 in seminal fluid.<sup>[89]</sup> Therefore, the concomitant administration of NSAIDs with carnitines may have a complementary mechanism that facilitates the positive effects of carnitine therapy while counteracting excess prostaglandin production. However, a certain level of prostaglandins and oxidative stress is required for physiologic sperm functioning. Although long-term treatment with NSAIDs at low doses has been associated with improved sperm quality and fertility,<sup>[88]</sup> *in vitro* experiments have illustrated that higher dosage of NSAIDs may inhibit sperm motility.<sup>[90]</sup> NSAIDs administered via a suppository are thought to exert a more pronounced and direct effect on seminal plasma due to rectal-prostatic lymphatic pathways.<sup>[91]</sup> A 30 mg suppository of the NSAID Cinnocicam combined with oral L-carnitine and acetyl-L-carnitine significantly increased sperm concentration, motility and morphology and pregnancy rates compared to placebo and to the two forms of carnitines alone.<sup>[75]</sup> The combination of cinnocicam and carnitine was proven to be significantly more active than placebo in two blind prospective controlled trials, and thus, this drug combination is likely to be considered effective.

### VITAMIN C AND E

Vitamin E (a-tocopherol) is one of the most important lipid-soluble antioxidant molecules, residing mainly in the cell membranes. It is thought to interrupt the chain reactions involving lipid peroxidation and enhance the activity of various antioxidants that scavenge free radicals generated during the univalent reduction of molecular oxygen and during normal activity of oxidative enzymes.<sup>[92,93]</sup> Vitamin E acts by breaking pathological, ROS-induced chain reactions. Therefore, this antioxidant confers its protective effects by shielding sperm membrane components from OS damage rather than by influencing ROS production itself. Vitamin E has been used extensively *in vivo* to treat a variety of diseases.<sup>[94]</sup> The results of *in vitro* experiments suggest that vitamin E may protect spermatozoa from oxidative damage and loss of motility as well as enhance the sperm performance in the hamster egg penetration assay.<sup>[95]</sup> In contrast to an earlier study by Giovenco *et al.*, in which patients failed to show any benefit from vitamin E, recent randomized control trials have reported vitamin E supplementation to be efficacious in treating infertility in males with oxidative stress.<sup>[24,96]</sup> Oral vitamin E significantly decreased spermatozoal MDA concentration and increased motility. Vitamin E treatment greatly decreased the MDA concentration in spermatozoa down to normospermic levels, and this change in levels seemed to be a good predictor of pregnancy in the patients' spouses.<sup>[24]</sup>

Vitamin C (ascorbic acid) is a water-soluble ROS scavenger with high potency. It is found in concentrations 10fold higher in seminal plasma than in serum.<sup>[97,98]</sup> Ascorbic

acid protects human spermatozoa against endogenous oxidative DNA damage.<sup>[99]</sup> Significantly reduced ascorbate concentrations have been observed in poor semen samples riddled with excess ROS.<sup>[100]</sup> Seminal plasma ascorbic acid concentrations have been positively correlated with percentage of morphologically normal spermatozoa.<sup>[101]</sup> Therefore, vitamin C supplementation has been assessed for its potential as an oral supplement, along with vitamin E, in the treatment of idiopathic male infertility. The combined hydrophilicity and lipophilicity of vitamins C and E has been hypothesized to act synergistically *in vivo* to reduce peroxidative attack on spermatozoa.<sup>[102]</sup> A randomized controlled double-blind study by Rolf *et al.*, showed that high-dose oral treatment with vitamin C and E for 56 days did not improve semen parameters, sperm survival or pregnancy rates in iOAT.<sup>[103]</sup> Although studies have not demonstrated vitamin E and C to be effective,<sup>[96,103-105]</sup> prospective controlled clinical studies should be carried out using selected patients with identified and known DNA damage for whom antioxidant treatment may be effective.

## SELENIUM

Selenium (Se) is an essential element for normal testicular development, spermatogenesis, and spermatozoa motility and function.<sup>[106]</sup> Se may protect against oxidative DNA damage in human sperm cells. However, the exact mechanism by which Se eliminates oxidative stress to improve male fertility and semen quality in humans is still controversial. The role of Se could be mediated via selenoenzymes, such as phospholipid hydroperoxide glutathione peroxidase (PHGPX)<sup>[107]</sup> and the sperm capsular selenoprotein glutathione peroxidase.<sup>[108]</sup> There are at least 25 selenoproteins in the human body, and they help maintain sperm structure integrity.<sup>[106,109]</sup> The best-characterized spermatozoal effects of Se deficiency are: important loss of motility, breakage at the midpiece level<sup>[110,111]</sup> and increased incidence of sperm-shape abnormalities, mostly of the sperm head.<sup>[112]</sup> This is evidenced by studies that reported a significant correlation between Se levels in seminal plasma and the percentage of morphologically normal sperm in a sample.<sup>[113]</sup> Other studies observed a significant positive correlation levels between sperm concentration and seminal plasma Se in patients consulting for infertility.<sup>[114,115]</sup> However, such relationships between Se levels in semen or seminal plasma and sperm concentration or motility were not observed by other investigators.<sup>[116,117]</sup>

The effectiveness of combined treatment with selenium and vitamin E in treatment of iOAT has been studied since Vitamin E is well known to work in synergy with selenium as an antiperoxidant.<sup>[118,119]</sup> A prospective, uncontrolled study reported that this drug combination led to statistically significant increases in motility and mean seminal plasma glutathione peroxidase activity. Although no improvements in sperm concentration were documented,

and no pregnancies were achieved, the better sperm motion characteristics may be explained by the amplified antioxidant enzyme activity.<sup>[120]</sup> These results were further confirmed by a more recent randomized controlled trial in which vitamin E and selenium improved sperm motility and lipid peroxidation markers.<sup>[121]</sup>

## N-ACETYL CYSTEINE

N-acetyl cysteine (NAC) is a derivative of the naturally-occurring amino acid L-cysteine and it exhibits antioxidant properties. As it is a precursor of glutathione (GSH), NAC works to increase the concentration of this endogenous reducing agent while also directly alleviating OS by scavenging free radicals.<sup>[122]</sup> The antioxidant actions of NAC have been observed to play an important role in germ cell survival in human seminiferous tubules *in vitro*.<sup>[123]</sup> Oeda *et al.*, found that incubating semen samples with NAC for 20 min significantly decreased ROS levels and led to improved sperm motility.<sup>[124]</sup> An uncontrolled study by Comhaire *et al.*, found that NAC improved sperm concentration and acrosome reaction, while reducing ROS and oxidation of sperm DNA. However, NAC did not appear to have an effect on motility and morphology.<sup>[125]</sup>

Researchers have studied the efficacy of NAC as part of a combined antioxidant regimen. A randomized controlled trial by Safarinejad *et al.*, reported that NAC with selenium has additive beneficial effects on mean sperm concentration and percent normal morphology. By the end of a 26-week treatment period, motility increased significantly in the combined treatment group and in those patients receiving selenium alone, compared to placebo. Furthermore, combination treatment led to significantly better sperm parameters than treatment with only selenium.<sup>[126]</sup>

## ZINC AND FOLIC ACID

Zinc therapy may reduce iOAT by preventing oxidative stress, apoptosis and sperm DNA fragmentation. Seminal plasma zinc concentrations have been demonstrated to differ significantly between fertile and subfertile men.<sup>[127]</sup> Zinc may promote male fertility by conferring protection to sperm structure. Zinc deficiency has been associated with grossly abnormal flagella showing marked hypertrophy and hyperplasia of the fibrous sheath, axonemal disruption and partial defects of the inner dynein arms of microtubular doublets, with distorted inner axonemal structure and a poorly formed or absent midpiece.<sup>[27]</sup> Prospective studies show an improvement of sperm concentration,<sup>[128-130]</sup> progressive motility, sperm integrity and pregnancy rates in subfertile iOAT patients after zinc supplementation. In a recent randomized controlled trial by Omu *et al.*, zinc therapy in 11 men with iOAT was shown to yield various benefits, including reduction of MDA and tumor-necrosis-factor (TNF), enhancement of antioxidant capacity in the

form of Zn-Copper-SOD, increased seminal Bcl-2, decreased expression of Bax, decreased titers of antisperm antibodies with immunoglobulin G, decreased DNA fragmentation and enhanced expression of the anti-inflammatory cytokine interleukin-4. Zinc therapy led to improved sperm parameters, although the improvements were not statistically significant.<sup>[27]</sup> Therefore, zinc may be useful in iOAT in reducing oxidative stress and the associated sperm membrane and DNA damage.

Zinc and folic acid are both essential for transfer RNA and DNA synthesis. However, the underlying mechanisms by which these micronutrients may affect spermatogenesis are not known.<sup>[131]</sup> According to the results of a study where this combined regimen led to an increase in sperm concentration, zinc and folic acid treatment were seen to have an endocrine-independent mechanism as FSH, testosterone and inhibin levels remained unaltered.<sup>[132]</sup> However, other studies have failed to demonstrate any significant difference in concentrations of zinc and folic acid between fertile and subfertile males.<sup>[133,134]</sup> Landau *et al.*, reported that daily supplementation with 10 mg of folic acid for 30 days had no beneficial effect on sperm concentration in normospermic or iOAT men.<sup>[135]</sup> Animal *in vivo* and *in vitro* studies have shown that zinc deficiency alters the absorption and metabolism of dietary folate.<sup>[136-138]</sup> A recent double-blind randomized controlled trial was conducted to assess whether zinc and folate supplementation work synergistically to improve semen quality.<sup>[139]</sup> Folic acid was given at a daily dose of 5 mg, and zinc sulfate was given at a daily dose of 66 mg. Subfertile men demonstrated a significant 74% increase in total normal sperm count and a minor increase of 4% in abnormal spermatozoa. A similar trend was observed in fertile men. However, whether the improvement in sperm concentration observed after administration of folic acid and zinc will lead to an increase in pregnancy rates remains to be established. Before widescale implementation of combined zinc and folic acid administration, we recommend that a larger randomized, placebo-controlled study on the efficacy and safety of these compounds be done.

### MAST CELL STABILIZERS

Maseki *et al.*, first reported mastocytosis in the testes of infertile males, indicating that there is a relationship between mast cell proliferation and testicular dysfunction.<sup>[140]</sup> Testicular biopsy samples show that mast cell counts are higher in men with idiopathic infertility than in normospermic men.<sup>[141,142]</sup> Hashimoto *et al.*, demonstrated that the number of mast cells was increased not only in the interstitium but also in the lamina propria of the seminiferous tubules in the idiopathic infertile testes.<sup>[143]</sup> The administration of a mast cell blocking agent was reported to have positive effects on the semen parameters of infertile men in a pilot study.<sup>[144]</sup>

Mast cell blocking agents inhibit the production or release of histamine, lipid mediators and cytokines from inflammatory cells and macrophages. These anti-inflammatory and immunomodulatory properties serve to decrease oxidative stress by suppressing the production of prostaglandins and interleukins and attenuating the pro-inflammatory activity of monocytes. Administration of the mast cell stabilizer, tranilast, has been reported to lead to the appearance of spermatozoa in the seminal fluid of men with idiopathic azoospermia within a year of treatment.<sup>[145]</sup>

Yamamoto *et al.*, conducted a controlled, single-blind study in which tranilast significantly increased the pregnancy rate from 0% in the placebo group to 26.8% in men with idiopathic severe oligozoospermia treated with 200 mg of tranilast per day for three months. The mast cell blocker group exhibited significantly higher levels of sperm density, sperm motility, and total motile sperm count. However, the two groups did not differ in terms of seminal volume and normal sperm morphology.<sup>[146]</sup> Hibi *et al.*, performed a double-blind controlled study to confirm these findings, using the same dosage and duration of tranilast treatment in 17 men with iOAT. Treatment resulted in significantly increased sperm concentration, although there was no change in motility, morphology or pregnancy rates when compared to placebo. Another study by Hibi *et al.* further confirmed that tranilast demonstrates a certain clinical benefit in iOAT. However, this improvement was not sustained after discontinuation of therapy.<sup>[147]</sup>

### LYCOPENE

Lycopene is a naturally synthesized carotenoid found in fruits and vegetables and is an important component of the human redox defense mechanism against free radicals. It has been found to have the highest physical quenching rate constant with singlet oxygen, and its plasma level is higher than that of beta carotene.<sup>[148]</sup> Lycopene is found in high concentrations in the testes and seminal plasma. Levels tend to be lower in men suffering from infertility. Gupta and Kumar<sup>[149]</sup> evaluated the effect of oral lycopene therapy in 30 men with iOAT. A 200 mg dose of lycopene, twice a day for three months, led to statistically significant improvements in the sperm concentration for 66% of patients and motility in 53%. However, those with baseline sperm concentration less than 5 million/ml did not exhibit significant improvement in response to therapy, whereas higher baseline concentrations were associated with significant improvement and resulted in six pregnancies in 26 patients. Therefore, larger randomized controlled trials are required to establish the indications for lycopene therapy in iOAT.

### PENTOXIFYLLINE

Pentoxifylline is a competitive nonselective phosphodiesterase inhibitor that raises intracellular cAMP and

reduces inflammation by inhibiting TNF- $\alpha$  and leukotriene synthesis. Pentoxifylline has been reported to decrease ROS production<sup>[150,151]</sup> and to preserve sperm motility *in vitro*<sup>[152]</sup> and improve semen parameters *in vivo*.<sup>[153,154]</sup> Tesarik *et al.*, demonstrated that in unselected asthenospermic patients, pentoxifylline improved sperm motion characteristics such as curvilinear velocity, path velocity and beat cross frequency but did not increase the percentage of motile spermatozoa.<sup>[155]</sup> Okada *et al.*, studied the effects of *in vitro* and *in vivo* pentoxifylline treatment on sperm motion parameters in select asthenospermic patients whose spermatozoa produced detectable steady state levels of ROS. Treatment decreased ROS formation and preserved sperm motion parameters *in vitro*. Orally administered pentoxifylline had no effect at a low dosage, whereas a high dosage was seen to increase sperm motility and some sperm motion parameters without altering sperm fertilizing ability.

### ADRENERGIC ANTAGONISTS

Although there is no clear pathophysiological basis for the use of  $\alpha$ -blocking agents, they have been studied for their effects in the treatment of iOAT.<sup>[156]</sup> Two placebo-controlled trials demonstrated an improvement in sperm concentration but not of ejaculate volume, morphology, motility or pregnancy rates in the treatment group.<sup>[157,158]</sup>

### CONCLUSION

Various methods of assisted reproduction have been proposed as a possible solution for male factor infertility. Although these techniques have motivated extensive research in sperm function, they have also hindered the development of new strategies for male factor infertility therapy. Assisted reproductive technology is not universally available, is expensive and has limited success. Therefore, any pharmacological agent that has a low cost and satisfactory effectiveness should be considered as first-line treatment in iOAT.

The pharmacological treatment of iOAT has yet to be standardized. Unfortunately, many drugs are currently used without any rationale: such therapies are often prescribed sequentially without any beneficial effect, and any improvements that do occur in semen parameters may be due to other unrelated reasons. Seasonal,<sup>[159]</sup> regional,<sup>[160]</sup> and racial<sup>[161]</sup> differences in sperm count and quality make it difficult to consider iOAT data as absolutely valid. Furthermore, the available forms of treatment have mostly produced only marginally satisfactory responses, even in the best of proper trials. This inadequacy relates to the fact that iOAT is a syndrome that has a number of different etiologies, and only some subsets will respond favorably to treatment, whereas others will experience no appreciable changes. Routine laboratory investigation and semen analyses are unable to delineate iOAT subgroups according to etiology.

Furthermore, conflicting results and conclusions in published studies could also arise from differences in the methodology of study design and semen analysis, andrological history and demographic characteristics of the study population. We sought to overcome this issue by employing a scoring system that allowed us to rank the quality of the overall evidence available on various antioxidant therapies for iOAT. Future randomized controlled trials are needed with larger sample sizes and with selection criteria to identify and study different iOAT subgroups.

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