New Developments for the Enhancement of Male Reproductive Health Using Antioxidant Therapy: A Critical Review of the Literature

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Key Points

- Oxidative stress is a significant cause of impaired sperm function resulting in infertility, miscarriage and possibly even long-term health consequences for the next generation.
- Optimal prevention of sperm oxidative stress should focus on reduction of leukocyte ROS production using anti-inflammatory therapies (NSAID, probiotics, omega-3 fish oils), neutralization of ROS by traditional antioxidants (vitamins C and E, lycopene, coenzyme Q10 and the like) and fortification of sperm against ROS-mediated damage (DNA protamination using zinc/selenium).
- The evidence for treatment of male infertility with antioxidants is difficult to critically interpret because of underpowered studies using a large number of different types and dosages of antioxidant, failing to screen for oxidative stress at enrolment, selective reporting on sperm quality rather than pregnancy as the primary endpoint and a lack of concurrent placebo controls.
- Direct antioxidants such as vitamin E, vitamin C, selenium, lycopene, coenzyme Q_{10} and astaxanthin all appear to improve sperm health by reducing seminal ROS levels and decreasing sperm membrane peroxidation and oxidative DNA damage; in addition, some evidence show that these antioxidants may improve natural and in vitro conception.
- Anti-inflammatory therapies such as omega-3 fish oil and probiotics/prebiotics have been shown in RCT to improve sperm function, but no study to date has analysed their ability to enhance pregnancy rates.

45.1 Introduction

Infertility is a condition that affects one in six couples, with impaired sperm quality playing a role in at least half of all cases of infertility. Of even more concern is the evidence suggesting that sperm quality has actually been decreasing over the past 50 years [1], leading to more and more couples requiring expensive fertility treatments such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). This trend has prompted researchers to focus more on identifying the underlying causes of male infertility, allowing treatments to be tailored to pathology, rather than a reliance on generic "mechanical" solutions such as ICSI.

While the identifiable causes of male infertility are many and varied (reviewed in Chap. 1), oxidative stress has been identified as a very significant cause.

Infertile men's semen contains higher levels of reactive oxygen species (ROS) and lower levels of protective antioxidants than fertile men, thereby placing these men's sperm at increased risk of oxidative damage. It has been estimated that between 30% and 80% of infertile men have some evidence of oxidative stress damage to their sperm, even when routine semen analysis results (concentration, motility and morphology) are within the normal WHO prescribed range [2]. Secondly, in vitro studies have confirmed that the direct application of ROS to sperm or the stimulation of sperm's own production of ROS can reduce sperm motility, membrane integrity and DNA quality, all linked with reduced male reproductive capacity. Finally, direct application of antioxidants in vitro can block the harmful effects of ROS on sperm motility and DNA integrity, confirming a causal association between oxidative stress and impaired male reproductive function. In summary, there appears to be a very sound scientific rationale to the use of antioxidants to treat male infertility.

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45.2 "Taming the Flames": A Holistic Path to Preventing Sperm Oxidative Stress

> "An ounce of prevention is better than a pound of cure"—Benjamin Franklin (American statesman, 1736)

While this famous axiom was made by Franklin in reference to fire prevention, it is equally true when applied to "taming the flames" of ROS damage to sperm. Oxidative stress is the net result between the production of potentially destructive ROS by sperm and seminal leukocytes and their neutralization by protective antioxidants [2]. As such, the optimal medical treatment of sperm oxidative stress should address both sides of this potentially destructive process. However, while a multitude of studies have examined the impact of antioxidant preparations that directly neutralize ROS, very little attention has been paid to therapies that may actually reduce the initial production of ROS or alternatively fortify the sperm against ROS attack through non-antioxidant mechanisms. The first part of this chapter will focus on the traditional antioxidant "ROS neutralization" approach, where the later parts will discuss some of the newer and less well-known therapies for reducing the production of ROS through anti-inflammatory action, plus fortifying sperm DNA against ROS attack.

45.3 Antioxidant Therapy for the Treatment of Male Infertility

This critical review of the literature will primarily focus on well-conducted randomized controlled trials (RCT) using various antioxidant and anti-inflammatory (indirect antioxidant action) agents in the setting of male infertility. The principal outcomes of interest are sperm quality, biochemical evidence of antioxidant effect and pregnancy outcome. Where appropriate, important non-randomized therapeutic and observational studies, plus supportive animal work, will also be outlined. Trials with poorly defined treatments such as the use of "general multivitamins" or studies that employed botanical preparations with poorly defined antioxidant action were excluded from this review.

Table 45.1 outlines the existing RCT evidence for the use of antioxidants to improve sperm function and treat infertility. With the existence of such a large body of evidence, one would expect that definitive conclusions on the value of antioxidant supplements in the treatment of male infertility would be able to be made. Unfortunately, this is not the case for several reasons. Firstly, there is a huge variation in the different types and dosages of antioxidants used in the published studies. Secondly, most of the studies are small and therefore underpowered, making meaningful analysis of differences in pregnancy outcomes with antioxidant therapy very difficult. Thirdly, many trials lacked appropriate inclusion criteria such as screening men for the presence of oxidative stress before enrolment. Finally, the paucity of clinically relevant endpoints such as live birth, rather than mere improvement in sperm quality, hampers the drawing of firm conclusions on the benefit of antioxidant preparations as a treatment for infertility [3].

The overview in Table 45.1 summarizes the methodological strengths and weaknesses of the available placebo-controlled antioxidant trials published up to January 2019. The finer detail of the various medications and trials is outlined below, thereby allowing the reader to make their own conclusions on the merits of antioxidant supplement therapy for the treatment of male infertility.

45.4 Vitamin E

Vitamin E is an essential fat-soluble vitamin, with α-tocopherol being the most common form of vitamin E available in food. Vitamin E is the major chain breaking antioxidant that directly neutralizes superoxide anions, hydrogen peroxide and the hydroxyl radical. As sperm membranes contain abundant phospholipids which are prone to oxidative damage, it is believed that vitamin E plays a critical role in protecting cellular structures from damage caused by free radicals and reactive products of lipid peroxidation. Furthermore, vitamin E exhibits some anti-inflammatory activity and, therefore, may also reduce leukocyte-initiated sperm oxidative stress.

The recommended dietary allowance (RDA) for vitamin E is suggested to be 15 mg (equivalent to 22.4 IU) of α-tocopherol per day for adult men, with the tolerable upper intake being suggested as 1000 mg (1500 IU) by the US National Institute of Health [4]. However, a meta-analysis of 19 clinical trials using long-term vitamin E supplementation in patients with chronic disease has reported that at dosages of 400 IU or greater per day, vitamin E may actually increase overall mortality compared to placebo [5]. Furthermore, it is known to inhibit platelet aggregation and has been linked with an increased risk of haemorrhagic stroke. Therefore, its use in infertile men on anticoagulants or at risk of serious haemorrhagic illness is probably contraindicated.

Two studies have analysed the ability of vitamin E to decrease sperm membrane oxidative damage by measurement of sperm malondialdehyde (MDA) levels before and after vitamin E supplementation. Geva et al. [6] reported that 200 mg a day of vitamin E was able to significantly reduce MDA levels within 1 month of therapy, while Suleiman et al. [7] found that the use of 300 mg of vitamin E per day for 6 months also produced a significant drop in MDA levels. Consistent with a reduction in sperm membrane oxidative damage, two studies have also reported an improvement in

Table 45.1 Randomized controlled studies examining the effect of direct antioxidant therapy on male reproductive health

Study reference	Therapy used per day	Duration of therapy (months)	Positive changes in semen quality	Positive changes in sperm OS endpoints	Positive changes in reproductive outcomes
[7]	Vitamin E 300 mg	6	↑ motility	↓ MDA	Pregnancy 17% active group vs. 0% placebo
[8]	Vitamin E 300 mg	3	Nil	Nil	Improved sperm-zona binding
[14]	Vitamin C (200 or 1000 mg)	1	↑ motility, morphology and viability	Not tested	Not reported
[16]	Vitamin C 500 mg	3	↑ motility, morphology	Not tested	Not reported
[17]	Vitamin E 800 mg, vitamin C 1000 mg	2	Nil	Not tested	None
[18]	Vitamins E and C (1000 mg each)	2	Nil	↓ sperm DNA damage	Not reported
[22]	Vitamin E 10 mg, vitamin C 5 mg, zinc 200 mg	3	Nil	Trend for ↓ MDA	Not reported
[24]	Coenzyme Q10 300 mg	6	↑ concentration and motility	Not tested	No difference in pregnancy rates
[25]	Coenzyme Q10 200 mg	3	Nil	↓ MDA	Not reported
[27]	Ubiquinol 200 mg	6	↑ concentration and motility and morphology	Not tested	Not reported
[31]	Sn 100 mg, vitamin A 1 mg, vitamin C 10 mg, vitamin E 15 mg	3	↑ motility	Not tested	No difference in pregnancy rates (11% vs. 0% placebo)
[32]	Sn 200 mg, NAC 600 mg	6	↑ concentration, motility and morphology	Not tested	Not reported
[33]	Glutathione 600 mg	2	↑ motility and morphology	Not tested	Not reported
[36]	L-Carnitine 2 g	2	Nil (raw data analysis)	No change in MDA	No difference
[38]	L-Carnitine 1 g	3	↑ concentration and motility	Not tested	Not reported
[39]	NAC 600 mg	3	↑ sperm concentration	Not tested	Not reported
[40]	NAC 600 mg	3	↑ motility	Not tested	Not reported
[41]	Astaxanthin 16 mg	3	↑ motility	↓ semen ROS ↓ MDA	↑ natural + IUI conceptions
[43]	Alpha-lipoic acid (ALA) 600 mg	3	↑ concentration and motility	↑ TAC	Not reported
[44]	Vitamin E 400 mg, Sn 225 mg	3	↑ motility	↓ MDA	Not reported
[45]	Vitamin C 30 mg, vitamin E 5 mg, β-glucan 20 mg, papaya 50 mg, lactoferrin 97 mg	3	↑ motility and morphology	No change in DNA quality	Not reported
[48]	Zinc sulphate 220 mg, folate 5 mg	4	Nil	Not tested	Not reported
[49]	Zinc sulphate 66 mg, folate 5 mg	6	↑ concentration and morphology (subfertile men only)	Not tested	Not reported
[51]	Menevit® (vitamins C and E, Sn, lycopene, folate, zinc and garlic oil)	3	Not reported	Not tested	↑ IVF-ICSI conceptions on active antioxidant (38.5% vs. 16% placebo)

OS oxidative stress, *MDA* malondialdehyde, *ROS* reactive oxygen species, *TAC* total antioxidant capacity, *Sn* selenium, *NAC N*-acetyl cysteine, *IUI* intrauterine insemination

in vitro sperm fertilization capacity assessed by routine insemination IVF or the use of sperm-zona binding assays [6, 8]. No study to date has analysed the ability of vitamin E monotherapy to improve sperm DNA quality.

Two small non-controlled studies of vitamin E supplementation have reported no effect on sperm count, motility or morphology [9, 10]. In addition, two well-conducted placebo-controlled trials of 3–12 months therapy using 600 mg of vitamin E per day also reported no significant effect on sperm concentration, motility or morphology [8, 11]. Conversely, another randomized controlled trial (RCT) using 6 months of vitamin E (300 mg/day) reported

a statistically significant improvement in sperm motility but no change in concentration or morphology [7]. However, in this last trial, the "dropout" rate for patients in the placebo arm was significantly greater than that seen in the active treatment arm (20 vs. 3 patients, respectively, from a starting number of 55 patients in each study arm). This selective "dropout" from placebo raises the possibility that patients or their treating physicians became unblinded to treatment allocation during the trial, biasing the final results.

No study to date using vitamin E monotherapy for the treatment of male infertility has been adequately powered to analyse pregnancy outcomes. While some studies have reported pregnancies [7, 8], the small number of pregnancies makes clear conclusions impossible. In the Suleiman study, 17% of patients allocated to vitamin E therapy achieved a live birth, compared to none in the placebo [7]. However, as previously outlined, the large "dropout" rate in the placebo arm suggests the potential for significant bias, thereby making it impossible to make firm conclusions on the value of vitamin E to assist pregnancy in the setting of male infertility.

45.5 Vitamin C

Vitamin C (ascorbic acid) is an important water-soluble antioxidant that competitively protects lipoproteins from peroxyl radical attack, while also enhancing the antioxidant activity of vitamin E by assisting in its recycling. Seminal plasma vitamin C levels are tenfold higher than serum [12], suggesting a very important protective role for vitamin C in the male reproductive tract. The RDA for vitamin C in the adult male is 75 mg, with the tolerable upper intake limit being suggested as 2000 mg/day [4]. However, the use of high dosages of vitamin C (≥1 g/day) may be harmful since at these high concentrations vitamin C can act as a pro-oxidant and may predispose to kidney stone formation [4, 13].

A small placebo-controlled randomized study of 30 infertile men allocated an equal number of participants to placebo, 200 mg or 1000 mg of vitamin C per day for a total of 4 weeks [14]. Both dosages of vitamin C were able to significantly increase seminal plasma vitamin C levels, with the magnitude of the increase being more significant in the group treated with 1000 mg. Sperm motility, morphology and viability all significantly improved within 1 week of vitamin C therapy. While the average sperm concentration doubled on vitamin C, this did not reach statistical significance. More critical analysis of the baseline characteristics in this study suggests that randomization may not have been successful in creating study groups that were equal. For example, at entry, the percentage of abnormal sperm morphology was 45, 64 and 62% for the placebo, 200 and 1000 mg vitamin C groups, respectively. At the end of the study, abnormal sperm morphology was 41% in the placebo group and 35 and 36% in the vitamin C groups, which was statistically significant. Critical analysis of these results suggests that the two vitamin C groups' morphology results were significantly inferior to the placebo at study entry, and that following 4 weeks of vitamin C treatment, these poor morphology results simply returned to levels equivalent to that seen in the placebo. This raises the possibility of selection bias or at least a "regression to the mean" spontaneous improvement in sperm morphology. Pregnancy outcomes were not reported in this trial, although in the introduction, the authors comment that a prior unpublished pilot study had achieved a 100% pregnancy rate with vitamin C therapy ($n = 20$ patients).

A larger study that randomly allocated 75 smokers to a placebo, 200 or 1000 mg vitamin C per day reported significant improvements in sperm morphology in the 1000 mg subgroup but no significant changes in the 200-mg-treated group [15]. As these men were not infertile and were not trying for pregnancy, the implications of this study for the infertile population are uncertain.

Finally, an RCT of 115 infertile men with probable oxidative stress (varicocele) to receive either vitamin C (250 mg/day) or placebo did report the ability of this antioxidant therapy to significantly boost sperm motility and morphology over a 3-month period but had no impact on sperm concentration [16]. Therefore, the overall impression on vitamin C monotherapy is that it may enhance sperm function (motility) but is unlikely to enhance sperm production (count).

45.6 Combined Vitamin C and Vitamin E Therapy

Two excellent placebo-controlled randomized studies have examined the ability of a combination of vitamins C and E to alter sperm quality. Rolf et al. [17] reported a small RCT in which infertile patients were allocated to either placebo ($n = 16$) or 2 months of treatment with 800 mg vitamin E and 1000 mg vitamin C ($n = 15$). The inclusion criteria for this study were impaired motility, not the presence of confirmed oxidative stress. No significant difference in sperm concentration, motility or morphology was observed during therapy, and no direct assessment of oxidative damage was made. Furthermore, no pregnancies were seen in either study group during the treatment period. A similar RCT using 2 months of therapy with 1000 mg of both vitamins C and E daily or placebo also found no significant changes in sperm count, motility or morphology [18]. However, this group did observe a very significant drop in sperm DNA damage. Unfortunately, pregnancy outcomes were not reported in this study, but were reported for a non-controlled study using the same treatment protocol by the same clinical group [19].

In this later report, patients who had failed to have a successful pregnancy after at least one cycle of IVF and who had documented elevated levels of sperm DNA damage were given 2 months of vitamins C and E combination therapy before a further cycle of IVF treatment. A total of 76.3% of participants experienced a normalization of their sperm DNA damage, and this "improved" subgroup achieved an implantation rate of 19.6%. As the study did not include a concurrent placebo control, firm conclusions on pregnancy effect are not possible.

Several small non-placebo-controlled trials have also examined the effect of vitamins C and E combinations on sperm quality. Kodama et al. [20] was able to show a significant drop in sperm DNA oxidative damage (8-OHdG) and MDA with 2 months of therapy (200 mg vitamin C, 200 mg vitamin E and 400 mg glutathione). They also reported a small but significant increase in sperm concentration but no effect of antioxidant supplementation on sperm motility or morphology. Menezo [21] observed a significant drop in sperm DNA fragmentation with 2 months of antioxidant therapy (400 mg vitamins C and E per day, plus low dosages of vitamin A, zinc and selenium) but no change in sperm concentration, motility or morphology. Interestingly, these investigators also noted a significant increase in sperm DNA decondensation. They believed that this was due to the high redox potential of vitamin C interfering with the reduction of cystine to two cysteine moieties, thereby opening protamine disulphide bridges. Decondensation of the sperm DNA may make the DNA more susceptible to ROS attack and may interfere with proper embryo development. Menezo, therefore, cautions against the use of antioxidant preparations containing high dosages of vitamin C in infertile men with sperm decondensation levels exceeding 20% at baseline. Finally, a small placebo-controlled study of 45 infertile men allocated to placebo, vitamin C 5 mg/vitamin E 10 mg/200 mg zinc or zinc alone observed a non-significant trend in improvement in sperm motility and a decrease in MDA in each treatment group [22]. The magnitude of improvement in MDA and motility was similar in the zinc-alone group compared to those treated with zinc and vitamins C and E. This observation suggests that the low dosages of vitamins C and E used in this study are likely to be subtherapeutic, with any improvement in sperm quality more likely to reflect the action of zinc.

Overall, the high-quality placebo-controlled studies suggest that vitamins C and E do not produce large improvements in sperm concentration, motility or morphology. The significant drop in sperm DNA damage seen in two trials [18, 21], together with observations of a drop in 8-OHdG and MDA in a non-placebo-controlled study [20], suggests that the combination of vitamins C and E can still have positive reproductive effects by enhancing sperm function even if they do not alter routine sperm parameters. The ability of vitamins C and E to improve pregnancy rates is still debatable until future adequately powered studies are conducted in this area.

45.7 Coenzyme Q_{10}

Coenzyme Q_{10} is primarily concentrated in the mitochondria of the sperm midpiece and plays an important antioxidant and energy production role in sperm. Coenzyme Q_{10} transports electrons from complexes I and II to complex III in the mitochondrial respiratory chain, leading to ATP synthesis in the mitochondrial membrane. In its reduced form (ubiquinol), coenzyme Q_{10} acts as a strong antioxidant preventing lipid peroxidation in biological membranes.

A small non-controlled study involving 38 men with male factor infertility and previous poor fertilization during IVF–ICSI therapy reported on the use of 60 mg of coenzyme Q_{10} per day for a period of 3 months [23]. This study found that coenzyme Q_{10} produced no significant changes in sperm concentration, motility or morphology, yet IVF–ICSI fertilization rates did improved significantly. This study did not measure sperm lipid peroxidation or DNA damage and offered no explanation on how coenzyme Q_{10} supplementation may boost fertilization without altering routine sperm parameters.

A large and very well-conducted placebo RCT recently reported on the use of 300 mg coenzyme Q_{10} or placebo per day for a period of 6 months in 212 men with male factor infertility [24]. At this dose of supplementation, a significant increase in seminal plasma coenzyme Q_{10} concentration was observed. Furthermore, significant improvements in sperm count and motility were also observed, together with an increase in serum inhibin B levels and a corresponding fall in FSH concentration. This would suggest that coenzyme Q_{10} therapy can enhance Sertoli cell function, not just sperm function. This study did not report on oxidative endpoints but did report a significant increase in the calcium ionophore-induced acrosome reaction, suggesting some improvement in sperm membrane function. Unfortunately, no significant difference in pregnancy rates was observed between the placebo and coenzyme Q_{10} supplement groups over a 12-month period of observation. This is not surprising when one recognizes that the magnitude of the statistically significant changes in sperm parameters observed were very small and unlikely to be of clinical significance. For example, total sperm motility after 6 months of coenzyme Q_{10} therapy was 27.6%, compared to 23.1% in the placebo group. While for the coenzyme Q_{10} group this was a statistically significant increase in sperm motility from baseline (22.2%), the final motility result was not significantly different from the placebo and highly unlikely to be of any clinical significance. As only men, who had partners with no evidence for female

factor infertility, were enrolled in the study, the lack of differences in pregnancy outcomes in such a large study suggests that coenzyme Q_{10} monotherapy is not of major benefit in assisting in vivo conception.

More recently an RCT using 200 mg of coenzyme Q_{10} or placebo over a 3-month period reported no significant change in routine sperm quality parameters but a significant decline in the lipid peroxidation marker MDA, confirming a biologically relevant antioxidant effect [25]. Another study of 300 mg of coenzyme Q10 daily did report some improvements in sperm parameters and a 34.1% pregnancy rate over the next 12 months [26]. However, as this later study had no concurrent placebo control, it is difficult to objectively assess coenzyme Q_{10}'s ability to boost natural conception.

Ubiquinol, the reduced more biologically active form of coenzyme Q_{10}, has been shown in an RCT (200 mg daily) to significantly increase sperm concentration, motility and morphology, while also boosting Sertoli cell health (reduction in FSH) [27]. These improvements in sperm quality with ubiquinol were greater than those observed by the same group using coenzyme Q_{10} [24], suggesting the former may be the optimal biologically active form of coenzyme Q_{10} therapy. Unfortunately, this RCT of ubiquinol did not assess pregnancy rate. Furthermore, a recent meta-analysis concluded that there is insufficient evidence that coenzyme Q_{10} boost the chances of successful conception [28].

45.8 Selenium

Selenium is an essential trace element required for normal male reproductive function. The antioxidant glutathione peroxidase 4 (GPX-4) is present within sperm and requires the presence of selenium to function. Not only does GPX-4 play an antioxidant role, it is also involved in augmenting sperm chromatin stability by acting as a protein thiol peroxidase. The adult male RDA for selenium is 55 μg/day, with the upper tolerable limit being 400 μg/day [4]. An individual's dietary intake of selenium depends on the selenium content in the local soil where food is grown. Men living in countries such as China where the soil is commonly selenium deficient are more likely to benefit from selenium supplementation. Conversely, excess supplementation of selenium may lead to toxicity and have detrimental effects on sperm quality [29].

Iwanier et al. [30] gave 200 μg of selenium per day to a group of men (33 infertile and 9 fertile) for a period of 2 months and measured sperm quality before and after treatment. The investigators observed a significant increase in seminal plasma selenium concentration and GPX activity during the trial but no significant improvement in sperm concentration, motility or morphology. In a small placebo RCT (n = 18 placebo, 46 active treatment), the supplementation of infertile men exhibiting low sperm motility with 100 μg of selenium (± very low dosages of vitamins A, C and E) produced no significant change in sperm concentration but a small significant improvement in motility (20.6–28.2%) [31]. The clinical significance of this improvement is questionable, as no significant difference in pregnancy rates was observed (no pregnancies in the placebo vs. 11% selenium group).

A very large placebo-controlled study randomized 468 infertile men to either placebo, 200 μg/day of selenium, with or without *N*-acetyl-cysteine, for a period of 6 months [32]. Sperm quality and male reproductive hormones were then assessed during supplementation and for a further 6 months. This study observed statistically significant increases in sperm concentration, motility and morphology in all treatment arms, together with an increase in serum inhibin B and testosterone. However, the magnitude of these improvements was again very small and unlikely to be of any clinical significance. Unfortunately, pregnancy outcomes were not reported for this study, making it impossible to draw any firm conclusions on the benefits of selenium supplementation to boost pregnancy rates.

45.9 Glutathione

Glutathione is an antioxidant released in large amounts by the epididymis that in turn can neutralize the damaging effects of superoxide anions, thereby preventing lipid peroxidation. Two trials by a single group of investigators have examined the effect of glutathione supplementation (600 μg intramuscular alternate days for 2 months) on two separate groups of infertile men. The first trial involved 20 infertile men with likely oxidative stress (past genitourinary tract infection with residual inflammation, varicocele) in a placebo crossover trial design [33]. This study observed no significant changes in sperm concentration, but significant improvements in sperm motility and morphology. These improvements were observed within 1 month of supplementation, suggesting an epididymal rather than a testicular mode of action. A second smaller non-controlled study using identical inclusion criteria examined changes in sperm lipid peroxidation with glutathione treatment [34]. This study observed improvement in all routine sperm parameters and a significant decrease in sperm MDA concentration, confirming an antioxidant effect. Neither study reported pregnancy outcome, making conclusions about the fertility promoting effect of glutathione treatment impossible. However, the requirement for intramuscular administration of glutathione therapy is certainly likely to limit its clinical application.

45.10 L-Carnitine

Carnitine is produced in the liver and then passes via the circulation to the epididymis, where it is taken up by the epididymal epithelium and actively transported into the luminal

fluid bathing sperm. In the epididymis carnitine is taken up by sperm, where it involves in energy metabolism by transporting fatty acids from the cytosolic compartment to the mitochondrial matrix.

Costa et al. [35] were the first to examine the effects of L-carnitine supplementation in the setting of male infertility. Their study group of 100 infertile men with unexplained impaired motility were given L-carnitine (3 g/day) for a period of 4 months, while measuring changes in sperm function. They reported small but statistically significant improvements in sperm concentration and motility but no changes in sperm morphology. Lenzi et al. [36] used an active medication/washout/placebo study design to determine if 2 months of L-carnitine therapy (2 g/day) could alter sperm quality. Analysis of the raw outcome data indicated no significant difference in sperm quality after L-carnitine therapy. However, when the researchers excluded several "outliers" from the analysis, a borderline statistically significant increase in sperm concentration and motility was reported. The subjective removal of "outliers" to create statistical significance, plus the failure of L-carnitine therapy to either improve epididymal function (alpha-glucosidase) or reduce levels of sperm lipid peroxidation, casts significant doubt on whether L-carnitine therapy has any beneficial effect on male reproductive performance.

Vicari et al. [37] studied the ability of L-carnitine in combination with non-steroidal inflammatory medication (NSAID) to alter sperm function in a group of 98 infertile men with confirmed oxidative stress. Two months of pretreatment with NSAIDs, followed by 2 months of L-carnitine (2 g/day), produced a significant reduction in seminal ROS production and an improvement in sperm motility and viability. A total of 23% of patients on NSAID/L-carnitine therapies achieved pregnancy, but the absence of a control group makes firm conclusions on these therapies effect on pregnancy rates impossible.

Finally, the most recent RCT of L-carnitine therapy (500 mg bd) over a 3-month period reported a significant improvement in sperm count and motility but no change in morphology [38]. This study did not report on pregnancy outcome, as was the case for all the previous L-carnitine studies. As such there is no convincing evidence that L-carnitine therapy actually translates into an improvement in chances of conception – the most clinically relevant endpoint.

45.11 *N*-Acetyl Cysteine

N-Acetyl cysteine (NAC) is believed to act as a precursor to glutathione, increasing the tissue concentration of this potent antioxidant. Recently, several good-quality placebo-controlled studies have examined the ability of NAC to alter sperm quality in infertile men with presumed oxidative pathology. Galatioto et al. [39] conducted a RCT in which 42 men with oligospermia were allocated to receive either 600 mg NAC a day plus a vitamin-mineral supplement for 3 months or no therapy at all. This small study reported a significant increase in sperm concentration but no change in sperm motility or morphology. A larger placebo-controlled study using 600 mg/day of NAC for a period of 3 months reported no change in sperm concentration or morphology but a small improvement in motility [40]. Finally, one arm of a multi-therapy RCT compared sperm quality between men with idiopathic male factor infertility on 600 mg NAC per day with placebo [32]. This study reported very minor, although statistically significant, improvements in sperm concentration and morphology but no changes in sperm motility.

The conflicting sperm quality outcomes for these three trials using an identical dose of NAC and the failure to report pregnancy outcomes make it impossible to conclude that NAC therapy has any clinically meaningful effect on male reproductive performance.

45.12 Miscellaneous Antioxidant Monotherapies

Astaxanthin is a carotenoid extract from the algae *Haematococcus pluvialis* with reported potent antioxidant qualities. A small placebo-controlled RCT reported on the effect of 3 months of therapy with this antioxidant in men with idiopathic male factor infertility [41]. Astaxanthin produced no change in sperm concentration or morphology but did produce a significant reduction in seminal ROS levels and improvement in sperm motility. Furthermore, the researchers observed a significant increase in natural or intrauterine insemination-assisted conceptions in the antioxidant-treated group, suggesting that the small improvement in sperm motility was of clinical significance.

Lycopene, an antioxidant found in high concentrations in fruits such as tomatoes and watermelon, is a powerful natural antioxidant. A non-controlled trial of 30 men with male factor infertility reported a significant improvement in sperm quality with 3 months of lycopene therapy at a dose of 4 mg/day [42]. However, upon further analysis of this study, it appears that the researchers only analysed sperm outcomes for the 14–20 men who showed an improvement in either sperm concentration, motility or morphology. Such an analysis is obviously flawed since excluding half the study participants who did not respond to treatment is clearly going to result in a significant difference being concluded. Therefore, this study provides no scientific support for the use of lycopene in male factor infertility.

Alpha-lipoic acid (ALA) is a sulphur-containing antioxidant involved in mitochondrial oxidative metabolism. A recent RCT using 600 mg of ALA per day reported a significant

increase in sperm count and motility, plus a reduction in biochemical markers of oxidative damage (MDA), but no improvements in sperm motility [43]. Unfortunately, pregnancy outcomes were again not reported.

45.13 Combination Therapies

The combination of vitamins C and E would appear to be the most commonly studied combinational antioxidant therapies for male factor infertility. However, other unique combinations have been trialed in the hope that using several different antioxidants with different modes of action may be more beneficial than antioxidant monotherapy.

The combination of vitamin E (400 mg/day) and selenium (225 μg/day) has been trialled in a placebo-controlled study of 54 men with male factor infertility [44]. Antioxidant therapy produced a small increase in sperm mobility and a drop in sperm MDA levels, confirming an antioxidant effect. No changes in sperm concentration or morphology were observed, and pregnancy outcomes were not reported. A significant weakness in this study was that out of a total of 54 initial participants, only 20 completed the study. This raises the possibility of bias and makes firm conclusions difficult.

Piomboni et al. [45] performed a controlled study comparing 3 months of therapy with an antioxidant combination (β-glucan 20 mg, papaya 50 mg, lactoferrin 97 mg, vitamin C 30 mg, vitamin E 5 mg) or no therapy. They observed a significant improvement in sperm motility, viability and morphology but no change in sperm DNA quality. Pregnancy outcomes were not reported in this study.

A small uncontrolled study of 33 men reported on the use of a combination of 600 mg NAC, 30 mg β-carotene, 180 mg vitamin E and a mixture of essential fatty acids for a period of 6 months as treatment for male factor infertility [46]. This combination produced no change in sperm concentration, motility or morphology, but a drop in seminal ROS levels, and sperm DNA oxidative damage (8-OHdG) was observed, together with an increase in the ionophore-induced acrosome reaction. A total of 22.2% of couples who completed the 6-month therapy did successfully conceive, but the absence of a control arm makes it impossible to determine if this is a clinical improvement above nontreatment levels.

A small case series reported on the success of using a combinational antioxidant (β-carotene 5000 IU, vitamin C 60 mg, vitamin E 30 IU, zinc 15 mg) for the treatment of early embryo loss related to sperm oxidative damage [47]. Out of the 17 men screened, 9 men were confirmed to have oxidative stress-related sperm pathology which could be amenable to antioxidant therapy. In six of these nine cases, the partners subsequently fell pregnant. When antioxidants had been taken by the male before conception, all pregnancies were viable (n = 4), whereas all the pregnancies conceived by men who refused antioxidant therapy miscarried. Such a small case series precludes definitive conclusions yet does suggest that oxidative pathology may be a significant cause of early pregnancy wastage.

Two RCTs of combinational zinc and folate therapy have been conducted, with one reporting no effect on sperm quality [48]. However, the earlier larger study involving 107 fertile and 103 subfertile men did report a significant increase in sperm concentration with a combination of 5 mg of folate and 66 mg of zinc sulphate for 6 months and a small (4%) increase in normal morphology, but only in the subfertile cohort [49]. Pregnancy outcomes were not reported.

One of the most widely studied combinational antioxidants in the field of male infertility is Menevit® (Bayer). This preparation consists of a combination of several natural antioxidants (vitamin C 100 mg, vitamin E 400 IU, lycopene 6 mg, selenium 26 μg, garlic oil 333 μg), anti-inflammatory action (garlic oil) and other ingredients involved in sperm DNA synthesis and packaging (zinc 25 mg, folate 500 mg). Three-month therapy with the Menevit® antioxidant has been reported to produce no significant change in sperm concentration, motility or morphology but did produce a significant reduction in seminal ROS levels and sperm DNA fragmentation [50]. Interestingly, while a dose of 400 mg of vitamin C has been shown to produce sperm chromatin decondensation by interfering with protamine disulphide bonds [21], the Menevit® antioxidant containing one-quarter dose of vitamin C has been reported to significantly increase sperm DNA protamination [50]. The Menevit® antioxidant has also been shown to improve pregnancy outcomes when compared with placebo in an RCT of 60 patients undergoing IVF-ICSI treatment [51]. Finally, recent preliminary studies have linked male infertility and sperm oxidative stress with impaired sperm DNA methylation, a possible risk factor for epigenetic disease in the next generation [52]. The treatment of infertile men with 3 months of Menevit® resulted in an improvement in the levels of sperm global DNA methylation [52]. This pilot study will require replication, and large epidemiological studies will need to confirm the link between sperm DNA methylation defects and childhood illness before definitive conclusions can be made regarding the utility of antioxidant supplements to prevent epigenetic disease in the next generation.

45.14 Therapies to Reduce Production of ROS Within the Male Reproductive Tract

While sperm numerically outnumber leukocytes by at least two orders of magnitude in the majority of men's semen, these leukocytes often play the dominant role in seminal ROS production [2, 53]. Activated leukocytes are professional

"oxidative killers" using ROS to destroy invading pathogens and clear damaged cells. In fact, on a per cell basis, leukocytes produce 1000-fold more ROS than sperm [2, 53], underlying the relative importance of inflammation in the male reproductive tract as a cause of sperm oxidative damage. Furthermore, studies have suggested that "extrinsic" oxidative stress initiated by leukocyte production of ROS can also increase the sperm's own "intrinsic" mitochondrial production of ROS – further potentiating oxidative damage to the sperm nucleus [54–57].

When in direct contact with Sertoli cells behind the protective "blood-testis" immunological barrier, sperm are relatively well protected from the ravages of leukocyte ROS production [58, 59]. However, once they pass to the epididymis, a non-immunologically privileged site [60], they can be damaged by leukocyte ROS production triggered by low-grade infection, sexually acquired or otherwise [61, 62]. Similarly, when sperm are expelled at the time of ejaculation, they may encounter leukocytes originating from the male accessory glands (MAG), with inflammation of these glands occurring with both infectious [62, 63] and non-infectious pathology (e.g. NIH class 3 nonbacterial chronic prostatitis) [64] – both leading to sperm oxidative stress [65–67].

Aside from infection, obesity has also been reported to produce a systemic state of chronic low-grade inflammation that extends to the male reproductive tract and can result in sperm oxidative stress [68, 69]. With two-thirds of the adult male population in the developed world now being overweight or obese [70], this is an increasing problem and may underline the decline in sperm health that has been observed in the past five decades [1]. The pathophysiology of obesity-related inflammation is multifactorial including the production of pro-inflammatory cytokines by adipose tissue [71] and disruption of the intestinal mucosal barrier with passage of gut bacteria into the circulation that triggers inflammation – the so-called metabolic endotoxemia [72, 73]. Furthermore, obesity is associated with lower serum testosterone and higher oestrogen levels compared with lean individuals. Given that testosterone is immunosuppressive and oestrogen pro-inflammatory [74, 75], it is not surprising that obese men's low serum testosterone to oestrogen ratio is associated with male accessory gland inflammation [76] and activated leukocyte-mediated sperm oxidative stress, even in the absence of infection.

The optimal approach to reducing leukocyte production of ROS and its associated sperm damage is fourfold.

45.14.1 Accurately Identify the Presence of Leukocytes in Semen

The traditional semen analysis often relies on identification of large number of round cells and altered semen viscosity and pH as potential signs of infection and leukospermia. However, definitive identification of pathological leukospermia requires additional testing with peroxidase staining or CD 45 immunohistochemistry [77] or alternatively chemical detection of neutrophil activation by quantification of seminal plasma elastase [78, 79]. Unfortunately, in the authors' experience, the majority of commercial pathology laboratories does not have the capacity or inclination to perform these confirmatory tests for leukocytospermia – resulting in a missed diagnostic opportunity. However, the increased adoptions of automated semen analysis platforms such as SQA-Vision, which routinely test for the presence of leukospermia using sensitive chemical test strips [80, 81], will result in better identification of pathological leukospermia in the future, with the hope for more informed treatment of this important trigger of sperm oxidative stress.

45.14.2 Treat Infectious Causes of Leukospermia

The presence of leukospermia should precipitate semen culture and screening for sexually transmitted diseases (STD) [82, 83]. Proof of effective antibiotic treatment should be sorted with repeat semen analysis and culture a few weeks after therapy, as many male accessory gland infections are quite resistant to antibiotic cure [63]. Failure to achieve cure should precipitate radiological assessment of the male accessory glands and referral to a urologist/andrologist experienced in the treatment of such matters [84].

45.14.3 General Anti-inflammatory Agents in the Absence of Active Infection

In many cases of leukospermia, no pathogenic bacteria are identified on repeated culture, or there is uncertainty whether the cultured bacteria indicate true infection or just skin contaminants, making directive antimicrobial therapy difficult. Altered semen viscosity or symptoms of perineal discomfort, pain on ejaculation and semen discolouration may all point to the presence of past MAGI and ongoing inflammation [63]. These men may also have increased MAG or epididymal blood flow on colour Doppler, altered prostate morphology or thickening of the seminal vesicle walls on ultrasound [84], plus biochemical signs of inflammation such as raised PSA levels [63]. Despite the absence of ongoing infection, the presence of activated leukocytes within prostatic or seminal vesicle secretions can still mediate oxidative damage to sperm [56, 65, 67]. However, this may be negated using anti-inflammatory agents such as NSAID [37], corticosteroids [85] or over the counter agents such as omega-3 fish oils [86]. None of these anti-inflammatory agents should be

Table 45.2 Randomized controlled studies examining the effect of anti-inflammatory therapies with antioxidant activity on male reproductive health

Study reference	Therapy used per day	Duration of therapy (months)	Positive changes in semen quality	Assessment sperm OS endpoints	Positive changes in reproductive outcomes
[37]	NSAID (nimesulide)	4	↑ sperm vitality ↓ seminal WBC count	↓ ROS (luminol)	Not assessed
[87]	Omega-3 (EPA/DHA) 1.8 gm	8	↑ sperm concentration, motility and morphology	↑ SOD ↑ catalase	Not assessed
[88]	Omega-3 DHA-enriched supplement (0, 0.5, 1, 2 gm)	3	↑ sperm motility and morphology	No change In LPO	Not accessed
[89]	Omega-3 DHA-enriched supplement (1.5 g)	2.5	↓ sperm DNA damage	↑ TAC	Not reported
[98]	Symbiotic prebiotic/ probiotic (Flortec)	6	↑ sperm concentration, motility and morphology ↓ sperm DNA damage	Not tested	25% pregnancy active arm, 0% placebo arm

NSAID non-steroidal anti-inflammatory drug, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *LPO* lipid peroxidation

initiated without first ruling out active infection, as suppression of the immune system with active infection could exacerbate the infection and aggravate ROS damage to sperm.

A 2–4-month course of NSAID after successful treatment of MAGI has been shown to boost sperm function and fertility [37]. Furthermore, a short course of prednisone (5–25 mg/day) is reported to boost sperm count and motility [85]. Finally, three recent RCT using 1.5–2 g of omega-3 fish oil per day have been shown to reduce oxidative stress and improve sperm quality [87, 88], including a significant reduction in sperm DNA damage [89]. It is well established that docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contained within fish oil have powerful anti-inflammatory capacity [86], plus documented systemic antioxidant capacity [90]. Furthermore, two RCT of omega-3 oils have confirmed enhanced antioxidant capacity in seminal plasma after therapy [87, 89], with the third showing no changes in a lipid peroxidation marker of oxidative stress [88] (Table 45.2).

45.14.4 Symbiotics: A New Potential Therapy for Male Oxidative Stress

A new development in the field of andrology is the recognition of the importance that the gut microbiome may play in testicular function and fertility, principally mediated by inflammation initiated by systemic exposure to gut bacterial endotoxin – the so-called GELDING theory (Gut Endotoxin Leading to a Decline IN Gonadal function) [75]. The human intestine contains 100 trillion bacteria, with these bacteria playing essential roles in digestion of dietary fibre and production of vitamins and short chain fatty acids that keep the bowel healthy [75]. A healthy intestine permits the selective passage of water and nutrients across the mucosal lining to sustain life, while excluding the passage of intestinal bacteria into the circulation. However, with adverse lifestyle choices such as obesity, a poor "western" high-fat low-fibre diet and excess alcohol consumption, there is a breakdown in this intestinal mucosal barrier function, allowing gut bacteria into the circulation where they initiate chronic low-grade inflammation – the so-called metabolic endotoxemia [72, 73, 91]. This metabolic endotoxemia state has now been linked with a reduction in testosterone production [92, 93] and an increase in sperm oxidative DNA damage [94] and is likely to be a significant cause of sperm oxidative stress seen in obese men. Therapies targeting "leaky gut"/metabolic endotoxemia include a reduction in dietary fat and alcohol intake [72] and the consumption of symbiotic therapies [95, 96].

Symbiotics are a combination of probiotic "beneficial bacteria" that enhance gut health, together with prebiotic fibre food to help sustain the growth of these prebiotic bacteria within the individual's intestine [96]. Probiotic bacteria include *Lactobacillus* and *Bifidobacterium*, two helpful strains of bacteria that keep pathogenic bacterial growth at bay and produce chemicals which enhance the intestinal barrier function, thereby reducing metabolic endotoxemia, inflammation and its associated production of ROS [95, 96]. Furthermore, probiotics have reported to have widespread antioxidant activity and benefits in many disease states [96, 97].

While still a relatively new emerging field, a recent RCT has shown that ingestion of a symbiotic product (Flortec; Bracco, Italy) for 3 months produced an improvement in sperm quality and quantity, plus an increase in testosterone production [98]. Furthermore, a small non-randomized study has recently reported that probiotic therapy was able to improve sperm motility and DNA integrity and reduce intracellular H_2O_2 levels, implying a probiotic antioxidant advantage [99]. Importantly, animal studies have also confirmed this beneficial effect of probiotics on sperm quality via an anti-inflammatory/antioxidant mechanism [100, 101]. The

additional benefit of this probiotic/symbiotic approach to management of infertility is that it is relatively inexpensive and free of side effects but may also treat other medical conditions known to be associated with metabolic endotoxemia in obese men such as improvement in insulin resistance [102]. However, further large RCT of probiotics/prebiotics will need to be conducted before they become a widespread treatment of inflammatory oxidative stress-mediated male infertility.

45.15 Therapies That Fortify Sperm Against ROS Damage

One of the primary mechanisms that oxidative stress results in infertility is oxidative damage to the sperm DNA, with the resulting fragmented DNA producing poor-quality embryos that either fail to develop to a blastocyst [103] or implant and miscarry [104]. Furthermore, oxidative damage to sperm has been implicated in alteration in sperm DNA methylation [52, 105], with both sperm DNA fragmentation and epigenetic modification of the paternal genome being associated with impaired health of the next generation [105]. While this adverse effect can be limited by reducing initial ROS production and neutralizing their action once produced using antioxidant therapy [50], the sperm has also developed a last resort defence mechanism to preserve its DNA integrity – protamination.

In somatic cells the strands of DNA are loosely intercoiled by histone packaging, thereby allowing the cellular machinery responsible for translating the genetic code into proteins to easily gain access to the DNA and allow gene transcription. However, as sperm are generally transcriptionally silent during the later stages of maturation, this loose DNA packaging is not necessary. Instead the paternal DNA is very tightly packaged by almost complete replacement of histones with protamines, leading to a sperm nucleus in which the DNA density is significantly greater than somatic cells [106]. This is a vital protective response as the surface area of DNA in direct contact with ROS is thereby minimized, reducing the amount of paternal DNA vulnerable to ROS attack [106].

Unfortunately, many infertile men have defective protamination of their sperm, resulting in decondensed sperm DNA that is vulnerable to oxidative attack [106, 107]. While it is presently uncertain why these infertile men have defective protamination packaging of their sperm, it is known that the micronutrients zinc and selenium play a vital role in forming these protamination cross links [108, 109]. Fortunately, studies using zinc and selenium supplements have been shown to improve protamination and reduce sperm DNA damage [50, 108, 109]. Conversely, other studies have shown free radicals to play an important role in maintaining the disulphide cross links between protamine bridges, with high-dose antioxidant therapy causing decondensation of the sperm and possibly increasing sperm oxidative damage vulnerability [21]. This is a salient cautionary tale emphasizing the centuries old therapeutic maxim that "the dose differentiates a poison from a remedy" (Paracelsus, sixteenth-century Swiss physician). While moderate doses of antioxidants are likely beneficial to sperm health, excessive doses may impair sperm health and functional activities such as capacitation, an ROS-dependent process vital to natural fertilization. Therefore, care should be exercised in all antioxidant therapies, with treatments backed up by well-conducted randomized controlled trials showing a positive risk/benefit profile (Fig. 45.1).

45.16 Conclusion

The current evidence clearly identifies oxidative stress as a major cause of impaired sperm function and male infertility. While many studies have been conducted examining the ability of various antioxidants to improve male reproductive function, it is still uncertain if many male preconception antioxidant therapies can actually improve a couple's chances of becoming parents. However, critical analysis of the higher-quality RCT trials suggests that combinational therapies using vitamin C, vitamin E, lycopene, coenzyme Q10, zinc, selenium and astaxanthin may be of benefit in improving sperm health and the chances of conception. Evidence supporting the use of other antioxidants such as glutathione, L-carnitine and NAC as effective therapies for male infertility is relatively weak. More recent novel approaches to managing oxidative stress by reducing leukocyte production of ROS include the use of omega-3 fish oil and probiotics/prebiotic therapy. These treatments have been shown in RCT to boost sperm health, but unfortunately no study to date has confirmed that this translates into a better chance of natural or IVF-assisted conception. The optimal future therapy for preventing sperm oxidative stress is most likely to combine these anti-inflammatory treatments and direct antioxidant agents (Fig. 45.1). Given the low cost and positive safety profile of these agents, we hope that large RCT targeting men with documented sperm oxidative stress are conducted, with the primary endpoint being the birth of a healthy baby, not improvements in semen analysis. Until these trials occur, physicians caring for infertile couples will remain sceptical regarding the benefits of male antioxidant therapy.

45.17 Review Criteria

An extensive search of studies examining the impact of antioxidant agents (direct antioxidants and indirect antioxidant action via anti-inflammatory action) on sperm health was per-

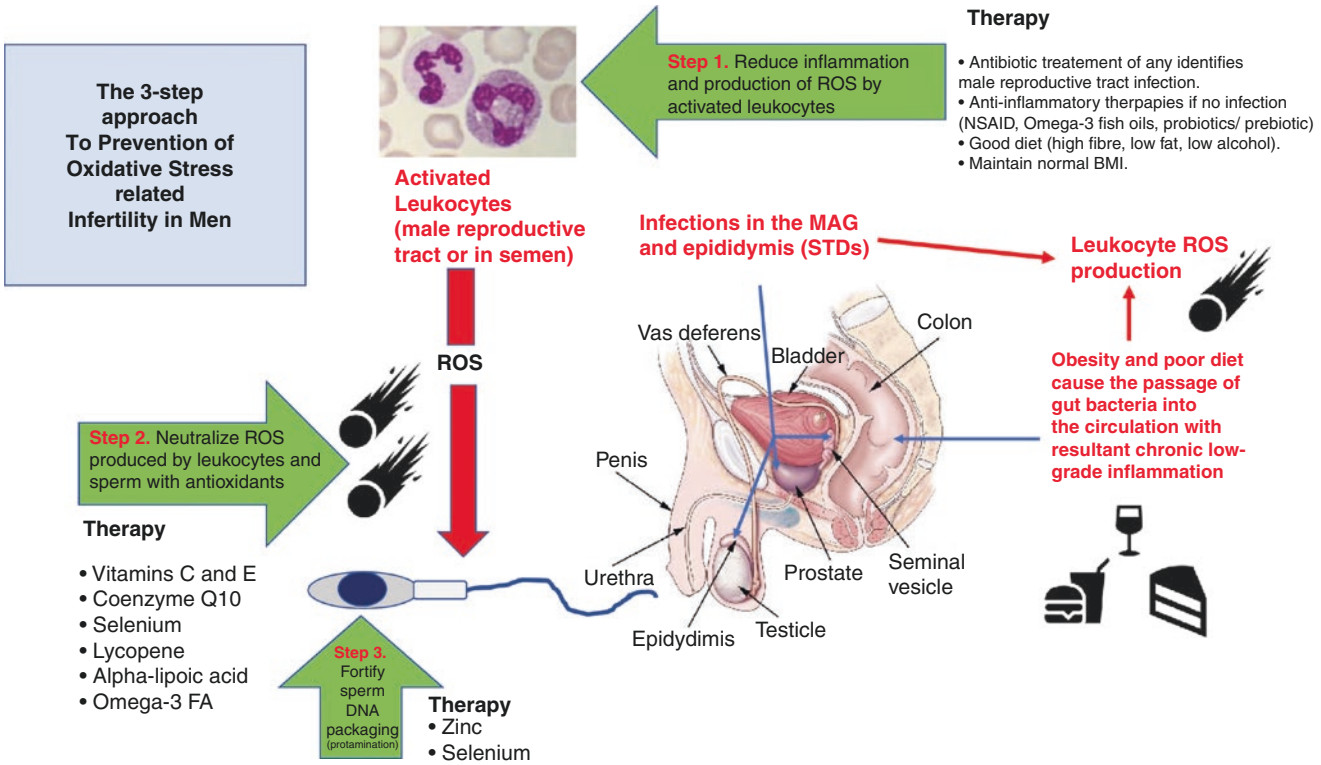

Fig. 45.1 Overview of the clinical management of male oxidative stress-related infertility. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2016–2019. All Rights Reserved)

formed using PubMed and Google Scholar. The completion date for this search was January 2019. The overall strategy for study identification was based on the keywords: "antioxidant", "oxidative stress", "reactive oxygen species", "infertility", "semen", "sperm", "anti-inflammatory", "pregnancy" and "randomized control trial (RCT)". Only peer-reviewed full-length articles published in English were considered, with a primary focus on randomized controlled studies conducted on men. Trials using "general multivitamins" and studies that employed botanical preparations with poorly defined direct antioxidant action were all excluded. Furthermore, studies which combined an antioxidant with an established endocrine therapy known to influence sperm production (clomiphene, aromatase inhibitors) were also excluded.

Findings published at conferences or on web sites that had not been later published in peer-reviewed journals were not considered.

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