

## Review Article

# Role of antioxidants in the treatment of male infertility

John C Kefer, Ashok Agarwal and Edmund Sabanegh

Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio, USA

**Abstract:** Male infertility continues to be a clinical challenge of increasing significance. While male factors such as decreased semen quality are responsible for 25% of all infertility issues, the etiology of suboptimal semen quality is poorly understood. Many physiological, environmental, and genetic factors have been implicated, including oxidative stress. Oxidative stress is induced by reactive oxygen species (ROS), or free radicals, and although ROS are required for critical aspects of sperm function, excessive levels of ROS can negatively impact sperm quality. The origin of ROS generation, and the etiologies of increased ROS in men with suboptimal sperm quality have only recently been elucidated, offering multiple targets for potential therapy. Here, we present a critical review of the literature describing the role of oxidative stress on decreased sperm function, as well as the role of antioxidants in the treatment of male factor infertility.

**Key words:** antioxidants, infertility, male, oxidants, quality, sperm.

## Introduction

The World Health Organization defines infertility as the inability of a couple to achieve conception or bring a pregnancy to term after 1 year or more of regular, unprotected sexual intercourse.<sup>1</sup> Infertility is a major clinical concern, affecting 15% of all reproductive-aged couples, and male factors, including decreased semen quality, are responsible for 25% of these cases.<sup>2</sup> Currently, the etiology of suboptimal semen quality is poorly understood, and many physiological, environmental, and genetic factors, including oxidative stress have been implicated.<sup>2–5</sup> Oxidative stress is induced by reactive oxygen species (ROS), or free radicals. While ROS have been shown to be required for sperm capacitation, hyperactivation, and sperm-oocyte fusion,<sup>6,7</sup> excessive levels of ROS can negatively impact sperm quality. Increased levels of ROS have been correlated with decreased sperm motility,<sup>8–10</sup> increased sperm DNA damage,<sup>11–13</sup> sperm cellular membrane lipid peroxidation<sup>14–16</sup> and decreased efficacy of oocyte-sperm fusion.<sup>17</sup> The origin of ROS generation, and the etiologies of increased ROS in men with suboptimal sperm quality have only recently been elucidated, offering multiple targets for potential therapy.

## Reactive oxygen species and antioxidants

ROS are products of normal cellular metabolism. The majority of energy produced by aerobic metabolism utilizes oxidative phosphorylation within mitochondria. During the enzymatic reduction of oxygen to produce energy, free radicals form as a byproduct.<sup>18</sup> A free radical is an oxygen molecule containing one or more unpaired electrons. Normally, molecular oxygen has two unpaired electrons, and this electronic structure makes oxygen especially susceptible to radical formation. For example, the addition of an extra electron to molecular oxygen ( $O_2$ ) forms a superoxide anion radical ( $O_2^-$ ), the primary form of ROS. This superoxide anion can then be directly or indirectly converted to secondary ROS such as the hydroxyl radical ( $\bullet OH$ ), peroxy radical ( $ROO\bullet$ ) or hydrogen peroxide ( $H_2O_2$ )<sup>19</sup> (Fig. 1). Free radicals induce cellular damage when they pass this unpaired electron onto nearby

**Correspondence:** Professor Ashok Agarwal MD PhD, Glickman Urological and Kidney Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA. Email: agarwaa@ccf.org

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cellular structures, resulting in the oxidation of cell membrane lipids, amino acids in proteins or within nucleic acids.<sup>20</sup>

Reactive oxygen species are formed as necessary byproducts during the normal enzymatic reactions of inter- and intracellular signaling.<sup>21</sup> Overproduction of ROS can be induced through physiological or pathological mechanisms, including ROS generation by leukocytes as a cytotoxic mechanism of host defense, during hypoxic states leading to a high burden of ROS, as well as by a wide array of drugs with oxidizing effects on cells. To counteract the possibility of significant cellular damage by excessive production of ROS, enzymatic and non-enzymatic antioxidant pathways scavenge excess ROS and allow a balance to be achieved between beneficial oxidant generation and damaging oxidative stress.

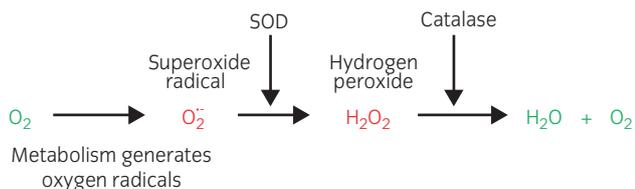
## Enzymatic antioxidants

Three groups of enzymes play significant roles as oxidant scavengers.<sup>18</sup> Superoxide dismutases (SOD) are metal-containing enzymes that catalyze the conversion of two superoxides into oxygen and hydrogen peroxide, which is less toxic than superoxide (Fig. 1). Catalase, an enzyme found in peroxisomes, degrades hydrogen peroxide to water and oxygen, thereby completing the reaction started by SOD (Fig. 1). Glutathione peroxidase also acts to degrade hydrogen peroxide (Fig. 2). Other enzymes, such as glutathione transferase, ceruloplasmin or hemoxigenase may also participate in enzymatic control of oxygen radicals and their products.<sup>18</sup>

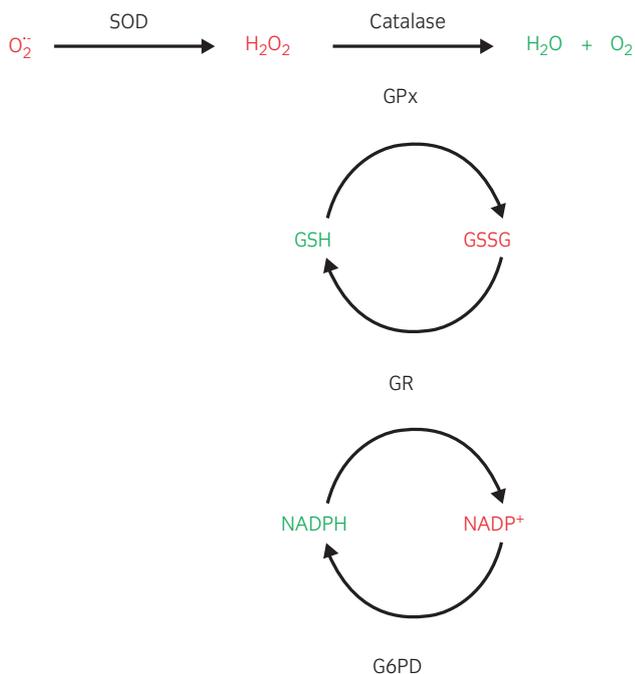
## Non-enzymatic antioxidants

The non-enzymatic antioxidant glutathione may well be the most important intracellular defense against ROS. Glutathione is a tripeptide, composed of glutamate, cysteine, and glycine. The cysteine subunit provides an exposed free sulfhydryl group (SH) that directly scavenges free radicals. Once oxidized, glutathione is then regenerated/reduced by glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) to complete the cycle<sup>17</sup> (Fig. 2).

Vitamins E and C also play critical roles as non-enzymatic antioxidants.<sup>17</sup> Vitamin E plays a vital role in protecting cell membranes from oxidative damage trapping and scavenging free radicals within cellular membranes. Vitamin C is a water-soluble antioxidant that reduces radicals from a variety of sources, and also serves to recycle oxidized vitamin E.



**Fig. 1** Antioxidant scavenging pathways of free radicals by superoxide dismutase (SOD) and catalase.



**Fig. 2** Glutathione peroxidase (GPx) also scavenges hydrogen peroxide ( $H_2O_2$ ), along with glutathione (GSH), which becomes oxidized and is reduced/regenerated by glutathione reductase (GR) to allow further antioxidant function. G6PD, glucose 6 phosphate dehydrogenase; GSSG, glutathione disulfide; NADPH, nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase.

Under normal conditions, these enzymatic and non-enzymatic antioxidants act to maintain an overall low level of oxidative stress in the semen, allowing for normal oxidant-dependent cell signaling processes and normal spermatid function, while concomitantly avoiding oxidant-induced cell damage. In contrast, the pathological effects of oxidative stress arise under conditions where levels of unscavenged ROS increase, or the antioxidant buffering capacity of the system decreases, thus perturbing the delicate oxidant/antioxidant balance. These free radicals induce sperm cell injury through several pathways, and can significantly impact both sperm quality and function.<sup>17,20,21</sup> This oxidative stress-induced sperm damage has been suggested to be a significant contributing factor in 30–80% of all cases of male infertility.<sup>20,21</sup>

### Oxidative stress impacts semen quality

The unique cellular structure of spermatozoa renders them particularly sensitive to oxidative stress. For example, while the antioxidants catalase, SOD and glutathione peroxidase are found in high concentrations

in the cytoplasm of most cells, sperm cells lack significant cytoplasm and therefore contain only minimal amounts of these critical ROS-scavenging pathways. The majority of the antioxidant enzymatic buffering capacity is instead contained in seminal fluid, which contains SOD, glutathione peroxidase and catalase, as well as the non-enzymatic antioxidants Vitamin E and C, and taurine and hypotaurine.<sup>17</sup> The high concentration of polyunsaturated fatty acids in sperm cell membranes makes sperm more susceptible to lipid peroxidation than other non-germ cells.<sup>14,15</sup> This combination of susceptibility to lipid peroxidation along with a relative lack of vigorous intracellular defense mechanisms is exacerbated by the autogenous production of ROS by spermatozoa.

Two principal sources of free radicals are found in the semen: leukocytes and spermatozoa. Most semen specimens contain variable numbers of leukocytes, with neutrophils noted as the predominant type.<sup>22–24</sup> Neutrophils function by generating and releasing high concentrations of reactive oxygen species to form cytotoxic reactions against nearby cells and pathogens. Over the last 10 years many studies have investigated the correlation between leukocytospermia and oxidative stress injury to sperm.<sup>20,22,25–33</sup> Nonetheless, the relationship between leukocytes in the semen and male infertility remains incompletely defined. Leukocytospermia has long been associated with decreased sperm concentration, motility, and morphology, as well as decreased hyperactivation and defective fertilization. In a thorough review of the literature, Wolff *et al.* delineated several studies describing the association between the presence of white blood cells (WBC) in semen and overall sperm quality.<sup>34</sup> The majority of these epidemiological, clinical and experimental studies all described a significant negative association between the number of WBC and overall sperm function. This relationship has most recently been addressed by Moskovtsev *et al.*, who analyzed the relationship between leukocytospermia and sperm DNA damage in 1230 unselected non-azoospermic infertility patients.<sup>35</sup> While the authors found no significant relationship between leukocytospermia and DNA integrity, a significant negative effect was again noted between the presence of leukocytospermia and corresponding sperm concentration, motility, and morphology. Nonetheless, this relationship is not definitive, as several other studies have found no evidence of a correlation between leukocytospermia and abnormal sperm parameters.<sup>25–27</sup> In a review of these studies, Aitken *et al.* emphasized that seminal leukocytes may not necessarily effect the fertilizing potential of spermatozoa, as not all men with leukocytospermia demonstrate abnormal sperm parameters.<sup>25</sup> The authors also postulate that the role of leukocytes on male fertility might instead be related to the etiology of the leukocytospermia or the degree of inflammation in the seminal fluid, as well as the leukocyte subtypes present. This relationship therefore remains controversial. While the significance of leukocytospermia on the fertility potential of the individual patient remains difficult to quantify, leukocytospermia can nonetheless be considered a marker of urological or systemic inflammation and possible sperm dysfunction.

### Internal sources of reactive oxygen species

#### Leukocytospermia

Part of the controversy regarding the role of leukocytospermia in sperm quality lies in the variability and reliability of leukocyte detection techniques. Conventional sperm staining techniques such as Papanicolaou and Giemsa staining cannot reliably differentiate between immature germ cells and WBC, leading to confusion between spermatozoa and lymphocytes or monocytes.<sup>34</sup> Immunocytological detection can be

used to detect all WBC types by utilizing a monoclonal antibody against CD45, however, this technique may overestimate seminal WBC counts.<sup>34</sup> Conversely, cytochemical detection utilizing peroxidase staining can underestimate the number of WBC present, as this stain does not accurately detect lymphocytes or monocytes.<sup>36</sup> Based on the staining technique utilized, these differences in WBC counts can result in the broad variability of results, leading to confusion when interpreting results from different studies of the significance of leukocytospermia on sperm quality. These differences must therefore be taken into account when comparing studies on leukocytospermia from different centers.

The variable significance of leukocytospermia on sperm quality may also be related to the activation state of the seminal leukocytes, which can result in increased production of pro-inflammatory cytokines, as well as increased levels of reactive oxygen species and sperm injury. Several authors have noted a correlation between decreased sperm function in the presence of high levels of seminal ROS or proinflammatory cytokines, including interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)- $\alpha$ .<sup>37-43</sup> For example, IL-6 has been shown to play a pivotal role in the induction of capacitation and the acrosomal reaction in sperm.<sup>39</sup> However, high levels of cytokines in the semen have been correlated with sperm injury, most notably cell membrane lipid peroxidation in the presence of elevated IL-6.<sup>38-40</sup> Further, increased levels of IL-6 have also been noted in the seminal fluid of infertile men.<sup>37</sup> Also, IL-1, IL-6, IL-8 and TNF have all been shown to induce increased ROS production, leading to increased sperm cell membrane lipid peroxidation.<sup>37,38,41</sup> This increased level of leukocyte activation leading to increased oxidative stress may provide an explanation for the differential levels of sperm dysfunction in men with similar concentrations of seminal leukocytes.

### Autogenous generation of ROS by sperm

Spermatozoa have also been noted to generate reactive oxygen species independent of leukocytes,<sup>42</sup> and the ability of sperm to generate ROS is dependent on the maturation level of the sperm. During spermatogenesis, cytoplasm is normally extruded from the spermatozoa prior to release of the germinal epithelium. Cytoplasmic residues contain high levels of the enzyme glucose-6-phosphate dehydrogenase, which generates NADPH. NADPH then generates reactive oxygen species via NADPH oxidase within the sperm membrane.<sup>43,44</sup> Abnormalities in sperm maturation may lead to increased levels of retained cytoplasmic residues in semen, thereby leading to increased seminal ROS production and subsequent sperm damage.

While both leukocytes and spermatozoa produce ROS, the concentration of ROS generated by each cell type varies greatly. Leukocytes have been reported to produce 1000 times more ROS compared to spermatozoa engaged in capacitation.<sup>43,45</sup> These disparate levels seem to implicate leukocytes as the offending agents of oxidative injury, but further evidence suggests that sperm injury by oxidative stress is also related to location as much as concentration. For example, Henkel *et al.* investigated the effects of ROS generated by leukocytes or spermatozoa on overall sperm quality. Levels of ROS production by seminal leukocytes were termed extrinsic ROS production, and ROS production by spermatozoa was termed intrinsic production. The origins and concentrations of ROS generation were compared to measures of sperm quality, including DNA fragmentation and sperm motility, counts and morphology.<sup>44</sup> The authors noted decreased sperm counts, motility and morphology in samples with high levels of extrinsic ROS production, however, increased levels of DNA fragmentation correlated more strongly with intrinsic ROS production. These intriguing data suggest

that specific sites of spermatid injury and function are not only related to levels of ROS produced, but also to the site of ROS generation. Low levels of intrinsic ROS production may substantially injure sperm DNA, whereas extrinsic ROS are more injurious to external spermatid structures, thereby impacting motility, counts and morphology.

### External sources of ROS

The generation of ROS can be exacerbated by a multitude of environmental, infectious, and lifestyle etiologies. A wide range of industrial byproducts and waste chemicals has been shown to negatively impact male infertility, both indirectly and directly. The increasing presence of these byproducts of manufacturing in the environment has been suggested to pose a serious threat towards the reproductive health of humans around the world.

### Industrial compounds

Industrial waste products have a wide impact on human and environmental health, and the impact of these compounds on fertility has been investigated by several groups. For example, the compound phthalate is found in a broad spectrum of plastics used in food packaging, as well as in beauty products and exposure to this chemical can occur via oral, skin, or inhalational routes. Phthalate exposure has been shown to cause DNA damage in sperm, as well as impaired spermatogenesis.<sup>46,47</sup> Heavy metals, such as cadmium and lead, have been shown to increase testicular oxidative stress,<sup>48,49</sup> and increased rates of infertility and miscarriage have been noted in welders as well as workers in paint and battery manufacturers.<sup>50,51</sup> Men in these industries can be exposed to heavy metals through skin absorption and inhalation during the manufacturing process. Patients employed in industries with high exposure risks should be tested for heavy metals and counseled to take aggressive steps to avoid further exposure.

Several pesticides have been linked to increased testicular oxidative stress in rodent models, including lindane, methoxychlor, and the herbicide dioxin-TCDD.<sup>52-54</sup> The nearly ubiquitous food preservative sulfur dioxide has been noted to directly increase intratesticular oxidative stress,<sup>55</sup> and diesel exhaust particulates have been shown to potentially activate leukocyte ROS production.<sup>56,57</sup>

### Cigarette smoking

Not surprisingly, exposure to cigarette smoke generates high levels of oxidative stress, directly increasing both seminal leukocyte concentrations and seminal ROS generation,<sup>58-62</sup> and decreasing seminal levels of the antioxidant enzyme SOD.<sup>63</sup> Men who smoke also have decreased measures of sperm quality, including decreased sperm counts, motility, and morphologically normal sperm.<sup>60-63</sup> Vine described a meta-analysis of 27 studies investigating the association between cigarette smoking and semen quality. Reductions of sperm concentrations and motility were seen in most studies, although the vast majority of these studies were performed on healthy men as opposed to men with infertility.<sup>60</sup> Smoking has been noted to decrease concentrations of the seminal plasma antioxidants Vitamin C and E,<sup>60-62</sup> thereby reducing the oxidant scavenging capacity of the spermatozoa and seminal fluid.

### Alcohol intake

Not surprisingly, high amounts of alcohol intake have also been shown to increase systemic levels of oxidative stress, and the effect of this oxidative stress can be further exacerbated by the low-nutrient diet

usually accompanying this high alcohol intake.<sup>64</sup> Nonetheless, despite the demonstrated link between alcohol and systemic oxidative stress, there are currently no studies described in the literature that have directly examined the relationship between alcohol intake and sperm oxidative damage, and this relationship therefore awaits further investigation.

### Exercise-induced oxidative stress

Interestingly, both lack of exercise and intensive levels of exercise have both been shown to generate high levels of oxidative stress. Some studies indicate that chronic intensive exercise training can lower testosterone levels, or interfere with the hypothalamic-pituitary-testis axis involved in reproduction.<sup>65-68</sup> As testosterone plays a major role in the development and maturation of sperm during spermatogenesis, this decrease in testosterone may decrease sperm quality. For example, Manna *et al.* have described in a rodent model that increased levels of exercise correspond to a reduction in sperm quality and testicular function, including decreased sperm counts, testosterone levels, and testicular oxidative stress. Further, the authors also found that daily administration of alpha-tocopherol to the exercising rodents prevented these reductions in sperm quality and testicular function.<sup>67</sup> In humans, most studies examining extreme levels of exercise have focused on increases in systemic oxidative stress,<sup>66,68</sup> and the relationship between intense exercise and sperm quality in humans has yet to be described.

### Elevated temperatures

Several researchers have demonstrated that increased intrascrotal temperatures in rodent models leads to decreased sperm counts and motility, and increased sperm DNA damage.<sup>69,70</sup> Increased intrascrotal temperatures have been suggested as a possible cause of poor sperm quality in humans as well. For example, Mieusset *et al.* measured scrotal temperatures, testicular volumes and sperm characteristics in infertile, nonazoospermic men versus fertile men. Measuring temperatures through scrotal skin contact, 30% of infertile men had scrotal temperatures + 0.5°C higher than fertile men.<sup>71</sup> Carlsen *et al.* also noted an association between hyperthermia and poor semen quality by following 27 healthy men with monthly semen samples and noting the occurrence of any febrile episode over a 16-month period.<sup>72</sup> The results demonstrate a 35% decrease in sperm concentration, a 7% decrease in normal morphology, and a 20% increase in immotile sperm following febrile episodes. Also, these parameters worsened with increased duration of the febrile episodes. Tiemessen *et al.* examined the relationship between underwear style and intrascrotal temperatures, and hypothesized that scrotal hyperthermia due to tight-fitting underwear may cause decreased sperm parameters. The authors followed 20 patients wearing loose-fitting underwear for 6 months and tight underwear for 6 months with semen samples analyzed every 2 weeks for one year.<sup>73</sup> Although 50% of patients did not complete the study, the results nonetheless demonstrated an approximately 50% decrease in sperm parameters in the group wearing the tight-fitting underwear, which improved after switching to loose underwear. This rapid reversal of sperm parameters following cessation of tight-fitting underwear is encouraging, and may be useful in the counseling of the infertile male.

### Identifying oxidative stress during clinical evaluation of the infertile male

Standard evaluation of the infertile man does not currently include measurement of oxidative stress levels. Excessive cost, inconvenience,

and most importantly the lack of a standardized measurement for oxidative stress all contribute to the lack of clinical utilization of these assays. Currently, over 30 assays have been described. These assays can be considered as one of three types of assays: direct assays, indirect assays, and implied assays.<sup>22,74</sup>

### Direct assays

As described above, oxidative stress results from an imbalance between ROS production and the intra- and extracellular antioxidants that scavenge ROS. Direct assays of oxidative stress measure the net oxidative sum of this balance by measuring oxidation of the sperm cell membrane. The most widely used assay measures malondialdehyde (MDA), one of the final products of sperm cell membrane lipid peroxidation.<sup>75,76</sup> Several authors have shown that increased levels of MDA correlate with decreased sperm parameters. Tavilani *et al.* noted that asthenozoospermic men had significantly higher levels of MDA compared to normozoospermic men.<sup>77</sup> Hsieh *et al.* compared normozoospermic men and oligoasthenozoospermic men, and also noted significantly elevated MDA concentrations in the oligoasthenozoospermic men.<sup>78</sup> Aitken *et al.* also demonstrated that sperm with decreased sperm-oocyte fusion capacity demonstrate elevated MDA levels.<sup>76</sup>

Quantification of sperm DNA damage has also been used as a direct assay of intracellular ROS-induced oxidant injury, however, previous studies were unable to distinguish oxidant-induced DNA damage from non-oxidative mechanisms. One possible solution is to measure a specific byproduct of oxidant-induced DNA damage. Loft *et al.* described a large, prospective clinical trial using oxidized deoxynucleotide, 8-oxo-7,8-dihydro 2' deoxyguanoside (8-OHdG) as a specific marker of oxidative injury to sperm DNA with the final end-point being conception.<sup>79</sup> The authors noted that rates of conception were inversely correlated with sperm 8-OHdG levels,<sup>79</sup> offering further evidence of the clinical utility of quantifying oxidant injury in men evaluated for infertility.

### Indirect assays

The most common method of measuring seminal ROS is via the indirect chemiluminescence assay. Luminol (5-amino-2,3-dihydro 1,4-phthalazinedione), or lucigen probes can be used for quantification of redox activities of spermatozoa.<sup>80</sup> Lucigen measures only extracellular superoxide radicals, and therefore luminol is more commonly used as it measures extracellular as well as intracellular levels of ROS. Quantification of oxidative stress using luminol can be performed using a luminometer, which is less expensive and easier to use compared to the instruments for the direct assays. Further, Luminol offers well established reported ranges of normal for fertile and infertile populations, bringing clinical relevance to its use.<sup>80</sup> Despite these significant advantages, the cost of luminometry remains a considerable challenge toward the widespread clinical utility of these assays.

An assay that has grown popular due to its inexpensiveness and ease of use is the nitroblue tetrazolium assay, which requires only a light microscope and allows for discriminating between spermatic ROS and leukocytic ROS without the additional steps required in chemiluminescence assays. Nitroblue tetrazolium interacts with superoxide radicals within sperm and leukocytes by changing to diformazan, a blue pigment. The concentration of diformazan seen using standard light microscopy has been shown to correlate with the concentration of intracellular ROS.<sup>80</sup>

The antioxidant levels of the semen can also be determined via the enhanced chemiluminescence assay, or through a colorimetric assay.

**Table 1** Supplementation with individual antioxidants may lead to improved sperm quality

Author	Year	n	Study population	Antioxidant measured	Quantification method	Outcome
Silver <i>et al.</i> <sup>82</sup>	2005	89	Non-smoking, healthy, no history of infertility, aged 20–80	Beta-carotene, Vit C and E	Dietary questionnaire Sperm DNA analysis	No correlation between increased antioxidant intake and improved sperm chromatin
Eskenazi <i>et al.</i> <sup>83</sup>	2005	87	Non-smoking, healthy, no history of infertility, aged 20–80	Zinc, Folate, beta-carotene, Vit C and E	Dietary questionnaire Semen analysis Sperm DNA analysis	High antioxidant intake associated with improved sperm parameters, no dose relationship
Song <i>et al.</i> <sup>94</sup>	2006	75	Infertile men	Seminal levels of vitamin C	Sperm ascorbic acid levels, semen and sperm chromatin analysis	Low levels of seminal Vitamin C correlate with increased sperm damage
Young <i>et al.</i> <sup>95</sup>	2008	89	Healthy, non-smoking men, no history of infertility	Zinc, Folate, beta-carotene, Vit C and E	Dietary questionnaire Sperm DNA analysis	High folate intake associated with low sperm aneuploidy, no correlation with other antioxidants
Piomboni <i>et al.</i> <sup>96</sup>	2008	51	Infertile men	Papaya, lactoferrin, Vit C and E	Sperm chromatin analysis	Increased dietary intake associated with improved semen parameters and decreased chromatin

Antioxidant levels are measured through the addition of a known concentration of ROS to the semen, leading to development of the chemiluminescence signal or a color change. This assay allows the antioxidants in the semen to scavenge the known ROS administered, and the residual ROS level is then measured. Thus, the intensity of the signal produced is inversely correlated with the total antioxidant capacity of the specimen.<sup>81</sup>

These tests, both direct and indirect have been designed to quantify the level of oxidative stress in men undergoing evaluation for infertility with the goal of deriving a therapeutic plan to decrease oxidative stress levels and improve overall sperm quality. While each test offers advantages, the overall cost and time-intensiveness of these devices has precluded their wide spread use in the clinical setting.

### Therapeutic management of oxidative stress in male infertility

As our clinical understanding of the impact of oxidative stress on male fertility increases, the desire to provide therapeutic options for these patients also increases. Many clinical trials have recently been performed to examine potential therapies for oxidant-stress-induced infertility. However, most of these studies demonstrate a suboptimal experimental design, making effective interpretation difficult. Nonetheless, despite the continued question of distinct clinical efficacy for these antioxidant therapies, the low cost and toxicity of most of these therapies offers a great deal of appeal for both patients and clinicians. Here, we present a critical review of the relevant data describing outcomes in the treatment of oxidative stress.

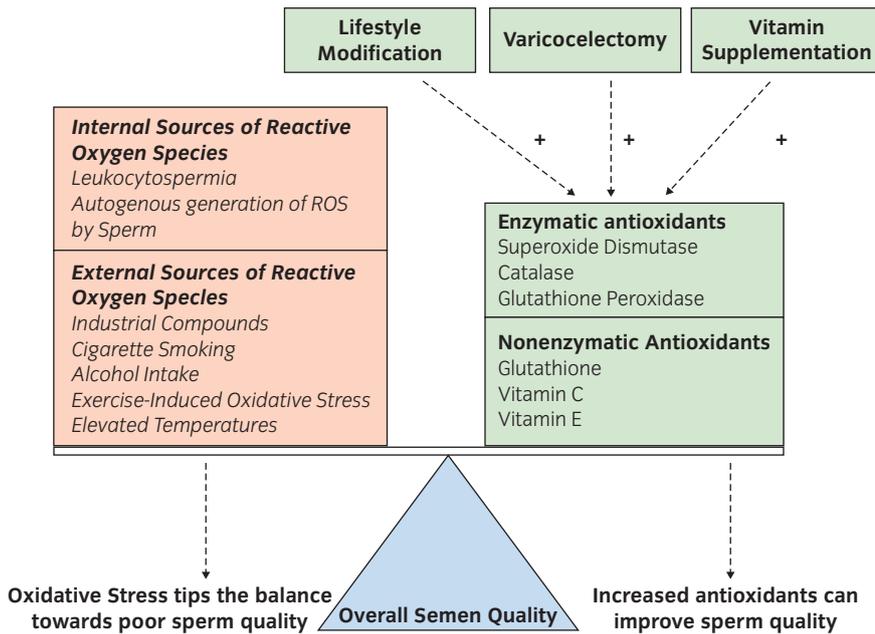
### Lifestyle modifications

As described above, lifestyle choices, including smoking, obesity, poor nutrition, and exposure to environmental toxins all lead to increased systemic or seminal oxidative stress. When considering therapeutic measures to decrease oxidative stress, counseling toward lifestyle

modifications must be considered first and foremost. An important challenge to proper counseling is the paucity of evidence supporting objective improvement in infertility following lifestyle modification. As an example, a diet rich in fruits and vegetables has been shown to offer considerable general health benefits, however, the potential health benefits from dietary supplements or individual vitamins remains controversial at best. Many of the reports in the literature have varying end-points, or use dietary questionnaires in lieu of direct quantification of oxidative stress levels before and after antioxidant supplements, making a comparative study of the literature difficult (Table 1). Nonetheless, considering the ease with which these modifications can be made as well as the profound systemic health benefits, these modifications should be strongly encouraged.

### Varicocele

Clinical varicoceles are one of the most common causes of male infertility. Varicoceles have been shown to increase oxidative stress levels in the testes as well as semen.<sup>84–86</sup> Many studies have concluded that surgical treatment of varicocele is highly effective, and varicocelectomy has been shown to decrease seminal oxidative stress, increase seminal concentrations of antioxidants, and also improve sperm quality.<sup>84–86</sup> A more controversial argument was posed by Evers *et al.*,<sup>86</sup> who performed a meta-analysis of seven randomized, controlled trials (RCT) of varicocele repair for male subfertility identified from the Cochrane Menstrual Disorders and Subfertility Group register of controlled trials. The authors concluded that treatment of varicocele in men from couples with otherwise unexplained subfertility could not be recommended.<sup>86</sup> However, this controversial conclusion was challenged by Ficcaro *et al.*, who argued that less than half of the RCT evaluated in the Cochrane meta-analysis included patients with abnormal semen analysis and palpable varicocele, and each of the studies contained significantly heterogeneous inclusion criteria and patient clinical characteristics.<sup>87</sup> Further, Ficcaro *et al.* described the



**Fig. 3** Balance of oxidants and antioxidants influencing overall sperm quality. In a system with high levels of oxidants, sperm quality will be poor overall. Conversely, a system with increased antioxidant capacity can keep the oxidants in balance, leading to improved sperm quality. ROS, reactive oxygen species.

methodology and statistical power of the Cochrane meta-analysis as poor, thereby minimizing the significance of the conclusions against varicocele repair.

The most recent meta-analysis on varicocelelectomy by Marmar *et al.* noted that men undergoing varicocelelectomy demonstrated lower oxidative stress as well as a significant benefit in spontaneous conception versus the control group.<sup>88</sup> Despite the controversy generated by the Cochrane meta-analysis, most well-designed studies demonstrate varicocelelectomy to be a highly effective treatment for clinical varicoceles.

### Vitamin/antioxidant supplementation

The antioxidants  $\alpha$ -tocopherol (Vitamin A), ascorbic acid (Vitamin C) and the retinoids (Vitamin A) are all potent scavengers of reactive oxygen species. Many studies have investigated the role of these and other antioxidants in improving sperm parameters. However, the majority of these studies are uncontrolled, focus on healthy men without infertility, or have indirect end-points of success. Several other studies are noted due to the quality of their study design, and demonstrate compelling evidence regarding efficacy of antioxidants towards improving semen parameters.

Silver *et al.* surveyed 97 healthy non-smoking men aged 20 to 80 years old regarding antioxidant intake using a dietary questionnaire, and subsequently examined semen samples. Those with a high daily intake of antioxidants were noted to have improved semen quality compared to men with low or moderate intake, thereby demonstrating some correlation between increased dietary antioxidant intake and improved semen parameters.<sup>82</sup> Keskes-Ammar *et al.* examined the therapeutic efficacy of increased antioxidant intake on semen parameters. They randomized 54 men to either Vitamin E and selenium or Vitamin B for 3 months, with examination of semen samples quantifying the lipid peroxidation marker, MDA, as well as measurement of serum Vitamin E levels. Although only 20 patients completed the study protocol, results indicated that Vitamin E and selenium supplementation produced a significant decrease in MDA concentrations with improved sperm motility, whereas Vitamin B showed no impact.<sup>89</sup> Sule-

iman *et al.* randomized their cohort of asthenozoospermic men with healthy female partners to vitamin E or placebo for 6 months, noting decreased MDA levels and increased motility, as well as increased pregnancy rates in the vitamin E arm.<sup>90</sup> Conversely, Rolf *et al.* randomized 31 men with asthenospermia to either 2 months of high-dose oral treatment with vitamins C and E or placebo and investigated semen parameters. The authors found no changes in semen parameters during treatment, and no pregnancies were initiated during this period.<sup>91</sup> Most recently, the best designed trial, by Greco *et al.*, examined the impact of increased antioxidant intake in a randomized, prospective manner. A group of 64 infertile men with >15% DNA-fragmented spermatozoa were randomized into two groups to receive either 1 gram of Vitamin C and E daily or placebo for two months. While no differences in basic sperm parameters were noted, the antioxidant cohort demonstrated a significantly reduced percentage of DNA-fragmented spermatozoa.<sup>92</sup> The authors further went on to demonstrate that supplementation with Vitamins E and C significantly increased rates of clinical pregnancy and implantation following intracytoplasmic sperm injection (ICSI).<sup>93</sup>

Although these data from different centers are potentially conflicting, direct comparison of these results is difficult given the varying nature of the dose, duration of treatment, and study end-points in each of these trials. Nonetheless, these studies provide compelling evidence toward the efficacy of vitamin antioxidants on improving overall sperm quality, and possibly improved pregnancy following ICSI.

### Conclusions

Our understanding of the role of reactive oxygen species on male fertility continues to increase supported by an expanding literature refining this relationship. The etiology of suboptimal semen quality due to oxidative stress is becoming elucidated. The origin of ROS generation, and the etiologies of increased ROS in men with suboptimal sperm quality are increasingly clear, offering multiple pathways for potential therapy (Fig. 3). Nonetheless, more well-designed randomized controlled trials will be required to assess the potential of these antioxidant regimens. Without further studies to test the treatments of best efficacy,

it is difficult to derive cohesive clinical guidelines of therapy from these studies. Nonetheless, the initial data demonstrating efficacy in improving sperm quality and conception rates is indeed encouraging.

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