Free Radicals in Andrology

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1.1 Introduction

Infertility is a condition associated with major medical and social preoccupation. A male etiology is responsible for nearly half the cases of infertility [1] and is caused by alterations in sperm concentration, motility, and/or morphology [2]. Recent advances in the field of infertility have greatly influenced our understanding of the different circumstances attributing to male factor infertility. While environmental, physiological, and genetic influences were recognized, at the molecular level, oxidative stress (OS) resulting from the imbalance between oxidants and reductants appears to be a common denominator impairing sperm function and delaying pregnancy.

Reactive oxygen species (ROS) are highly reactive oxidizing agents that can, at supraphysiological levels, have a potential toxic effect on sperm quality and function [3]. Like other free radicals, ROS contain unpaired electrons triggering a tendency for strong reactivity with other compounds. Moreover, they typically incite a chain reaction exposing a vicious circle type of activity. Under normal physiological circumstances, ROS are products of natural oxygen metabolism acting as vital signaling molecules. However, excessive levels of ROS can be
produced secondary to a variety of environmental exposures and pathologic processes (Fig. 1.1) resulting in several disease entities such as neurodegenerative disease, vascular disease, cancer, and infertility. To minimize the hazardous effects of excessive ROS levels, a number of endogenous enzymatic and nonenzymatic antioxidants exist scavenging or neutralizing excess ROS.

Fig. 1.1 Factors contributing to oxidative stress-induced male infertility (Copyright license provided)
Like all other living cells, spermatozoa require oxygen for survival. However, excessive exposure to oxygen metabolites can alter normal sperm function and vitality [3, 4]. Several reports have confirmed the presence of high ROS levels in the semen of 25–40% of infertile men [5, 6]. Negative correlations were detected between ROS levels and normal sperm morphology as well as with measures of sperm DNA fragmentation [7, 8]. Spermatozoa are predominantly vulnerable to the damage caused by excessive ROS because their plasma membranes contain extraordinary large amounts of polyunsaturated fatty acids (PUFA) [9], and their cytoplasm contains very low concentrations of scavenging enzymes [10]. These intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to depend on the protection provided by the seminal plasma.

This review discusses the mechanisms by which ROS develop in semen and their role in the pathophysiology of male infertility. Topics include the clinical implications of high levels of seminal ROS, the different methods available for ROS detection, and a treatment strategy for infertile men in whom OS is found to be influential.

1.2 ROS Generation

The family of ROS includes oxygen-centered radicals such as the superoxide anion radical (·O$_2^-$), hydroxyl radical (OH·), and nitric oxide radical (NO·), in addition to non-radical derivatives, such as hydrogen peroxide (H$_2$O$_2$), peroxynitrite anion (ONOO$^-$), and hypochlorous acid (HOCl) [11].

A variety of semen components, including sperm with abnormal morphology, germ cells, and leukocytes, are capable of generating ROS. Seminal leukocytes and morphologically abnormal spermatozoa are the main sources of ROS in human ejaculates [12].

1.2.1 Reactive Oxygen Species Production by Spermatozoa

Human spermatozoa can produce ROS in one of two ways: (1) through the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the level of the sperm plasma membrane and (2) through the NADH-dependent oxidoreductase (diphorase) system at the level of mitochondria [13]. Damaged spermatozoa are an established source of excessive ROS production [14]. The increase in ROS generation is thought to result from excess residual cytoplasm (cytoplasmic droplets) typically present in abnormal spermatozoa. During spermatogenesis, defects in cytoplasmic extrusion result in the development of immature sperm containing a surplus of residual cytoplasm [15]. ROS generation has been positively correlated with the extent of residual cytoplasmic retention. This observation is thought to be mediated by the concurrent increase in the enzyme glucose-6-phosphate-dehydrogenase (G6PD) activity seen in abnormal spermatozoa. G6PD stimulates...
glucose influx through the hexose monophosphate shunt raising the intracellular availability of NADPH, a major source of electrons for spermatozoa used to fuel the generation of ROS [16].

Creatine kinase (CK), a biochemical marker of cytoplasmic space, has been correlated with the degree of oxidative damage in human sperm. Alani and El Yaseen [17] have found a significant positive correlation between CK activity and malondialdehyde (MDA) formation in sperm fractions from infertile men. Hallak et al. demonstrated an inverse relationship between CK levels and sperm morphological forms suggesting that CK levels can be used to reliably predict sperm quality and fertilizing potential in men with infertility [18].

Since spermatozoa are rich in mitochondria in order to supply the necessary energy needed for motility, the major source of ROS in spermatozoa in infertile men originates from the mitochondria. Mitochondrial dysfunction has been associated with increased ROS production [19], which results in more damage to the mitochondrial membrane and consequently fuels more ROS production.

Superoxide is the primary ROS generated in human spermatozoa [20]. This one-electron molecule is capable of reacting with itself in a dismutation reaction to generate $\text{H}_2\text{O}_2$ and $\text{O}_2^-$. In the presence of transition metals such as iron and copper, it can interact to generate OH· through the Haber-Weiss reaction ($\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}_2 + \text{OH} + \text{O}_2$). Alternatively, OH· can be produced from $\text{H}_2\text{O}_2$ through the Fenton reaction, which requires ferrous ions as reducing agents ($\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}_2 + \text{OH}$). The OH· is thought to be the most hazardous ROS initiating the lipid peroxidation (LPO) cascade and causing loss of sperm functions [21].

### 1.2.2 ROS Production by Leukocytes

Leukocytospermia has been largely considered as a major cause of male infertility [22]. Peroxidase-positive leukocytes include polymorphonuclear leukocytes and macrophages which represent 50% and 30% of all seminal leukocytes, respectively [23], while T lymphocytes constitute the remaining 10% of seminal leukocytes [23]. They are mainly released with prostatic and seminal vesicle secretions and are capable of producing large amounts of ROS in response to infection or inflammation [22]. Once activated, the leukocytes’ myeloperoxidase system is stimulated leading to an oxidative burst that provides the early defense system against microbes in cases of infection.

Sperm damage from leukocyte-produced ROS can occur after removal of seminal plasma during preparations performed for assisted reproduction or when leukocytospermia exists. A recent study by Lobascio et al. [12] demonstrated the presence of a positive correlation between the number of seminal leukocytes and ROS levels ($p < 0.001; n = 125$). Moreover, they confirmed the negative consequence of such a relationship through finding significant negative correlations with sperm concentration ($p = 0.01$) and motility ($p = 0.02$) and a positive correlation with sperm DNA fragmentation (SDF) ($p = 0.08$) [12]. Mupfiga et al. investigated semen samples from 60 infertile patients and found higher ROS production and caspase activity, a marker of apoptosis, in samples containing a higher number of leukocytes [24]. Saleh et al. [25] compared semen samples from 48 infertile men with those from
healthy volunteers. To objectively assess leukocyte-ROS production, samples from patients with no evidence of leukocytospermia \((n = 32)\) were further incubated with blood neutrophils. In comparison with samples from healthy volunteers and non-leukocytospermic infertile men, significantly higher levels of ROS were detected in samples from leukocytospermic infertile men or after incubation with blood neutrophils. Furthermore, the authors demonstrated a significant decrease in sperm motility and increase in sperm DNA damage in leukocytospermic samples [25].

Sperm damage from leukocyte-derived ROS may happen even at leukocyte concentrations below the World Health Organization’s cutoff value for leukocytospermia, that is, greater than \(1 \times 10^6\) peroxidase-positive leukocytes/mL of semen [26]. In a comparative study, Agarwal et al. divided semen samples from 472 infertile men into three groups: group 1, no seminal leukocytes; group 2, low-level leukocytospermia \((0.1–1.0 \times 10^6\ \text{WBC/mL})\); and group 3, frank leukocytospermia \((>1.0 \times 10^6\ \text{WBC/mL})\). Results revealed significantly higher levels of ROS and sperm DNA fragmentation in group 2 samples (ROS, 1839.65 ± 2173.57RLU/s; DNA damage, 26.47 ± 19.64 %) compared with group 1 samples (ROS, 1101.09 ± 5557.54 RLU/s; DNA damage, 19.89 ± 17.31 %) \((\text{ROS}, p = 0.002; \text{DNA damage}, p = 0.047)\), without significant differences detected between groups 2 and 3. Finally, seminal leukocytes may induce ROS production by human spermatozoa through a mechanism that is not clearly understood [25].

### 1.3 ROS and Sperm Physiology

At optimal levels, ROS exhibit favorable effects that can potentiate sperm fertilizing capabilities. Fertilization is key to normal conception. It requires ideal oocyte and sperm conditions to successfully occur. After spermiation, the spermatozoa must mature within the male genital tract and undergo capacitation and acrosome reaction during their passage in the female tract. Such steps are necessary before penetrating the zona pellucida of the ova and fusing with the female pronucleus. Studies have shown that the incubation of spermatozoa with \(\text{H}_2\text{O}_2\) stimulates sperm capacitation, hyperactivation, acrosome reaction, and oocyte fusion [27–29].

Other ROS, such as \(-\text{O}_2^-\) and NO, have also been shown to promote sperm capacitation and acrosome reaction [30]. Capacitation usually starts after an increase in intracellular calcium, followed by an increase in cyclic adenosine monophosphate (cAMP), activation of protein kinase A, and consequent tyrosine kinase activation resulting in the development of a highly vigorous form of motility known as hyperactivation. Studies have shown that \(\text{H}_2\text{O}_2\) is specifically involved in capacitation through its ability to increase cAMP levels [31]. Upon approaching the oocyte, a sudden influx of extracellular calcium into the acrosomal region occurs due to the influence of progesterone. This causes the spermatozoa to become highly sensitized and ready to undergo the acrosome reaction [28]. Sperm-oocyte fusion was found to be mediated by signaling events that occur in low concentrations of \(\text{H}_2\text{O}_2\) [32]. Aitken et al. revealed that low levels of ROS augment the ability of spermatozoa to bind with the zona pellucida, an effect that was reversed by the addition of vitamin E [33].
1.4 Consequences of Excessive Generation of ROS

Almost every human ejaculate contains potential sources of ROS, namely, leukocytes, immature spermatozoa, and precursor germ cells. It is generally accepted that OS and the consequent loss of sperm function occur in every ejaculate, however, to a variable extent that depends on the nature, amount, and timing of ROS exposure. Excessive seminal ROS levels have been implicated in the pathophysiology of male infertility through their detrimental effects on the following:

1.4.1 ROS and Apoptosis

Apoptosis or programmed cell death is a noninflammatory response to tissue injury characterized by a series of biochemical changes leading to changes in cellular morphology and death. A stringently regulated apoptosis is essential for normal development of spermatozoa as well as the adjustment of the number of sperm cells that are produced [34]. On the other hand, a process termed “abortive apoptosis,” occurring during spermatogenesis, has been associated with male infertility [35–38].

While apoptosis may be triggered by several intrinsic and extrinsic factors, ROS levels appear to be directly correlated with the extent of sperm cell death. Mostafa et al. demonstrated the presence of higher levels of ROS which correlated with a higher percentage of apoptosis in the seminal plasma of infertile men compared to healthy volunteers [8]. After adding 100 μmole of H₂O₂ to 12 semen samples to induce OS, Mahfouz et al. reported a higher percentage of apoptosis after H₂O₂ exposure [39]. Wang et al. compared semen samples from 35 patients with idiopathic infertility with 8 samples from healthy volunteers. The authors detected significantly higher levels of ROS (4.15 × 10⁶ counted photons per minute [cpm] vs. 0.06 × 10⁶ cpm; P < 0.01), cytochrome C (2.78 vs. 1.5; P < 0.01), caspase 9 (2.52 vs. 6; P < 0.006), and caspase 3 (0.56 vs. 1.69; P < 0.01) in infertile men vs. healthy volunteers, respectively [40].

1.4.2 ROS and Lipid Peroxidation

Lipid peroxidation (LPO) is the most extensively studied manifestation of ROS in biology. LPO is generally defined as oxidative induced damage of fatty acids that contain more than two carbon double bonds, also known as PUFA [41]. This is because most PUFA contain a double bond next to a methylene group weakening the methylene-carbon-hydrogen bond. As a result, free radicals are capable of capturing the hydrogen moiety from PUFA leaving an unpaired electron on the fatty acid that can be oxidized to form a peroxy radical (Fig. 1.2). Lipid peroxides are unstable and decompose to form a complex series of compounds, which ultimately end up with MDA.
LPO measurement relies on the interaction of MDA with thiobarbituric acid (TBA). While this method is controversial in that it is quite sensitive, but not necessarily specific to MDA, it remains the most widely used means to determine LPO. The resulting membrane structure disturbance affects vital functions such as signal transduction and maintenance of ion and metabolite gradient necessary for optimal sperm function. Unlike other cells, spermatozoa are unable to overcome the resulting damage since they lack the necessary cytoplasmic enzymes involved in the repair process [42].

1.4.3 ROS and Sperm Motility

Normal sperm motility is an integral requirement for male fertility. The free radical’s ability to reduce sperm motility was first described by Jones et al. in 1979 [43], who linked ROS-induced LPO to reduction of sperm tail motion. ROS illicit a cascade of events that result in a decrease in axonemal protein phosphorylation and sperm immobilization [44]. H$_2$O$_2$ can inhibit the activity of G6PD decreasing the availability of NADPH thereby causing an accumulation of oxidized glutathione. The latter can reduce the antioxidant defenses of the spermatozoa which ultimately aggravate peroxidation of membrane phospholipids [44]. A direct cytotoxic effect for ROS on sperm mitochondria was also recognized as another cause for impaired sperm motility [27, 45, 46].
Clinical studies evaluating the link between ROS levels and defects in sperm motility have confirmed the presence of a negative association. du Plessis et al. [47] examined the influence of exogenous H$_2$O$_2$ addition to semen samples on sperm motility parameters and intracellular ROS and nitric oxide (NO) levels. After incubating human spermatozoa from ten donors with different exogenous H$_2$O$_2$ concentrations (0, 2.5, 7.5, and 15 μmole), they detected a significant inversely proportional relationship with sperm total motility (7.5 μmole 28.3 ± 4.01, $P < 0.001$; 15 μmole 16.67 ± 3.33, $P < 0.001$, versus control, 60.33 ± 6.86) and progressive motility (2.5 μmole 14 ± 1.65, $P < 0.01$; 7.5 μmole 10 ± 2.28, $P < 0.001$; 15 μmole 6.33 ± 1.68, $P < 0.001$, versus control, 29.33 ± 4.56). Higher concentrations of H$_2$O$_2$ significantly increased both NO (172.40 ± 22.341 % versus control; $P < 0.05$) and ROS levels (130.40 ± 7.108 % versus control; $P < 0.05$). The percentage of total motility was also inversely correlated with both endogenous NO ($r^2 = 0.99$, $P = 0.0041$) and ROS ($r^2 = 0.965$, $P = 0.017$) levels [47].

Urata et al. [48] incubated sperm from 37 healthy volunteers with lipopolysaccharide (LPS), an OS inducer, with and without antioxidant scavengers. Sperm motility was inhibited by 15 % in the presence of 0.1 μg/mL, 21 % in the presence of 1 μg/mL, and 50 % in the presence of 10 μg/mL dose of LPS after 60 minutes of incubation, compared with the control groups ($P < 0.05$). LPS-treated groups had a significantly higher ROS production in comparison to the control groups ($P < 0.05$). The addition of ROS scavengers such as superoxide dismutase and glutathione restored the motility index and suppressed ROS production in the LPS-treated semen samples [48]. Kourouma et al. [49] examined the in vitro administration of nonylphenol, a chemical known to induce OS by generating H$_2$O$_2$ and O$_2^-$, on epididymal sperm from 24 Sprague Dawley rats. Results showed a significant decline in the percentage of motile spermatozoa ($P < 0.001$) in a dose-related manner [49].

### 1.4.4 ROS and Sperm DNA Damage

Sperm DNA is uniquely structured to keep its nuclear chromatin highly stable, compact, and protected against assaults. It undergoes timely de-condensation to ensure the proper transfer of the packaged genetic material to the ovum during the fertilization process. Sperm DNA damage can occur as a consequence of OS and is thought to occur secondary to dysregulated apoptosis factors (Fig. 1.3). ROS can alter DNA integrity through modification of nucleic bases resulting in deletions, cross-links, frameshifts, and chromosomal rearrangements [50–52]. Such changes destabilize the DNA backbone, causing single- and double-strand DNA breaks [53]. The significance of sperm DNA fragmentation (SDF) has been acknowledged in male infertility. High SDF has been found to decrease the chances of natural pregnancy [54], increase the likelihood of miscarriages [55], and decrease the outcomes of assisted reproductive techniques, specifically intrauterine insemination [56] and conventional in vitro fertilization [57].

The relationship between OS and DNA damage has been proven in numerous reports. Lommiello et al. investigated semen samples from 56 infertile men and
revealed a significant positive correlation between ROS levels and the degree of SDF ($p = 0.037$) [58]. In another study, semen collected from 63 patients attending an IVF unit was tested for physical characteristics along with ROS levels and SDF. The authors reported a strong positive correlation between intrinsic ROS levels and SDF measurements [59].

1.4.5 ROS in Varicocele

Varicocele is a common medical condition that has long been implicated as a major cause of male infertility [60, 61]. It is prevalent in about 15% of the general male population, 35% of men with primary infertility, and up to 80% in men with secondary infertility [62, 63]. OS is now widely believed to be the common underlying pathophysiology causing infertility in varicocele patients [64, 65]. Studies involving OS markers in the semen of men with varicocele detected a significant increase of such markers in varicocele patients compared to controls [66].

Seminal ROS levels measured by chemiluminescence were significantly higher in infertile men with varicocele than fertile controls. Moreover, Allamaneni et al. [67] reported a positive correlation between seminal ROS levels and varicocele grade meaning that men with larger varicoceles had significantly higher seminal ROS levels than men with small varicoceles. Among healthy fertile men, the
presence of varicocele was associated with a significant increase in ROS levels in comparison to men without varicocele [68]. Higher seminal levels of specific free radicals, namely, NO and NO synthase, have been detected in infertile men with varicocele compared with fertile men without varicocele [69–72]. Seminal levels of H₂O₂ and extracellular seminal O₂ were also found to be significantly higher in infertile men with varicocele in comparison to fertile healthy controls [73, 74].

Interestingly, surgical treatment of varicocele has been shown to reduce seminal OS in varicocele patients [75]. In one study, Sakamoto et al. [69] found that a time lag of approximately 6 months is required to achieve a marked improvement in seminal ROS markers after varicocele repair. Mostafa et al. [76] demonstrated a significant reduction of markers of seminal OS and an elevation of antioxidant levels 3 and 6 months after varicocele ligation. Finally, Hurtado de Catalfo et al. [77] reported normalization of seminal levels of antioxidant enzymes compared with age-matched fertile controls after varicocele ligation.

### 1.5 Measurement of ROS

Screening for OS is increasingly gaining attention in the evaluation of infertile men as current evidence has confirmed its utility in various clinical presentations [78]. ROS can be tested through direct or indirect assays (Table 1.1). Direct assays measure the amount of oxidation within the sperm cell membrane [79], while indirect assays estimate the detrimental effects of oxidative stress, such as DNA damage or lipid peroxidation levels [79]. The most commonly utilized methods for detection of ROS levels include:

### 1.5.1 Chemiluminescence Assay

This assay measures the oxidative end products of the interaction between ROS and certain reagents, which results in an emission of light that can be measured with a luminometer (Fig. 1.4) [80]. Two reagents are available, luminol and lucigenin. In contrast to lucigenin, which detects only the superoxide anion [81, 82], luminol has few advantages such as: (1) it has the ability to react with different ROS, including superoxide anion, hydroxyl radical, and hydrogen peroxide; (2) it measures both intra- and extracellular free radicals; and (3) it conducts a fast reaction allowing rapid measurement [83]. To ensure accurate readings, semen samples should contain sperm

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<th>Table 1.1 Types of ROS measurement assays</th>
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<td>Flow cytometry</td>
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<td>Nitroblue tetrazolium test</td>
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<td>Thiobarbituric acid assay</td>
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<td>Cytochrome C reduction test</td>
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concentration 1 × 10⁶/mL or greater and be analyzed within the first hour of collection. Despite its clinical applicability, the widespread use of the chemiluminescence assay has been hampered by equipment expenses and the presence of assay confounders such as incubation time, leukocyte, and seminal plasma contamination [84].

1.5.2 Flow Cytometry

Flow cytometry is an alternative method that can also be used to measure intracellular sperm ROS [85]. It quantifies the amount of fluorescence per cell. Excited by a light source, cells emit light that is passed through optical filters before reaching optical detectors. Optical filters allow light of specific wavelengths to pass, thereby producing waves of specific colors. Flow cytometry is also an expensive tool that is not practical for widespread clinical use.

1.5.3 Nitroblue Tetrazolium (NBT) Assay

NBT is a cost-effective user-friendly assay. Using a light microscope, it can accurately predict ROS levels and provide insight on the potential source of OS, i.e., spermatozoa or leukocytes. It is based on the ability of NBT to interact with the superoxide present within spermatozoa or leukocytes converting into a blue pigment called diformazan, which can be measured and correlated with intracellular ROS concentration [78, 86].

1.5.4 Measurement of MDA Levels

The most commonly used method of assessing sperm membrane peroxidation is MDA level measurement via the TBA assay. Sensitive high-pressure liquid chromatography (HPLC) equipment [87, 88] or spectrofluorometric measurement of
iron-based promoters [89] may be utilized for detection of the low sperm MDA concentrations. On the other hand, seminal plasma MDA levels are five to tenfold higher than sperm, making measurement with standard spectrophotometers possible [90]. The clinical relevance of MDA measurement emerges from its significant positive correlation with seminal ROS levels in men with infertility, compared with fertile controls or normozoospermic individuals [90–92]. Furthermore, in vitro studies have linked ROS-induced abnormalities in motility, sperm DNA integrity, and sperm-oocyte fusion with an increase in MDA concentration [33, 89].

1.5.5 Measurement of Oxidation-Reduction Potential

Oxidation-reduction potential (ORP), also known as the redox potential, is a measure of the potential for electrons to move from one chemical species to another [93]. ORP is a measure of this relationship between oxidants and antioxidants, providing a comprehensive measure of OS. Recently, a novel technology based on a galvanostatic measure of electrons has been developed (MiOXSYS) and utilized to evaluate changes in OS in trauma patients and as a function of extreme exercise [94–96]. The MiOXSYS is a simple, rapid, and inexpensive system composed of the analyzer and disposable test sensor (Fig. 1.5). It measures the electron transfer from reductants (antioxidants) to oxidants under a steady low-voltage reducing current. Thus, it provides an aggregate measure of all current oxidant activity and antioxidant activity in a sample. Higher ORP values (millivolts, mV) indicate a higher

![Image of MiOXSYS System](https://via.placeholder.com/150)

Fig. 1.5 The MiOXSYS System comprises an (a) analyzer and (b) a disposable sensor (Copyright license provided)
oxidant activity relative to the antioxidant activity and therefore greater state of OS. Recent studies have confirmed the reliability of the MiOXSYS System in measuring ORP levels in semen and seminal plasma demonstrating the presence of significant negative correlations between ORP results and abnormalities of semen parameters [97].

1.5.6 Predictors of OS in Semen Studies

A number of routine laboratory tests have been suggested as possible predictors for the presence of OS in the semen [98]. While any abnormality in routine semen parameters (count, motility, morphology) may be associated with OS, asthenozoospermia is probably the best surrogate marker for OS in a routine semen analysis [99, 100]. The presence of an exaggerated number of round cells in the semen may represent leukocytes and hence possible OS [26]. These cells, however, may represent immature germ cells and hence need to be tested with ancillary tests such as peroxidase test, CD45 staining, or measurement of seminal elastase activity [101, 102]. Poor sperm viability detected by the hypoosmotic swelling test or dye exclusion assays has been linked with the presence of sperm OS [103]. Additionally, macroscopic semen parameters have also been considered. Hyperviscosity of the seminal plasma, an observation that is commonly seen with infection, has been linked to increased levels of seminal plasma MDA [104] and reduced seminal plasma antioxidant status [105].

1.6 Management of OS

OS management is based on alleviating potential causes of excessive ROS production or boosting the patient’s antioxidant system to counterbalance the hazardous effects of ROS.

1.6.1 Lifestyle Modification

The accompanying stresses of the modern world have caused an increase in negative behaviors such as smoking, substance abuse, and obesity, all of which have been shown to contribute to OS, and, therefore, minimizing such unfavorable behavior is likely to aid in alleviating OS [106].

Environmental exposures to heat, pollution, toxins, and heavy metals should be minimized as these can result in development of OS. Moreover, activities capable of increasing scrotal temperature such as hot baths, saunas, extended periods of driving, and long and sedentary office hours should be avoided. Finally, adequate protective equipment and aeration should be ensured at workplaces to limit exposure to any chemical or vapor that may cause OS.
1.6.2 Treatment of Potential Sources of ROS

As stated previously, leukocytospermia is a significant source of ROS in seminal plasma. A significant association between leukocytospermia and genitourinary infection has been acknowledged [107], which, if confirmed by a positive culture, should be treated with appropriate antibiotics. Controversy regarding the value of treating asymptomatic leukocytospermia in infertile men exists and stems from the fact that spontaneous improvement of seminal WBC levels has been witnessed in about 40% of cases [108]. Anti-inflammatory medications (Cox-2 inhibitors) and broad-spectrum antibiotics (doxycycline, erythromycin, and trimethoprim/sulfamethoxazole) have been tried successfully reducing seminal WBC levels and improving semen parameters [109, 110].

Varicocele is another condition eliciting OS [66, 75]. In addition to the favorable influence varicocele ligation has on semen parameters [111], a decrease in seminal ROS levels has invariably been demonstrated, thereby protecting the sperm membrane and DNA from oxidative damage [66, 98].

1.6.3 Antioxidant Supplementation

Antioxidants work by stopping the oxidation cascade through scavenging, neutralizing, or reducing the formation of ROS [45]. There are two types of antioxidants: (1) preventive antioxidants, which are chelators or binding proteins that prevent the formation of ROS, and (2) scavenging antioxidants, such as vitamins C and E, which can quench the ROS that is already present [112].

A number of systematic reviews have explored available evidence on antioxidant use yielding variable conclusions. A Cochrane review of 48 randomized controlled clinical trials including 4179 subfertile men was recently performed [113]. Live birth and pregnancy rates were reported in four and seven trials, respectively. Despite a considerable variability in the reported antioxidant effect on semen parameters, a statistically significant improvement in live birth rate (OR 4.21, 95% CI 2.08 to 8.51; *P* < 0.0001) and clinical pregnancy rate (OR 3.43, 95% CI 1.92 to 6.11; *P* < 0.0001) was detected [113].

Different selection criteria were utilized in other literature reviews. Ross et al. [114] analyzed 17 randomized trials, including a total of 1665 infertile men on whom oral antioxidants were compared to placebo or no treatment. Semen parameters and reported pregnancy rates were the outcome measures analyzed. Despite the methodological and clinical heterogeneity, an improvement in sperm after antioxidant therapy was reported in 14 out of 17 trials. Pregnancy rate was reported in seven trials, six of them showed a significant improvement after antioxidant therapy. The authors concluded that the use of oral antioxidants in infertile men may have a beneficial effect on sperm quality and pregnancy rates.

In an attempt to evaluate the impact of oral antioxidants on measures of sperm oxidative stress and DNA damage, Gharagozloo and Aitken selected 20 trials that assessed such an association [115]. Analysis showed that 19 out of the 20 studies
reported a significant reduction of oxidative stress or DNA damage after treatments with antioxidants. Moreover, an improvement in sperm motility, particularly in asthenozoospermic patients, was significantly observed [115]. In addition to addressing the effect of oral antioxidants on sperm dysfunction and DNA damage, Zini and Al-Hathal also investigated the in vitro use of antioxidants prior to assisted reproduction revealing a protective effect for antioxidants against exogenous ROS, sperm cryopreservation, and thawing [112].

Although many reviews generally demonstrate a favorable influence for antioxidants on male fertility, the ideal regimen of antioxidants is still unknown. This is mainly due to the lack of knowledge of what is a normal redox level in the human body. Currently, no generally accepted normal values for this important parameter exist. In addition, many patients are taking antioxidants in an uncontrolled manner, which in turn might shift the fine balance that is essential for normal sperm function from the OS or normal levels into the so-called reductive stress, which is as dangerous as OS and can be the cause of cancer and heart or neurological disease including infertility among others [116–119]. Many experts suggest an individualized treatment approach where the dose and type of antioxidant should be adjusted according to the clinical presentation and/or the level of seminal oxidative stress.

**Conclusion**

ROS play a vital role in male reproduction. At low levels, they ensure optimal sperm function necessary for fertilization and embryo development. However, when excessive amounts of ROS are produced, they can inflict undesirable effects such as aggravated apoptosis, lipid peroxidation, and DNA damage ultimately worsening sperm function and causing infertility. Measuring seminal ROS levels should become an integral part of male fertility evaluation as it can guide clinicians with their management strategies and provide a sound modality for patient follow-up.

**References**


