REVIEW

Role of oxidative stress in male infertility

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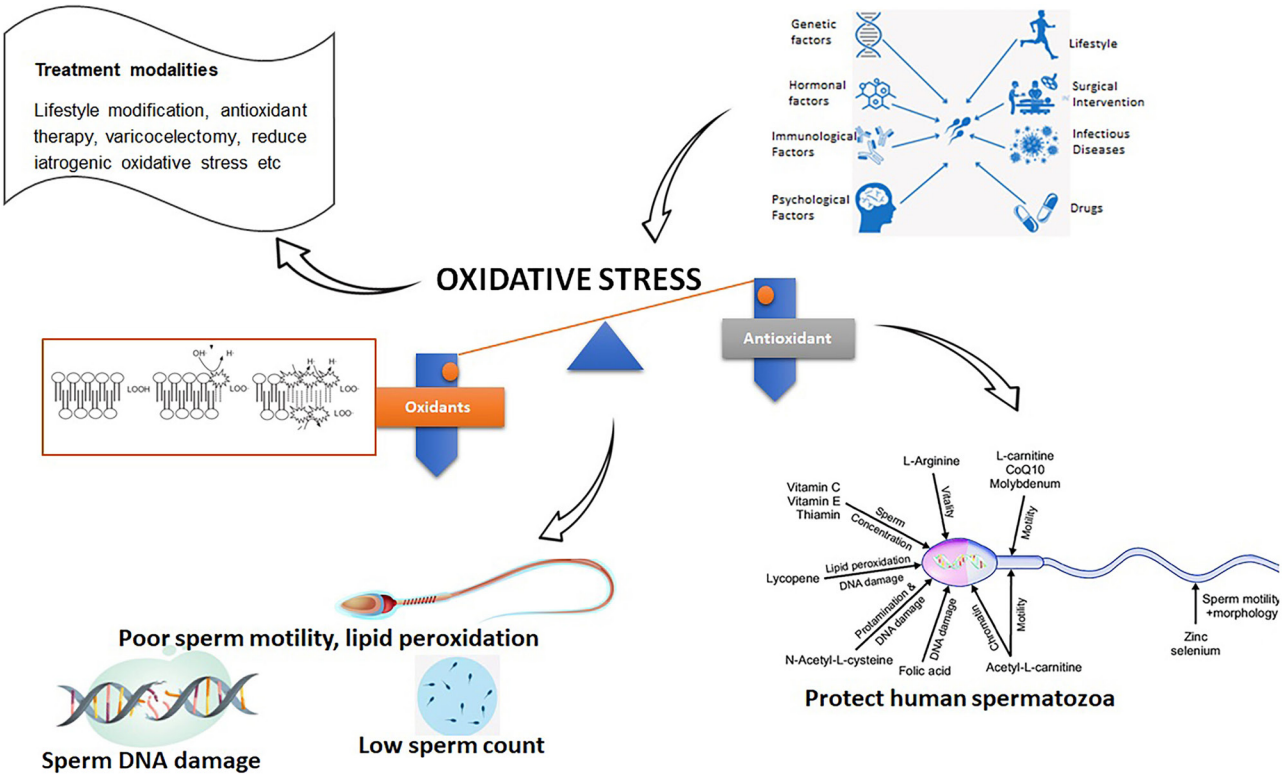
Graphical abstract



Abstract

Infertility affects millions of couples worldwide. Oxidative stress (OS) causes peroxidation of lipids and damage to spermatozoa, thus, reducing the quality of seminal parameters. In addition, the differences in the levels of antioxidants and reactive oxygen species (ROS) caused by intrinsic and extrinsic variables linked to lifestyle, diet, genetics, and OS also contribute to male infertility. High levels of ROS result in sperm damage of sperm parameters due to lipid peroxidation and oxidation of proteins. Other significant causes of ROS include changes in sex hormone levels, sperm DNA damage, including mutations, and immature spermatozoa. Treating the root causes of OS, by changing one's lifestyle, as well as antioxidant therapy, may be helpful strategies to fight OS-related infertility. However, the determination of male infertility

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induced by OS is currently a challenge in the field of reproductive health research. This review intends to describe the role of oxidative stress on male infertility and the current understanding of its management.

Lay summary

The inability to conceive affects many couples globally. Oxidative stress refers to imbalances between different oxygen species which can lead to male fertility problems by damaging sperm and semen. Oxidative stress may be caused by several factors, including diets high in fats, sugars and processed foods, lifestyle (including smoking, alcohol consumption and having a sedentary lifestyle), and genetics. Treatment that focuses on the root cause may help combat male infertility. However, there is currently no consensus on the best way to treat male fertility problems, particularly those associated with oxidative stress. This paper describes the role of oxidative stress on male infertility and discusses the current techniques employed in treating male fertility issues.

Keywords: ▶ oxidative stress ▶ reactive oxygen species ▶ antioxidant ▶ male infertility ▶ oxidation–reduction potential ▶ sperm cells

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Introduction

Infertility is defined as the failure to achieve pregnancy conceive following after 12 months of regular, unprotected sexual intercourse without contraceptives (Larsen 2005). About 40–50% of male infertility is caused by 'male factor' infertility, with 2% of these exhibiting suboptimal sperm parameters (Kumar & Singh 2015). Infertility has been associated with emotional, sociocultural, and physical difficulties (Larsen 2005). Infertility is also associated with a high prevalence of sexually transmitted infections, underdeveloped or undeveloped testes, and hypothalamic and pituitary abnormalities (Agarwal *et al.* 2021).

Furthermore, about 186 million people and 48 million couples globally are infertile (Boivin *et al.* 2007, Mascarenhas *et al.* 2012). In addition, the prevalence of infertility is highest in South Asia, sub-Saharan Africa, North Africa/Middle East, Central/Eastern Europe, and Central Asia (Mascarenhas *et al.* 2012). Although not accurately representing the global statistics, males are found to be solely responsible for 20–30% of infertility cases and contribute to 50% of overall cases (Vander Borght & Wyns 2018). Indeed, as many as 2% of all men will exhibit suboptimal sperm parameters (Kumar & Singh 2015). Roughly 50% of cases associated with infertility are influenced by poor seminal parameters referred to as male factor infertility; this however, varies from region to region (Agarwal *et al.* 2015).

Infertility is classified as primary and secondary (Larsen 2000). Primary infertility refers to a couple who have not achieved pregnancy after 1 year of regular sexual intercourse within their childbearing age (Larsen 2000). Secondary infertility is identified after 6–12 months of unsuccessful attempts to get pregnant. The inability to conceive or bring a pregnancy to term after giving birth is referred to as secondary infertility (Larsen 2000). Also, prior pregnancy must have occurred naturally, without using fertility drugs or procedures like *in vitro* fertilization, for it to be classified as secondary infertility (Katib *et al.* 2014). A study on the longitudinal trend between 1993 and 2017 demonstrated that the primary and secondary infertility prevalence rate globally was lower among men than women and also decreased in high-income countries (Borumandnia *et al.* 2022).

The World Health Organization reports that half of the incidence of infertility is caused by male factor infertility (World Health Organization 2018), which is characterized by poor sperm quantity, poor sperm motility, and morphological defects in at least one sample of two semen analyses and collected in an interval of 1 to 4 weeks apart (Patel *et al.* 2017). Roughly a quarter of men experiencing infertility present cases of teratozoospermia, asthenozoospermia, oligozoospermia, or a combination of all these anomalies, termed oligoasthenoteratozoospermia (Gatimel *et al.* 2017, Alahmar 2019). Idiopathic male infertility is diagnosed when there are unexplained sperm abnormalities, with no female factor infertility, in contrast to unexplained male infertility, where there are normal sperm parameters (Table 1) (Agarwal *et al.* 2019). Oxidative stress has been identified as one of the mechanisms for idiopathic male infertility. Previous studies have reported that spermatozoa with morphological defects are prone

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Table 1 WHO 2010 (5th edition) and WHO 2021 (6th edition) report on male semen parameters.

Semen parameter	WHO 2010	WHO 2022
Semen volume (mL)	1.5 (1.4–1.7)	1.4 (1.3–1.5)
Total sperm count (10^6 per ejaculate)	39 (33–46)	39 (35–40)
Overall motility (%)	40 (38–42)	42 (40–43)
Progressive motility (%)	32 (31–34)	30 (29–31)
Non-progressive motility (%)	1	1 (1–1)
Immotile sperm (%)	22	20 (19–20)
Vitality (%)	58 (55–63)	54 (50–560
Normal forms (%)	4 (3–4)	4 (3.9–4)

WHO, World Health Organization.

to producing excessive reactive oxygen species (ROS) and have reduced antioxidant capacity (Agarwal *et al.* 2019). Furthermore, oxidative stress is commonly detected in the male population with idiopathic infertility having an imbalanced level of ROS and antioxidant capacity compared to fertile male counterparts (Ayad *et al.* 2022).

Despite the association between oxidative stress and idiopathic infertility, there is still a lack in its definite treatment (Ayad *et al.* 2022). For instance, there are no conclusions about which patients should be screened for oxidative stress or what tests should be performed to measure the amount of ROS in the semen sample. Also, controversy exists regarding the type, dose, and duration of antioxidant treatment for patients with excessive ROS levels (Wagner *et al.* 2018). This review intends to describe the role of oxidative stress on male infertility, and the current understanding of its management.

Seminal ROS and male infertility

Human spermatozoa are highly vulnerable to oxidative stress due to the extraordinarily high content of polyunsaturated fatty acids (PUFA) in the plasma membrane and the absence of cytoplasmic antioxidant enzymes (Henkel 2011). Excessive ROS and its metabolites damage lipids, proteins, and DNA, causing apoptosis, altering enzyme activity, and affect sperm parameters which are a prerequisite for fertilization (Alahmar 2019).

An imbalance between ROS production and the body's antioxidant defense mechanisms results in oxidative stress, resulting in a disturbance in cellular functions. ROS are defined as oxygen-containing species (Li *et al.* 2016), which includes ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), peroxyl radical ($LOO^{\bullet}$), alkoxyl radical ($LO^{\bullet}$), lipid hydroperoxide (LOOH), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), and ozone (O_3) (Li *et al.* 2016).

The human semen sample contains a variety of cells, including immature and mature spermatozoa, round-shaped cells of different phases of spermatogenesis, epithelial cells, and leukocytes (Long & Kenworthy 2022). The leukocytes (particularly macrophages and neutrophils are usually activated in response to stimuli during infection and inflammation) and immature, morphologically abnormal spermatozoa are the primary sources of ROS (Agarwal *et al.* 2014). However, the rate of production of ROS is up to 1000 times more in the leukocytes (extrinsic source) compared to the spermatozoa (intrinsic source) (Alahmar 2019). The mitochondrial oxidoreductase and the oxidase in the sperm plasma membrane, both dependent on sperm-specific NADPH, have been proposed to be responsible for ROS production (Gavella & Lipovac 1992, Aitken 1999). In addition, unhealthy lifestyle-related factors such as cigarette smoking, excessive alcohol consumption, unhealthy diet, psychological stress, sedentary lifestyle, and environmental factors (e.g. radiation, metals, and environmental toxicants) are shown to increase the level of ROS in spermatozoa, thereby contributing to the risk of male infertility (Agarwal *et al.* 2014, Durairajanayagam 2019).On the other hand, low levels of ROS generated by spermatozoa play a crucial role in the optimal functioning of spermatozoa. ROS are also involved in physiological processes, such as tyrosine phosphorylation, capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion (Castellini *et al.* 2021). For example, during capacitation, there is an increased ROS level, intracellular calcium, and tyrosine kinase, which result in increased cyclic AMP and, subsequently, hyperactivation (de Lamirande *et al.* 1997).

The etiology of male infertility is heavily influenced by oxidative stress (Agarwal *et al.* 2019). High seminal ROS levels exist in 30% to 80% of infertile men (Agarwal *et al.* 2014). Therefore, the seminal oxidative stress must be considered to measure male reproductive potential correctly. Formerly referred to as idiopathic male infertility, male oxidative stress infertility (MOSI) is a new term for infertile males with abnormal semen features and oxidative stress (Agarwal *et al.* 2014, 2019).

Measurement techniques for oxidative stress in human semen

ROS can be measured using direct and indirect assays. The indirect assays (such as myeloperoxidase, 8-hydroxy-2-deoxyguanosine, thiobarbituric acid reactive substances test, and total antioxidant capacity (TAC)) measure the extent of ROS-induced adverse effect, while the direct

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assays quantify the level of ROS directly (Katerji *et al.* 2019). Chemiluminescence, dihydroethidium probe, nitroblue tetrazolium test (NBT), electron spin resonance, and cytochrome c reduction analysis are some of the methods used in the direct measurement of ROS in semen samples (Agarwal *et al.* 2004). Tables 2 and 3 summarize the indirect and direct testing methods for oxidative stress and ROS in human semen.

In addition, intracellular ROS levels are recognized as a crucial method for detecting changes in redox status and oxidative stress because of its susceptibility to ROS-induced oxidation by peroxynitrite, hydrogen peroxide, hydroxyl radicals, peroxide ions, and H2DCF-DA (Schieber & Chandel 2014). Therefore, cytochrome c centers are being used to grow cells with the fluorescent probe H2DCF-DA (2.5 M) to study intracellular ROS generation in spermatozoa, leukocytes, and blood cells other cellular components (McCarthy *et al.* 2010).

The thiobarbituric acid reactive substance assay (spectrophotometry or fluorometry probe) is frequently used to measure malondialdehyde and 4-hydroxyalkenals as an indication of lipid peroxidation (Seljeskog *et al.* 2006). Sensitive high-pressured liquid chemigraphy is recommended for low malondialdehyde levels, while mass spectrometry signifies another method to investigate lipid peroxidation product as isoprostanes (Ito *et al.* 2019). Sperm MDA levels positively correlate with ROS production in the semen of infertile men. Chemiluminescence quantifies seminal ROS levels and involves using a luminometer and a chemiluminescent probe, such as luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Dutta *et al.* 2019). This assay directly measures intracellular and extracellular ROS (Agarwal *et al.* 2015). Light signal is emitted after free radicals in the semen samples react with luminol, which is converted to an electrical signal by the luminometer. The ROS level in the sample is represented as relative light units (RLU) per second per 10^6 spermatozoa per milliliter (RLU/s/10^6 sperm/mL) (Dutta *et al.* 2019, Dias 2021). The usual range of ROS levels in washed sperm suspensions is 0.10–1.03 × 10^6 counted photons per minute per 20 × 10^6 sperm (Agarwal & Majzoub 2017). The chemiluminescence assay is reported to be a reproducible and reliable assay for measuring seminal ROS and used for diagnosing male infertility (Dutta *et al.* 2019). More so, it is extremely sensitive and reacts with various ROS at neutral pH and should be measured within 4 h of sample collection (Kobayashi *et al.* 2001). A significant limitation of this assay is that luminol can act as a source of O_2^- in the presence of other univalent oxidants(Khan *et al.* 2014).

TAC measures the total amount of antioxidants in seminal plasma to inhibit ABTS 2,29-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) oxidation to $ABTS^+$ after incubation with metmyoglobin and hydrogen peroxide (Miller *et al.* 1993). The TAC may be examined by using improved chemiluminescence or colorimetric methods. ROS–TAC scores were suggested as a new approach to assessing the impact of redox status on infertility (Sharma *et al.* 1999). In particular, the ROS–TAC score might be used to identify oxidative damage in semen samples from asthenozoospermic men (Sharma *et al.* 1999). A continued focus on a particular global index that can easily distinguish between fertile and infertile males rather than using ROS or TAC alone is sought (Robert *et al.* 2021).

Flow cytometry is a technology that analyzes single cells in solution in real time using multiple parameters. Lasers are used as light sources in flow cytometers, producing both scattered and fluorescent light signals that are read by detectors such as photodiodes or photomultiplier tubes (McKinnon 2018). Flow cytometry measures intracellular ROS by quantifying a cell's fluorescence amount (Agarwal & Majzoub 2017).

The NBT is colorimetric marker of superoxide and is based on the use of NBT, a yellow, water-soluble, nitro substituted aromatic tetrazolium salt (2,20-bis(4-nitrophenyl)-5,50-diphenyl-3,30-(3,30-dimethoxy-4,40-diphenyl) ditetrazolium chloride), with the ability to interact with intracellular superoxide to produce formazan (purple), which can then be measured spectrophotometrically or microscopically (Armstrong *et al.* 2002, Esfandiari *et al.* 2003). The NBT test measures intracellular ROS levels and gives insight into the potential source of oxidative stress with the aid of a light microscope (Agarwal & Majzoub 2017).

Measurement of oxidation–reduction potential

Oxidation–reduction potential (ORP, redox potential) is a practical and straightforward direct assay for measuring oxidative stress. It measures the balance between oxidants and antioxidants (Agarwal *et al.* 2016). The ORP measures the levels of antioxidants and oxidants in several biological fluids (Okouchi *et al.* 2002). In addition, ORP could supplement semen analysis due to its strong association with impaired sperm function (Agarwal *et al.* 2019).

The Male Infertility Oxidative System (MiOXSYS) is a novel, user-friendly, and less expensive system that is used to evaluate the ORP in human semen. It expresses the ORP as the static ORP (sORP; mV). The reading is then

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Table 2 Pros and cons of direct tests used to measure oxidative stress levels in the seminal plasma.

Assay	Description	Pros	Cons	Reference
Chemiluminescence assay	The emission of electromagnetic radiation brought on by a chemical process that results in the production of light is known as chemiluminescence (CL). Chemiluminescence immunoassay (CLIA) is a test that combines immunochemical responses with the chemiluminescence method. CLIA uses chemical probes that can label the antibody by a chemical reaction, much as other labeled immunoassays (RIA, FIA, and ELISA).	• Extremely specific and responsive	• Constrained in time • Large, high-maintenance, and expensive machinery. • Requires a significant sample volume. • Does not allow for the measurement of reactive oxygen species in samples that are frozen, gelatinous, or azoospermia. • Changes in pH, centrifugation, and the presence of NADPH, cysteine, ascorbic acid, or uric acid may affect the results.	Agarwal *et al.* (2015), Li *et al.* (2016)
Cytochrome c reduction test	This test gauges how much cytochrome c is reduced by NADPH-cytochrome c reductase when NADPH is present. Cytochrome C's oxidation/reduction status affects its absorption spectrum. At 550 nm, a significant absorption peak is seen after reduction.	• It is effective for measuring a high level of reactive oxygen production and can quantify the amount of oxygen released by neutrophils during their respiratory burst.	• Ferricytochrome c can be reduced by electron transfer from enzymes and other molecules • The change in absorbance for O2• is unclear. • In circumstances where the enzyme activity is limited, it is impossible to identify the presence of NADPH oxidase activity. • As a result of the enzyme's lack of access to intracellular spaces, only the quantity of reactive oxygen species present outside of the cell can be determined	Melendez-Ferro *et al.* (2013)
Nitroblue tetrazolium (NBT)	The nitroblue tetrazolium measures intracellular ROS level as well as give insight to the potential source of OS with the aid of a light microscope. NBT is converted into a blue pigment (diformazin) following its interaction with the superoxide released from the leukocytes or spermatozoa. The concentration of diformazin is positively correlated with the concentration of intracellular ROS.	• Always on the market, less expensive, and very effective • Give specifics regarding the distinct roles that damaged spermatozoa and leukocytes play in the production of reactive oxygen species. • Identifies intracellular ROS in a diverse population of cells	• The levels of NBT reduction are influenced by variations in the cellular material of various oxidation–reduction • Due to the assay's focus on the reduction of NBT, there are discrepancies in the detection of reactive oxygen species.	Tvrdá *et al.* (2011), Agarwal & Majzoub (2017)
Oxidation–reduction potential (ORP)	The interaction between oxidants and antioxidants is measured by ORP, which offers a thorough assessment of oxidative stress. Higher ORP readings distinguish the level of oxidative stress caused by an imbalance in the activity of oxidants and antioxidants. reproductive issues caused by men	• Simple method that takes less time. • Favors both fresh and frozen samples without being altered or treated in any way. • Results remain stable for up to 2 h. • It can isolate sperm samples based on seminal parameters or fertility status. • It can quantify the number of antioxidants and oxidants in a sample.	• Viscous samples may affect the reading. • Azoospermia samples are not supported.	Agarwal *et al.* (2017)

(Continued)

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Table 2 Continued.

Assay	Description	Pros	Cons	Reference
Electron spin resonance	A spectroscopic method called electron spin resonance (ESR) uses a static magnetic field to detect transitions between the energy levels of electron spins caused by electromagnetic radiation.	• Oxidation of hydroxyl spin probes in hydroxylamine spin results in the formation of extremely stable nitroxide with a half-life of several hours. • Spin trap distinguishes between various oxidative molecules.	• Basic analyses in hydroxylamine spin are partially inactive at biological pH. • In both methods, the scavenging action of antioxidants may hinder the formation of adducts.	Kopáni *et al.* (2006)

normalized to the sperm concentration and expressed as mV/10^6 sperm/mL (Agarwal *et al.* 2018*a*). The MiOXSYS analyzer consists of an ultrahigh impedance electrometer and a sensor with working electrodes and references (Agarwal & Bui 2017). The test is done by adding a small volume (30 µL) of the liquefied neat semen on a pre-inserted sensor. The electrochemical circuit is completed when the sample fills the sensor's reference terminal, and the test begins. The principle of this methodology is based on comparing the electrical conductance of an internal reference standard to the Nernst equation:

$$E(ORP) = E0 - RT/nF$$

E is the redox potential or ORP.

E0 is the standard potential of a redox system measured concerning hydrogen electrons, which is arbitrarily assigned an E0 of 0 volts.

R = gas is constant.

T = absolute temperature measured in degrees Kelvin.

n = number of moles of electrons transferred in the balanced equation for the reaction occurring in the cell.

F = Faraday's constant.

A cutoff value of 1.34 mV/10^6 sperm/mL for sORP is set to demonstrate the quality of seminal parameters (Agarwal *et al.* 2016, 2017). A higher sORP indicates an imbalance between oxidants and antioxidants in favor of the oxidants, thus indicating oxidative stress (Agarwal *et al.* 2017).

A good correlation has been found between the ORP and male semen parameters such as sperm motility and morphology, indicating ORP as a marker of seminal oxidative stress (Agarwal *et al.* 2019). Semen from the male partners of fertile couples tends to have lower sORP than those from infertile male partners (Agarwal *et al.* 2016). These findings suggest that ORP measurement may be the most effective method for predicting and managing sperm infertility (Aitken 2022, Castleton *et al.* 2022, Joao *et al.* 2022). Additionally, research confirmed an association between DNA fragmentation and seminal ORP (Panner Selvam *et al.* 2022). Furthermore, it was also noted that ORP is a more precise method compared to chemiluminescent ROS assessment for determining the redox status in male infertility (Vassiliou *et al.* 2021).

The available assays for the measurement of seminal ORP are a reliable marker for assessing the overall redox state is seminal ORP. These assays are trustworthy and user-friendly with great potential of being used in clinical andrology settings (Panner Selvam *et al.* 2022). In addition, they usually provide one single marker of oxidative stress, that is, oxidant level, antioxidant level, or posthoc damage (Agarwal *et al.* 2018*b*).

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Table 3 The advantages and disadvantages of indirect tests used to measure oxidative stress levels in the seminal plasma.

Assay	Description	Advantages	Disadvantages	Reference
Myeloperoxidase	Myeloperoxidase, which is found in the leukocyte granules, converts substrates from an insoluble blue/brown derivative to a colorless form in the presence of hydrogen peroxide. As substrates, benzidine, 3,3′-diaminobenzidine, or p-phenylenediamine dihydrochloride are used.	• Quick, simple, and inexpensive to operate • Suggested by WHO for the purpose of examining leukocytospermia in sperm samples • >1 × 10^6 peroxidase positive WBC/mL of semen (leukocytospermia)	• PMNs and macrophages, both peroxidase-positive leukocytes, make up between 50 and 60% of all seminal leukocytes. • Unable to determine whether spermatozoa produce ROS.	Shekarriz *et al.* (1995)
Total antioxidant capacity	Antioxidant capacity (AC) provides an integrated metric rather than the mere total of measured antioxidants by taking into account the cumulative activity of all antioxidants present in plasma and bodily fluids.	• Automatically measures the total antioxidant content of seminal plasma. • Recommended cut off ≥1950 µM Trolox significative of good antioxidant reserves	• Makes use of a costly assay kit and microplate reader	Robert *et al.* (2021)
HNE-HIS adduct ELISA	The protein-HNE adduct ELISA is a technique for detecting HNE bound to proteins, which is thought to be the type of HNE occurrence in biological systems	• Fast	• Cross-reactivity	Agarwal *et al.* (2017)
Malondialdehyde assay	This assay is based on the formation of the MDA-TBA2 adduct, a strong absorber at 532 nm, by the interaction of malondialdehyde (MDA) with thiobarbituric acid (TBA). The most widely used technique for calculating MDA in biological samples is this reaction.	• Easy to use, • Capable of determining lipid peroxidation, • Able to identify the MDA-TBA adduct using either fluoroscopy or colorimetry	• Expensive materials • Thorough controls required • Generic for MDA	Khoubnasabjafari *et al.* (2015)
DNA fragmentation	TUNEL is a technique for identifying apoptotic DNA fragmentation that is frequently used to recognize and measure apoptotic cells or to find cells that have excessively broken DNA. The test depends on the enzyme terminal deoxynucleotidyl transferase (TdT), which is used to attach deoxynucleotides that have been dyed or otherwise marked to the 3'-hydroxyl termini of DNA double-strand breaks. Additionally, it may identify cells whose DNA has been damaged in ways beyond apoptosis.	• Different methods are available (TUNEL, SCSA, Comet, SCD, and 8-OHdG) • Comet assay is easy to carry out, flexible, sensitive, and rapid assay and has shown some similarities with other assays like the SCSA and TUNEL • SCSA and TUNEL using a flow cytometry is robust and sensitive assay • TUNEL assay is very direct in terms of measuring single- and double-strand DNA breakage	• Lack of standardized reference value • The 8-OHdG method may itself trigger DNA oxidation tempering with basal level • Cost is a major concern in TUNEL and SCSA assays	Homa *et al.* (2019)
Protein alterations ASSA	Determine the quantity or concentration of a particular protein or a variety of distinct proteins in a sample using a protein test. Many clinical and scientific procedures include the isolation and detection of proteins.	• Incredibly precise and sensitive • Western blot, ELISA, or immunochemistry can be used to verify certain proteins. It is possible to identify changes in sperm protein that are specific to male infertility conditions.	• It takes a long time and requires expensive instruments.	Agarwal *et al.* (2017)

8-OHdG, 8-hydroxy-2-deoxyguanosine; ELISA, enzyme linked immunosorbent assay; MDA, malondialdehyde; OS, oxidative stress; PMN, polymorphonuclear neutrophils; ROS, reactive oxygen species; SCD, sperm chromatin dispersion; SCSA, sperm chromatin structure assay; TBA, thiobarbituric acid; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; WBC, white blood cell; WHO, World Health Organization.

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Furthermore, conventional semen analysis is criticized for its poor reproducibility, subjectivity, and fertility prediction. Consequently, ORP is recommended as a clinical biomarker for MOSI in men with abnormal semen analysis and male infertility based on the limitation of conventional semen analysis, pathological consequences, and ubiquity of oxidative stress among the subfertility male population (Nago *et al.* 2021, Henkel *et al.* 2022, Kavoussi *et al.* 2022, Niu *et al.* 2023).

Management of MOSI

Despite the progress made to measure oxidative stress in semen analysis, well-defined procedures for treatment associated with male infertility oxidative stress as a result of undefined aetiologies of male infertility are still lacking (Ayad *et al.* 2022). Therefore, this section will review the literature regarding treatment options, including empirical treatment for men with elevated ROS, specific treatments, and procedures for reducing iatrogenic oxidative stress.

Empirical medical treatment for men with elevated ROS: evidence for antioxidants

Empirical medical treatment (EMT) is widely used in men with idiopathic infertility. Based on the mode of action, EMT is categorized into two groups: hormonal therapy and antioxidant supplementation (Jung & Seo 2014). The agents of the hormonal treatment target the hypothalamic–pituitary–gonadal axis to correct subclinical endocrinopathy and includes aromatase inhibitors, gonadotropins, androgens, and selective estrogen receptor modulators (Dabaja & Schlegel 2014, Jung & Seo 2014). Although hormonal therapy in male counterparts with detectable aberrations like hypogonadotropic hypogonadism is well articulated, 10% of infertility cases in men are culminated by an imbalance in the endocrine system (Ko *et al.* 2012). Therefore, studies recommend using EMT to combat idiopathic infertility; however, there is a lack of evidence to validate successful birth outcomes in *in-vitro* (Thaker *et al.* 2020, Ayad *et al.* 2022). On the other hand, it is suggested for males with no genetic aberration, bacterial infections, and imbalanced endocrine system, identification of the primary source of MOSI rather than EMT should be employed to treat male infertility (Thaker *et al.* 2020).

Antioxidant therapy

An imbalance between ROS production and antioxidant level is implicated as the primary cause of idiopathic male infertility. Scavenging enzymes in the cellular cytoplasm and antioxidants in the seminal fluid play a crucial role in the antioxidant defense mechanism to counteract the adverse effects of ROS (Agarwal *et al.* 2014). However, the relatively low concentration of the cytoplasmic scavenging enzymes and the high amount of PUFA within the plasma membrane of human spermatozoa make them vulnerable to ROS from lipid peroxidation (Sanocka & Kurpisz 2004). Antioxidant defense mechanisms against ROS may be enzymatic or non-enzymatic (Sies 1997).

Several studies have been undertaken to determine the effectiveness of various antioxidants such as vitamins C and E, zinc, selenium, L-carnitine, folic acid, or coenzyme Q10 on seminal fluid oxidative stress as well as sperm parameters (Alahmar & Sengupta 2021). A few of these reported improved semen parameters such as DNA fragmentation, sperm motility, morphology, and concentration (Alahmar & Sengupta 2021, Gupta *et al.* 2021). Furthermore, significant improvements in sperm redox status as well as a good correlation with pregnancy outcomes were demonstrated (Gharagozloo & Aitken 2011). In addition, oral administration of antioxidants for 3 months significantly improved sperm health and count in idiopathic infertile male counterparts (Agarwal *et al.* 2019). This improvement might increase the likelihood of conceiving naturally (Ko *et al.* 2012). Other studies also support antioxidants' efficacy in increasing live birth rates and semen parameters (Showell *et al.* 2020). Although antioxidant therapy in male infertility is still debated, once a patient has been diagnosed with infertility caused by oxidative stress, treatment should focus on amelioration and classification of the leading cause before resorting to antioxidant therapy (Lanzafame *et al.* 2009). The combination of MOSI diagnosis with ORP monitoring may be a reliable strategy in antioxidant therapy (Agarwal *et al.* 2019). Compared with hormonal EMT and assisted reproductive technology (ART), antioxidants are generally safe, affordable, and widely accessible (Agarwal *et al.* 2019). Table 4 summarizes a wide range of antioxidants used in a clinical trial to combat male infertility.

Non-enzymatic antioxidants such as (vitamin C and vitamin E complex, GSH, coenzyme Q10, carnitine, and minerals such as zinc, copper, selenium, and chromium) have been reported to be crucial in maintaining sperm physiology (Collins & Rossi 2015, Ahmad *et al.* 2017). GSH is a tripeptide thiol that is derived from cysteine, glutamine, and glycine and has many biological functions such as maintaining the redox state and detoxifying endogenous and exogenous compounds (Aquilano *et al.* 2014). Vitamin C is considered the most important

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water-soluble antioxidant in the extracellular fluid as it inhibits the formation of ROS (Aquilano *et al.* 2014) and protects sperm from DNA damage by inhibiting the harmful effects of ROS before activating LPO. This is accomplished by lowering oxidized tocopherol and reversing the hydroxyl effect, which protects human germ cells from oxidative damage (Aquilano *et al.* 2014). A study demonstrated that spermatozoa with excessive ROS had lower levels of vitamin C (Rahimlou *et al.* 2019). Tocopherols and tocotrienols are the components of vitamin E (Rahimlou *et al.* 2019). Vitamin E can be found in wheat germ, avocados, palm oil, and veggie oils. Tocopherol and ROS interact during LPO to produce lipid radicals that stop the degradation of cell membranes (Bartolini *et al.* 2022). Vitamin C and E taken together have been shown to protect sperm from peroxide damage and DNA damage (Rahimlou *et al.* 2019). Zinc, the is the second most prevalent in the human body, is involved in protein synthesis during DNA transcription, which is crucial for reproduction (Fallah *et al.* 2018). Zinc plays a role in several biological reproduction processes, including germ cell development and the synthesis of luteinizing, follicular, and testicular hormones (Fallah *et al.* 2018). Zinc in combination with other antioxidant enzymes helps men with sperm deficiencies increase their fertility and sperm health (Fallah *et al.* 2018). Another essential micronutrient that can end up in sperm and testosterone production is selenium (Fallah *et al.* 2018). About 25 selenoproteins have been found in animals and humans, significantly affecting sperm integrity (Rahimlou *et al.* 2019).

Enzymatic antioxidants (SOD, GPx, and CAT) are another category of antioxidants known to scavenge ROS from the gonads and seminal fluids (Ahmad *et al.* 2017). Glutathione peroxidase is a selenium-containing antioxidant enzyme found in the cell's mitochondria and cytoplasm (Lubos *et al.* 2011) and exists in two forms, selenium-dependent and selenium-independent enzymes (Lubos *et al.* 2011). Superoxide dismutase in the prostate gland and seminal vesicles is a first-line defense against oxidative stress in reproductive cells and a regulator of ROS production (Lubos *et al.* 2011). Additionally, SOD works well with CAT and GPx due to its scavenging property (Lubos *et al.* 2011). Catalase is an antioxidant enzymatic found in (Ohta *et al.* 1996, Rubio-Riquelme *et al.* 2020).

The B-type vitamins folate, vitamin B6, and vitamin B12 enhance the enzymatic activity of the methylenetetrahydrofolate reductase (Serapinas *et al.* 2017). Compounds of the cystathionine b-synthase family are responsible for eliminating homocysteine from the plasma (Jhee & Kruger 2005). It is suggested that any male with hyperhomocysteinemia and oxidative stress should take a B vitamin supplement (5 mg folate, 100 mg vitamin B6, and 100 mg vitamin B12) since it is affordable and has no significant adverse effects (Kaye *et al.* 2020).

Several studies have been published to date examining the effects of various antioxidant therapies on sperm parameters and pregnancy outcomes (Sies 1997, Smits *et al.* 2018, Agarwal *et al.* 2023). Conclusions regarding the clinical viability of oral antioxidant therapy on sperm functionality and pregnancy outcomes should be readily available, given the abundance of research references (Arafa *et al.* 2020, Scaruffi *et al.* 2021). However, this is not the case due to heterogeneous data such as differences in dosage of the antioxidants, absence of appropriate viewpoint placebo-controlled research design, and small scale-sample size (Martin-Hidalgo *et al.* 2019). A preliminary report revealed a significant improvement in sperm quantity, motility, and sperm morphology following antioxidant therapy (Wang *et al.* 2019). Antioxidants such as astaxanthin, carnitine, or a blend of antioxidants, for example, acetylcysteine, β-carotene, vitamin E, and unsaturated fatty acids have all been shown to decrease ROS levels (Tremellen 2008, Kefer *et al.* 2009, Zhaku *et al.* 2021). In a placebo-controlled study, a combination of 225 mg of selenium, 400 mg of vitamin E, or 300 mg of vitamin E significantly reduced malondialdehyde levels in spermatozoa (Ammar-Keskes *et al.* 2003). A randomized clinical trial revealed a positive effect toward improvement in the health of the sperm DNA following 2 months of treatment with 1 g of vitamins C and E (Ammar-Keskes *et al.* 2003). Another study also reported on the reduction of sperm DNA aberration after treatment with a variety of vitamins E and C (400 mg each), β-carotene (18 mg), zinc selenium, or a mixture of 30 mg β-carotene, 180 mg of vitamin E and fatty acids (Punjabi *et al.* 2022). Furthermore, several studies have revealed antioxidant therapy to improve sperm quantity and quality (Büyükleblebici *et al.* 2014, Moichela *et al.* 2021, Takalani *et al.* 2021, Setumo *et al.* 2022).

However, the most reported parameter which seems to have been improved through antioxidant supplementation and reported frequently is sperm motility (Agarwal & Said 2004). For instance, significant positive effects on the motility of sperm were observed following antioxidant therapy using carnitine, selenium, vitamin E, glutathione, and a combination of selenium and vitamin E supplements (Pehlivan 2017). On the other hand, randomized clinical trials assessing vitamin C and E therapy with placebo revealed antioxidants to have no capacity for sperm improvement (Bartolini *et al.* 2022).

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Table 4 Clinical trials conducted using different antioxidants to combat male infertility.

Type of antioxidant or ingredient	Evaluated semen parameter/ fertility condition	Dosage	Outcome	Reference
Vitamin C–E	Human sperm DNA fragmentation, sperm motility, morphology,	One gram of vitamin E and 1 g of vitamin C. 200 μg vitamin E	The intervention group had less DNA damage after 2 months. However, major semen parameters like motility and concentration were found to have no significant relationship with vitamin E or C intake. According to Greco and colleagues, a 2-month course of treatment with 1 g of vitamin E and C reduced the amount of DNA damage and improved ICSI success in patients with sperm DNA damage. 690 infertile men with idiopathic asthenoteratospermia who received daily supplement of selenium (200 μg) in combination with vitamin E (400 IU) for at least 100 days. They reported 52.6% (362 cases) total improvement in sperm motility, morphology, or both, and 10.8% (75 cases) spontaneous pregnancy in comparison with no treatment.	Greco *et al.* (2005), Moslem & Tavanbakhsh (2011), Lobascio *et al.* (2015)
Vitamin E	Motility and viability in asthenoteratozoospermic men	2, 4, and 6 h. that the test group was incubated with VE (2 mM)	By maintaining normal antioxidant processes, *in vitro* vitamin E supplementation may shield spermatozoa from the negative effects of oxidative stress during sperm preparation.	Ghafarizadeh *et al.* (2021)
Vitamin C	Sperm parameters of teratozoospermic semen samples	600 μm ascorbic acid	Vitamin C improves sperm motility, progressive motility, and acrosome reaction	Fanaei *et al.* (2014)
Zinc	Asthenoteratospermia	220 mg per capsule/ per day for 3 months	Zinc supplementation increased the volume of sperm, progressive sperm motility percentage, and total normal sperm count.	Hadwan *et al.* (2012)
Folic acid	Subfertility parameter	5 mg/day	Treatment significantly increased sperm concentration in sub fertile males. Other semen and endocrine parameters were not affected by intervention treatment.	Ebisch *et al.* (2006)
CoQ10	Semen parameters in infertile men	150 mg CoQ10/day	Because CoQ10 increases total antioxidant capacity, it was found to be associated with important sperm parameters like concentration, motility, and morphology. Thakur suggested that infertile men's sperm parameters could be improved by taking 150 mg of CoQ10 daily.	Thakur *et al.* (2015)
Selenium and NAC	Idiopathic oligo-asthenoteratospermia	100 μg selenium orally daily, 600 mg NAC orally daily	The parameters of the sperm and the concentrations of selenium and NAC in the seminal plasma showed a strong positive correlation. The sum of the selenium and NAC levels was found to have a strong correlation with the mean sperm concentration ($r = 0.67$, $P = 0.01$), sperm motility ($r = 0.64$, $P = 0.01$), and percentage of normal morphology ($r = 0.66$, $P = 0.01$).	Safarinejad & Safarinejad (2009)

CoQ10,Coenzyme Q10; NAC, *N*-acetyl-cysteine.

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Matorras *et al.* (2020) reported that treatment with vitamin E brought about a considerable decrease in the damaging effect of ROS on sperm and an improvement in unconstrained pregnancy rates during the preceding 6 months (21% pregnancy rate in the vitamin E group vs 0% placebo). On the other hand, Rolf *et al.* (1999) detailed no improvement in spontaneous gestation resulting from 2 months of treatment with a blend of vitamin C and E. However, a new randomized clinical trial contrasting the antioxidant formulation Menevit and placebo treatment detailed a significant rise in clinical pregnancy in circumstances where the antioxidant treatment was administered for 3 months before IVF-ICSI therapy (Tremellen *et al.* 2020). Menevit, a nutraceutical hypothesized to improve sperm health through three correlative systems, contains antioxidant agents like vitamins C and E, selenium, and lycopene to shield sperm from previously generated ROS (Tremellen *et al.* 2020). Lastly, it contains zinc, selenium, and folate, which play a significant role in enlarging the protamine bundling of sperm DNA (Arafa *et al.* 2020) and protecting the sperm against the damaging effect of ROS. While it is yet to be demonstrated that combinational treatment like Menevit has a positive impact on DNA structure, it is suggested that the use of different antioxidants with a variety of modes of action together with an agent to limit the production of leukocytes ROS is probably going to bring about a beneficial impact (Beltrán *et al.* 2018).

Procedures for reducing iatrogenic oxidative stress

In andrology, centrifugation of sperm samples before use can exacerbate oxidative stress (Marzano *et al.* 2020); this can be mitigated by reducing centrifugation time, using non-centrifuge partitioning procedures such as 'swim-up' or glass wool filtration, and reducing the time interval in which human sperm are cultured in a media separate from seminal plasma (Martí *et al.* 2006). Furthermore, cultivating spermatozoa in low oxygen tension settings has been shown to improve sperm health by decreasing seminal leukocytes during ROS formation (Agarwal *et al.* 2022). Furthermore, avoiding cryopreserved sperm for fertilization is advised because ROS are produced during sperm banking, lowering the quality of the sperm cells (Tafuri *et al.* 2015, Agarwal *et al.* 2022). Also, throughout the sperm preparation process, the media used may be supplemented with various antioxidants to protect the cells from oxidative damage. For example, supplementing sperm medium with vitamin C, vitamin E, catalase, ferric acid, EDTA, albumin, and glutathione has been shown to protect against oxidative damage (Fanaei *et al.* 2014). However, aside from amino acids and albumin, currently employed sperm preparation media do not contain any type of antioxidant supplement (Bui *et al.* 2018). Unfortunately, better sperm media still lack features of the complicated sequential media produced for embryos, which needs extensive research (Bui *et al.* 2018).

Oxidative pathology direct treatment

Varicocele surgery has been shown to effectively reduce oxidative stress and enhance sperm DNA integrity (Kavoussi *et al.* 2022). Even though the most recent meta-analysis examining the effect of varicocelectomy on spontaneous conception found a significant benefit, the Cochrane Database claims no benefit. Randomized trials evaluating oxidative stress (sperm lipid peroxidation and oxidative DNA damage) and pregnancy rates must be carried out before varicocelectomy may be considered in males with oxidative stress.

Conclusions

The physiological level of ROS has been reported to be essential in the fertilizing capacity of spermatozoa, including capacitation and acrosome reaction. However, a high level of ROS results in oxidative stress, resulting in sperm membrane lipid peroxidation, damage of seminal parameters (sperm motility, viability, morphology), and poor pregnancy and artificial reproduction outcomes. Nevertheless, non-diagnostic and therapeutic methods have been developed to combat infertility and oxidative stress. However, profound evidence to recommend a suitable oxidative stress test is still lacking. Further research is required to overcome the current limitations.

Declaration of interest
RH is employed at LogixX Pharma. The other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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N B Takalani contributed to conceptualization, drafted the manuscript, and created the tables; C S Opuwari contributed to conceptualization, manuscript writing, and editing and provided supervision. T K Monsees, R Henkel, E Monaneng, and K Mohlala contributed to manuscript editing.

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