The Impact of Oxidative Stress on Male Infertility

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Reactive oxygen species, also known as oxygen and oxygen-derived oxidants, cause cellular damage at a higher rate under the term "oxidative stress" (ROS). Oxidative stress is typically present as a background factor in all live aerobic cells. This oxidative process can be accelerated and lead to cell damage by several stress-related situations, including chronic disease states, aging, toxin exposure, physical injury, and exposure to a variety of foods. One of the main contributing factors to infertility is thought to be the malefactor. A new and significant reason, oxidative stress, has been discovered in addition to the traditional causes of male infertility, such as varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma, and tumors. Reactive oxygen species (ROS) and antioxidants in the body are out of balance, which leads to oxidative stress. This potent mechanism can cause damage, deform, and ultimately result in male sterility in sperm. The physiological significance of ROS, their function in healthy sperm function, and the pathophysiology of ROS in the male reproductive system are all covered in this book.

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The Impact of Oxidative Stress on Male Infertility

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List of contents:

Abstract:

Reactive oxygen species, also known as oxygen and oxygen-derived oxidants, cause cellular damage at a higher rate under the term "oxidative stress" (ROS). Oxidative stress is typically present as a background factor in all live aerobic cells. This oxidative process can be accelerated and lead to cell damage by several stress-related situations, including chronic disease states, aging, toxin exposure, physical injury, and exposure to a variety of foods. One of the main contributing factors to infertility is thought to be the malefactor. A new and significant reason, oxidative stress, has been discovered in addition to the traditional causes of male infertility, such as varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma, and tumors. Reactive oxygen species (ROS) and antioxidants in the body are out of balance, which leads to oxidative stress. This potent mechanism can cause damage, deform, and ultimately result in male sterility in sperm. The physiological significance of ROS, their function in healthy sperm function, and the pathophysiology of ROS in the male reproductive system are all covered in this article.

Keywords: Antioxidants, Male infertility, Oxidative stress, Sperm, ROS.

Introduction:

Medically and psychosocially, infertility is a serious clinical issue that affects many people. Oxidative stress (OS) has been receiving considerable attention recently because it has been recognized as one element that influences reproductive status. One of the main reasons for male infertility is oxidative stress, which is defined as an imbalance in the amounts of reactive oxygen species (ROS) and antioxidants. For sperm to operate physiologically, especially for capacitation, hyperactivation, and acrosomal response, a tiny level of ROS is required. Yet, large amounts of ROS can also result in infertility by inactivating enzymes and oxidizing proteins in spermatozoa, in addition to lipid peroxidation and DNA damage. By World Health Organization **[1]** recommendations, when an "alteration in sperm concentration, motility, and/or morphology is observed in at least one sample of two sperm analyses, collected 1 to 4 weeks apart," the male factor is the factor that causes infertility **[2].** The problem is exacerbated when there is no obvious cause. Oxidative stress (OS) is currently considered to be a substantial and possible contributing factor to male infertility. "OS is a situation that represents an imbalance between a biological system's ability to rapidly detoxify (antioxidant defenses) the reactive intermediates or to repair the ensuing damage" **[3,4].** Pro-oxidants and antioxidants coexist in harmony in a healthy organism. Spermatozoa feature antioxidative mechanisms and are thought to dampen ROS, preventing oxidative damage to mature spermatozoa and gonadal cells **[5].** Under pathological circumstances, unchecked ROS generation outpaces the seminal plasma's antioxidant activity, leading to OS **[5,6].** Since physiological quantities of reactive oxygen species (ROS) are required to maintain proper cell function, oxygen is crucial for maintaining life. On the other hand, oxygen breakdown products such as ROS can be harmful to cell survival and function **[7].** Free radicals, or

reactive oxygen species, are present. ROS are extremely reactive oxidizing agents that fall under the category of free radicals. Any substance (not necessarily produced from oxygen) with one or more unpaired electrons is referred to as a free radical. Superoxide (02 -) anion, hydrogen peroxide (H202), peroxyl (R00) radicals, and the extremely reactive hydroxyl (OW) radicals are the most prevalent ROS that may have an impact on reproductive biology **[8].** It has been established that increased ROS production is a component of the suggested mechanism for the loss of sperm function caused by oxidative stress **[9].** H202 can affect sperm in both positive and negative ways, which can affect the process of fertilization. Nitric oxide (NO-), a free radical produced from nitrogen, also seems to be important for reproduction and fertilization as an intermediary in many number of physiopathological events, including the control of vascular tone, neurotransmission, apoptosis, and inflammatory processes **[10].** NO's concentration and interactions with H202 determine its final effects. As a result, free radicals and ROS are linked to oxidative stress and probably have a variety of important and varied roles in reproduction. Vitamin C, vitamin E, pyruvate, glutathione, and carnitine are just a few of the nonenzymatic antioxidant compounds found in semen **[11].** As the cytoplasm is extruded during spermiogenesis, these antioxidants make up for the loss of sperm cytoplasmic enzymes, which reduces endogenous repair mechanisms and enzymatic defenses **[12].** The evaluation of such OSS and the involvement of antioxidants may aid in the medical therapy of this male factor infertility. To better understand how ROS affect human spermatozoa, they can be artificially produced under predetermined experimental settings. Superoxide (O2) and hydrogen peroxide (H202), respectively, are produced when xanthine and xanthine oxidase combine, resulting in the univalent and divalent reduction of dioxygen. These

radicals produce the extremely reactive hydroxyl radical (OH-), which is particularly harmful to spermatozoa when ferric ions are present. Under specific circumstances, the electrolysis of physiological buffer also produces ROS, which might impair sperm motion **[13].**

Role of ROS and Antioxidant System in Male Fertility

Oxidative stress is a significant cause of male infertility due to detrimental alteration during spermatogenesis, epididymides maturation, and sperm capacitation, which can lead to infertility **[14].** The hormone-controlled process of spermatogenesis results in the production of spermatozoa in the testes. The crucial stage for achieving motility, complete maturity, and the ability to fertilize occurs in the epididymis **[14,15].** Spermatozoa are physiologically exposed to ROS at this period, which are also engaged in physiological processes such as sperm capacitation and acrosome response. They demand a lot of energy, which is produced by metabolic processes like glycolysis or oxidative phosphorylation, and are essential for effective fertilization (OXPHOS). Spermatozoa can bind to the oocyte's zona pellucida through several cellular events known as capacitation. This causes the acrosome reaction, which results in the production of proteolytic **[14].** Another factor to consider is the high concentration of polyunsaturated fatty acid in sperm membranes, this ensures the flexibility needed for fertilization. Due to PUFA's vulnerability to lipid peroxidation (LPO), spermatozoa are at risk from this high concentration of PUFA **[15].** The oxidative damage that can happen, which is connected to the loss of membrane lipids, mitochondrial dysfunction, change of morphology, decrease in vitality, as well as other disturbances **[16],** can result in the loss of implantation. Likewise, because haploid and very compressed nucleus no longer transcribes at all, the structure of sperm cells cannot react to stress stimuli. At the last phases of spermatogenesis, the most of spermatozoa's cytoplasmic, which is the primary antioxidant source, has been eliminated **[17].** Hence, DNA takes up most of the tiny amount of cytoplasm in spermatozoa **[15].** Although healthy levels of ROS are required to control spermatic activities, an excessive amount can overcome the antioxidant

defenses that protect spermatozoa. The antioxidant system in the seminal fluid, which is responsible for maintaining normal cellular activity, is made up of enzymatic and non-enzymatic components that work in concert to provide the best possible defense against ROS. The enzyme trinity of glutathione peroxidase (GPx), catalase (CT), and superoxide dismutase (SOD) plays significant roles in these variables (GSHPX) **[14,18].** SOD is a metalloprotein that acts as a catalyst for superoxide anion dismutation activities and is particularly important for protecting PUFA, which are cytoplasmic membrane components, as well as for DNA fragmentation **[15].** Although the majority of the enzymatic activity is concentrated in the cytoplasm of the cells, it can carry out its activities both outside and within the cell **[20].** Hydrogen peroxide is converted into water and molecular oxygen by the enzyme catalase (CT). It is distinguished by the heme system, which has an iron atom at its center. Its action has been discovered in a variety of organelles, including peroxisomes, mitochondria, endoplasmic reticulum, and the cytoplasm of many cell types **[20].** The sperm antioxidant system also includes glutathione peroxidase (GSHPX), whose active site contains selenocysteine. In general, this enzyme is responsible for reducing organic and hydrogen peroxides **[20].** There are three isoforms: nuclear, mitochondrial, and cytosolic. Particularly, sperm quality and motility depend on the mitochondrial isoform **[19].** Cell death happens when the antioxidant system fails to stop the excessive rise in ROS. Consequently, ROS play a dual role: physiologically, they help spermatozoa mature and/or fertilize, but when they are produced in excess, they can be detrimental to cell structures, functions, and survival **[16].**ROS levels may rise as a result of both endogenous and external factors producing it. The ejaculate of fertile males often contains a variety of cell types, including mature spermatozoa, immature sperm cells, leukocytes, and

epithelial cells **[21-23].** The main endogenous sources of ROS among these are leukocytes (especially neutrophils and macrophages) and defective or immature spermatozoa **[22,24,25].** The levels of ROS in the seminal fluid are also modulated by several environmental and lifestyle factors. For instance, there are more than 4,000 chemical components in tobacco smoke, many of which are ROS and reactive nitrogen species (RNS). This ROS and RNS of tobacco smoke result in greater rates of DNA sperm fragmentation and axoneme damage as well as a lower concentration of spermatozoa in semen **[26-28].**

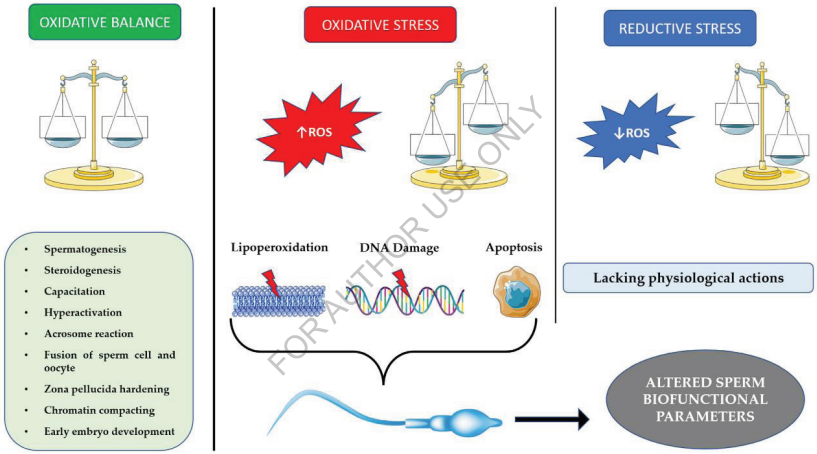


Figure 1. Left panel: redox homeostasis with positive effects of ROS; central panel: oxidative stress situation due to increased ROS production and consequential oxidative damages; right panel: reductive stress situation with low ROS levels and consequential detrimental effects.

Reactive oxygen species function

When present in the right amounts, ROS have a significant physiological impact, controlling processes such as fertilization, acrosome response, hyperactivation, motility, and capacitation **[21,27,29,30].** When ROS levels are elevated, they harm spermatozoa's biomolecules, such as lipids, proteins, and DNA.Polyunsaturated fatty acids (PUFA) are abundant in the Sperm plasma membrane, making it susceptible to ROS oxidation **[27,31,32].** The peroxidation of phospholipids encourages changes in membrane fluidity, which alters the characteristics of sperm motility. Malondialdehyde (MDA), a consequence of lipid peroxidation, is frequently examined in labs to assess the peroxidative damage to spermatozoa **[27,33,34].** In human spermatozoa, ROS can also fragment DNA bases and phosphodiester backbones, causing mitochondrial and nuclear DNA damage **[35,36].**With the activation of the apoptotic process, ROS also causes a reduction in the number of sperm cells. Because of the release of cytochrome c and the apoptosis-inducing factor (AIF) into the cytosol and the subsequent activation of caspases, changes in the fluidity of the mitochondrial membrane cause apoptosis **[37,38].**Protein oxidation, which impairs structural protein function or enzymatic activity, is also brought on by ROS. Although the oxidized proteins are inactive and rapidly eliminated, some may accumulate in the body and cause several diseases **[21,28,39].**Nitric oxide at high concentrations can cause protein nitration in tyrosine residues. This procedure results in the creation of 3-nitrotyrosine by adding a NO2 group to the third position of either free or protein-bound tyrosine (3-NT). In the presence of CO2, the reactions between nitric oxide and superoxide anion (O2•) result in the highly reactive peroxynitrite (ONOO). This

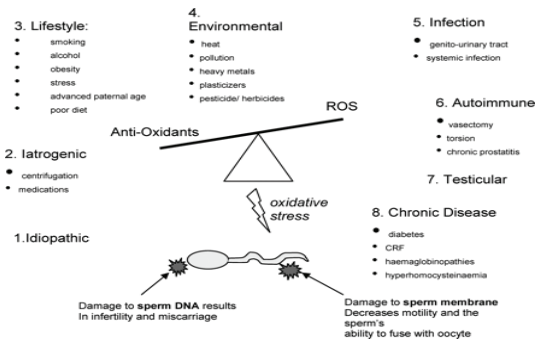
chemical reacts with proteins to produce 3-NT or S-nitrosylation **[40,41,42].**

Reactive Oxygen Species and Seminal oxidative Stress

Little levels of ROS produced by spermatozoa are crucial for numerous functions of sperm **[43,44].** To keep only a small amount required to keep cells functioning normally, ROS must be continuously inactivated. Due to its unique structural makeup, excessive ROS production in semen can damage spermatozoa. The main source of antioxidants is the cytoplasm that the spermatozoa release throughout the maturation phase. A cytoplasmic droplet forms in the middle of the sperm when this process is slowed down. These spermatozoa having to carry cytoplasmic droplets are thought to be immature as well as functionally defective **[45].** Some cytoplasmic enzymes (G6PDH, SOD), which are also a generator of ROS, are concentrated in high concentrations in the remaining cytoplasm [46]. Reduced antioxidant defense is the outcome of cytoplasm deficiency. Poor sperm quality and high ROS are related to this mechanism.Leukocytes, epithelial cells, mature and immature spermatozoa, round cells from various phases of the spermatogenic process, and other cell types can all be found in the human ejaculate. They include defective spermatozoa that continuously create free radicals and peroxidase-positive leukocytes **[47,48].** Due to the high concentration of polyunsaturated fatty acids (PUFA) in their plasma membranes, which are easily subjected to lipid peroxidation by ROS, spermatozoa are also particularly vulnerable to the harm brought on by excessive ROS **[49,50].** In sperm, there are primarily two methods for producing ROS. The nicotinamide adenine dinucleotide-dependent oxidase system in the sperm plasma membrane and the NADH-dependent oxidoreductase (diaphorase) system in the mitochondria **[51].** Immature spermatozoa exhibit a substantial positive connection with ROS generation, which is inversely linked with sperm quality **[52,53].**

Oxidative Stress and its Effect on Sperm Motility

When present in excess, seminal ROS levels can have deleterious consequences on sperm quality and function **[55,56].** Reduced sperm motility, faulty acrosome reactions, and lower fertility have all been linked to increased seminal ROS generation **[56].** The kind, volume, and length of ROS exposure all affect how sperm cells behave as a result of ROS damage. The quantities of molecular components including ions, proteins, and ROS scavengers as well as environmental variables like oxygen tension and temperature can affect how much damage is caused by ROS **[57].**Low hydrogen peroxide concentrations do not affect sperm motility, but they do inhibit human sperm competence during oocyte fusion, according to Aitken et al **[58].** It's possible that ROS levels are not high enough to impact the typical seminal parameters, but they can lead to errors in other fertilization-related procedures, like sperm-oocyte contact. These results point to a possible explanation for the phenomenon of idiopathic infertility in patients with normal semen characteristics. A chain of events, including lipid peroxidation (LPO) of the sperm plasma membrane, which ultimately affects the phosphorylation of the axonemal protein and results in sperm immobility, is the cause of decreased motility **[59].**



Role of Oxidative stress in Sperm DNA Damage as Related to Male Infertility

The fluidity of the sperm plasma membrane and the integrity of the DNA in the sperm nucleus are both negatively impacted by the overproduction of ROS in the reproductive tract. Oxidative damage to DNA bases can lead to base modification, strand breakage, and chromatin cross-linking. DNA damage brought on by oxidative stress results in pro-mutagenic transformation, which in its most severe form degrades the germ line's quality and inhibits conception. When there is less oxidative damage, fertilization can proceed, but the oocyte must repair the DNA strand breaks before the first cleavage begins. DNA damage in the germ line is mediated via apoptosis and OS **[60].** Due to its genetic makeup and inability to repair double-stranded DNA deletions, the Y chromosome is particularly susceptible to DNA damage. Whereas infertile men, especially those with defective seminal parameters, have a higher fraction of sperm DNA damage, fertile healthy men with normal seminal parameters nearly always have minimal levels of DNA breakage **[61].** Idiopathic infertile men may have normal seminal parameters (concentration, motility, and morphology) along with defective DNA integrity **[61-63].** The fact that the most effective ART methods for treating male factor infertility have a high level of sperm DNA damage is extremely concerning. To lower the possibility of introducing spermatozoa with strand breaks during ICSI, it is always preferable to use spermatozoa with normal morphology **[64].** This isn't always the case, though, as research has shown that standard sperm metrics including sperm count, motility, and shape aren't necessarily associated with the presence of DNA damage **[65,66].** Furthermore, this has important therapeutic ramifications since employing spermatozoa with DNA damage for in vitro fertilization may result in the paternal transmission of

defective genetic material, which would be harmful to embryo development. These results imply that calculating the percentage of DNA-damaged spermatozoa in fertile and infertile men may be significant and that methods for identifying and choosing spermatozoa with intact DNA during IVF/ICSI operations will need to be developed. Sperm from infertile males with varicoceles have recently been linked to very high levels of DNA damage **[67].** The discovery of increased seminal OS in varicoceles patients may suggest that OS plays a significant role in the aetiology of sperm DNA damage in these patients. While Zini et al. found that varicocelectomy can enhance human sperm DNA integrity in infertile males with clinical varicoceles **[68].** Studies on potential remedies to minimize sperm DNA damage are few and far between. Avoiding gonadotoxins has been recommended for therapeutic purposes **[69].** (Smoking, medications) and hyperthermia **[70].** (Saunas, hot tubs) might reduce sperm DNA damage. Based on the data that leukocytospermia generates ROS production and possibly DNA damage, treatment of GU infection may also be beneficial **[71].** According to studies, oral antioxidants given over a short period may be able to lessen sperm DNA damage **[72].** These suggestions, however, have been based on tiny, uncontrolled research, and no treatment for abnormal DNA integrity has yet been demonstrated to have positive clinical outcomes **[63].**

Internal Sources of Reactive Oxygen Species

The unpredictability and dependability of leukocyte detection methods contribute to the debate about the contribution of leukocytospermia to sperm quality. Traditional sperm staining methods such as Papanicolaou and Giemsa staining cannot reliably distinguish between immature germ cells and WBC, causing misunderstanding between spermatocytes and lymphocytes or monocytes **[73].** Immunocytological detection can be

used to identify all WBC types using a monoclonal antibody against CD45. Nonetheless, this method might overstate seminal WBC counts **[73].** On the other hand, because peroxidase staining does not reliably detect lymphocytes or monocytes, cytochemical detection using this dye can overestimate the quantity of WBC present **[74].** The seminal leukocytes' activation status, which can lead to increased production of pro-inflammatory cytokines, elevated levels of reactive oxygen species, and sperm damage, may also play a role in the varying relevance of leukocytospermia on sperm quality. Some investigators have found a link between high levels of seminal ROS or proinflammatory cytokines, such as interleukin IL-6, IL-8, and tumor necrosis factor (TNF)-, and impaired sperm function **[75-81].** For instance, it has been demonstrated that IL-6 is essential for causing sperm capacitation and the acrosomal response **[77].** Nevertheless, higher IL-6 and cell membrane lipid peroxidation have been linked to sperm damage when large quantities of cytokines are present in the semen **[76-78].** Moreover, elevated IL-6 levels have been found in the seminal fluid of infertile men **[75].** Moreover, it has been demonstrated that IL-1, IL-6, IL-8, and TNF all increase the formation of ROS, which increases the lipid peroxidation of the sperm cell membrane **[75,76,79].** The variable levels of sperm dysfunction in males with comparable quantities of seminal leukocytes may be explained by the greater amount of leukocyte activation resulting in enhanced oxidative stress.

Autogenous Generation of ROS by Sperm

Reactive oxygen species (ROS) can also be produced by spermatozoa without the help of leukocytes **[80],** and the amount of sperm maturity affects the sperm's capacity to produce ROS. Before the germinal epithelium is released during spermatogenesis, the cytoplasm is often extruded from the spermatozoa. The enzyme glucose-6-phosphate

dehydrogenase, which produces NADPH, is found in large concentrations in cytoplasmic residues. The sperm membrane's NADPH oxidase then produces reactive oxygen species **[81,82].** Increased levels of retained cytoplasmic residues in semen may result from abnormalities in sperm maturation, which could then enhance the formation of seminal ROS and cause further harm to the sperm.Although spermatozoa and leukocytes both create ROS, the amount of ROS produced by each cell type varies substantially. According to some reports, leukocytes create 1000 times more ROS than spermatozoa that are capacitating **[81,83].** These discrepant levels appear to point to leukocytes as the causative agents of oxidative injury, but additional data suggests that location is just as important as a concentration in the oxidative stress-induced harm to sperm. For instance, Henkel et al. looked at the effects of ROS produced by leukocytes or spermatozoa on the general quality of sperm. Extrinsic ROS production refers to levels of ROS produced by seminal leukocytes, whereas intrinsic ROS production refers to levels of ROS produced by spermatozoa. Measures of sperm quality, such as DNA fragmentation and sperm motility, counts, and morphology, were compared to the sources and concentrations of ROS generation **[82].** Extrinsic ROS are more harmful to external spermatic structures than intrinsic ROS, which can significantly harm sperm DNA at low levels. This can influence sperm motility, counts, and morphology.

External Sources of ROS

Many environmental, viral, and lifestyle factors can make ROS production worse. It has been demonstrated that a variety of waste chemicals and industrial byproducts have a deleterious impact on male infertility, both directly and indirectly. It has been suggested that the

growing environmental presence of these production byproducts poses a severe hazard to the reproductive health of people all over the world.

Lifestyle

To the best of our knowledge, several lifestyle factors are recognized as having a significant impact on human health. Much research on lifestyle and male subfertility has been conducted. Because of an imbalance between ROS generation and the antioxidants' defending function, oxidative stress is thought to harm male fertility. Increased ROS production is more likely to be the cause of the oxidative stress found in the semen of infertile men that reduced antioxidant levels **[84].**

Industrial compounds

Numerous groups have looked into how these substances affect fertility because they have a significant impact on both human and environmental health. For instance, the chemical phthalate is present in a variety of polymers used in food packaging as well as beauty goods, and exposure to this chemical can happen orally, topically, or inhaled. Phthalate exposure has been linked to decreased spermatogenesis and DNA damage in sperm **[85,86].** Heavy metals like cadmium and lead have been demonstrated to cause testicular oxidative stress **[87,88],** as well as those in the paint and battery industries, have a higher incidence of infertility and miscarriage **[89,90].** Throughout the manufacturing process, men working in these fields may be exposed to heavy metals by inhalation and skin absorption. Heavy metal testing and proactive action advice should be given to patients working in industries with high exposure concerns.In rat models, lindane, methoxychlor, and the herbicide dioxin-TCDD have all been connected to an increase in testicular oxidative stress **[91–93].** Sulfur dioxide, a nearly universal dietary preservative, has been observed

to directly increase intratesticular oxidative stress **[94],** and diesel exhaust particulates have been demonstrated to potently activate leukocyte ROS generation **[95,96].**

Cigarette Smoking

Smoking causes significant levels of oxidative stress, which directly raises seminal leukocyte concentrations and ROS generation **[97-101].** While lowering seminal levels of the antioxidant enzyme SOD **[102].** Males who smoke had lower sperm counts, motility, and morphologically normal sperm **[99-102].** Vine discussed a meta-analysis of 27 research looking at the relationship between smoking and the quality of semen. Although the bulk of these investigations were conducted on healthy men as opposed to infertile men, reductions in sperm concentrations and motility were observed in the majority of them **[100].** Smoking has been shown to lower levels of the antioxidants Vitamin C and E found in seminal plasma **[99-101],** which lowers the ability of spermatozoa and seminal fluid to scavenge free radicals.Europeans are the biggest tobacco consumers, accounting for around one-third of daily users worldwide **[103-105].** Nicotine, hydroxy cotinine, alkaloids, cadmium, cotinine, lead, and other carcinogenic chemicals are all present in large quantities in cigarette smoke. ROS is elevated and antioxidant levels are decreased as a result of these chemicals' effects on the oxidative/antioxidant balance **[103,106,108].**A recent meta-analysis of several publications found a significant negative impact of smoke on sperm characteristics such as sperm count, motility, and morphology. This study used the WHO 2010 methodologies for sperm analysis. Although there has been no discernible change in semen volume, those adverse effects are particularly prominent in infertile men. Smoking cigarettes has harmful side effects that are dose-dependent. Smokers who smoke moderately (10–20 cigarettes per

day) and heavily (>20 cigarettes per day) will experience different effects on the quality of their semen **[103].**Due to an excess of ROS in human spermatozoa, smoking also damages DNA. It can disrupt the histone-to-protamine transition, disrupt the expression of micro-ribonucleic acids (miRNAs), and inhibit protein phosphorylation. All of those do significantly affect gene regulation, complicating fertilization **[108,109].** In addition to the studies on humans that we have already covered, there are also significant insights into this subject from studies on animals **[110].** Consuming cigarette smoke also increases oxidative stress in the testes and contributes to lipid peroxidation. In a 2014 study by Oyeyipo et al., rats were given nicotine orally for 30 days, and the results showed higher testicular lipid peroxidation and increased oxidative stress. Moreover, lowered testicular antioxidant levels have been found **[111].** Both show that increased ROS generation in rats causes a decrease in spermatogenesis. Adenosine triphosphate, a chemical energy source, is produced by mitochondria in the middle of spermatozoa (ATP). Sperm motility is significantly influenced by ATP. Increased lipid peroxidation was linked to a decrease in ATP levels, as demonstrated by Gogol and his colleagues **[112].** Smoking males had decreased CK activity in their spermatozoa, which has the potential to influence sperm energy homeostasis and particularly sperm motility **[113].** As a result, smoking can be considered a cause of male infertility indirectly.Smoking causes a persistent inflammatory reaction that has been linked to an increase in seminal leukocyte concentrations of 48% and an increase in reactive oxygen levels of 107% **[104].** Proinflammatory leukocytes are drawn into the semen plasma by the inflammatory reaction brought on by smoking. It connects to male subfertility since leukocytes have been identified as one of the major producers of ROS **[114].**In addition to traditional smoking, new delivery methods for tobacco and its derivatives are emerging and

are being used more frequently, such as e-cigarettes. Although the majority still include nicotine, those do contain different ingredients than traditional cigarettes. A cross-sectional research of men from the general population discovered e-cigarette use had an impact on semen plasma. Sperm count and concentration have been found to have significantly decreased **[105].**

Alcohol intake

Excessive alcohol consumption has been demonstrated to raise systemic levels of oxidative stress, and the low-nutrient diet that typically goes along with excessive alcohol consumption might increase the effects of this oxidative stress **[115].**In the western world, drinking alcohol is very common **[116].** Nothing is known regarding the direct pathways of alcohol-induced oxidative stress as a cause of male subfertility. Acetaldehyde is created when ethanol is metabolized, which releases free radicals into the body. The ethanol metabolism has been connected to changes in ROS generation and the antioxidant system **[117].** According to evidence in the literature, drinking alcohol increases the body's generation of ROS, particularly in the liver. In addition, researchers hypothesize that drinking alcohol increases the creation of ROS, which affects male infertility **[118].**Alcohol use and oxidation were found to have an impact on semen in two animal experiments. There have been discovered to be changed in the membrane and lipid peroxidation end products, such as MDA on testicles **[119].** These are consistent with studies that demonstrate elevated levels of lipid peroxidation and testicular antioxidants **[118].** These elements show that ethanol causes oxidative damage within the testis.Alcohol use is linked to modifications in semen properties. A recent meta-analysis of 15 cross-sectional studies were included evaluate alcohol intake in various ways. Even after modest

alcohol consumption (4–7 units/week) **[120].** Rats given alcohol showed a substantial decrease in sperm motility and sperm concentration in their testicles **[118].** The discrepancy between the researches could be caused by different classifications, variations in alcohol intake intensity, or other aspects of lifestyle.

Exercise-induced oxidative stress

It's interesting to note that significant amounts of oxidative stress can be produced by both inactivity and intense exercise. According to certain studies **[121–124],** long-term, intense exercise training may suppress testosterone levels or affect the hypothalamic–pituitary–testis axis, which is important in reproduction. Because testosterone is essential for the growth and maturation of sperm during spermatogenesis, its decline may result in lower-quality sperm. For instance, Manna et al. has shown in a mouse model that increasing levels of exercise are associated with poorer sperm quality and testicular function, including lower sperm counts, lower testosterone levels, and increased testicular oxidative stress. Additionally, the scientists discovered that giving the exercising rodents daily doses of alpha-tocopherol protected these declines in sperm quality and testicular function **[123].** The association between high exercise and sperm quality in people has not yet been described, with the majority of studies studying extreme levels of exercise in humans concentrating on increases in systemic oxidative stress **[122,124].**

Psychological Stress

Any culture experiences psychological stress, a sense of emotional pressure, and strain. It has also been connected to male subfertility and elevated levels of cortisol, adrenaline, and norepinephrine (NE) **[125].** A study revealed negative impacts on semen parameters **[126,127].** In healthy medical students taking exams, two prospective studies have

connected psychological stress to sperm quality. Students having additional stressors beyond the exam period were excluded from the study, which examined their psychological stress using the widely used State-Trait Anxiety Inventory questionnaire. Eskiocak et al. (2005) demonstrated decreased antioxidant levels and sperm quality **[128].** Compared to three stress-free months after the assessment period, sperm motility has decreased. Higher levels of nitric oxide, a highly reactive radicals free, they determined in another study with a comparable technique, which revealed considerably decreased sperm motility, sperm concentration, and seminal plasma arginase activity. Both studies showed reduced sperm effectiveness, which has been brought on by an imbalance in antioxidant and ROS levels **[128,129].** Also, among healthy males who had more than two recent stressful life events, sperm concentration, motility, and morphology declined **[126].** This outcome is in line with a cross-sectional study of Danish men who self-reported having psychological stress due to idiopathic subfertility **[130].**According to recent studies, meditating and practicing yoga reduce reactive oxygen species (ROS) levels as well as nuclear and mitochondrial DNA damage, which enhances sperm motility and count **[131,132].** With declining cortisol levels, anti-inflammatory cytokines, cell cycle control, and modulation of the immunological response, mind-body exercises like yoga and meditation have been associated with lower levels of psychological stress **[106,131,132].** A Danish cross-sectional research of 953 healthy men identified a connection between sleep disruptions and sperm count, morphology, and concentration **[133].** A recent study looked at the reproductive health of men who were experiencing "circadian desynchrony," or operating inconsistently with their biological clock. They discovered a connection between rotating shift employment and reduced sperm levels. They also discovered adverse impacts on sperm

count from non-work-related factors, such as prolonged mobile phone use **[134].**

Diet:

Data supports the significance of nutrition, its impact on male fertility, and its influence on the level of oxidative stress **[135-137].** Throughout the past few decades, society's eating habits have undergone a significant shift. The 'Western diet' is consumed by the majority of developing and developed nations. This diet is characterized by high-energy carbohydrates, hydrolyzed and trans fatty acids, omega-6 polyunsaturated fatty acids, processed foods, and low intakes of omega-3 polyunsaturated fatty acids, vegetables, fruits, and essential minerals. Higher levels of oxidative stress are linked to this unhealthy hypercaloric diet **[135].** A study found a substantial dose-response relationship between dietary saturated fat intake and a decline in sperm concentration and sperm count **[136].** In comparison to men in the lowest quartile of saturated fat intake, those in the highest quartile had a lower sperm concentration and a lower total sperm count. Also, it has been discovered that overweight and obese subfertile males have decreased sperm concentration and motility as well as an increase in DNA damage, which explains the association between BMI and oxidative stress **[137].** So, maintaining the equilibrium between the amounts of ROS and antioxidant defense levels may require a balanced diet. According to Oostingh et al. (2017), there is a link between men's semen quality and their commitment to a healthy diet. Increased sperm concentration, sperm count, and gradually greater motility were detected in a healthy diet pattern group, particularly in men with a total motile sperm count of 10 million spermatozoa, suggesting a larger effect in subfertile individuals (>10 million spermatozoa are considered normal). Consuming fewer dairy products, sweets, and saturated fats

while increasing the intake of fruits, vegetables, and healthy unsaturated fats is considered to be a healthy diet pattern **[138].** The consumption of fruits and vegetables is inversely related to spermatozoa DNA damage, according to a cross-sectional study of 161 infertile males **[139].**Antioxidant supplementation appears to reduce DNA damage and is linked to improved semen quality **[135].** As a defensive and preventive mechanism against oxidative stress, antioxidants are essential **[140].** The majority of antioxidants are found in the semen, not in the spermatozoa **[141].** Zinc, vitamins C and E, carnitine, folic acid, and N-acetylcysteine are the antioxidant supplements that are most widely utilized, both separately and in combination **[135,138].** Infertile men's semen contained less zinc and selenium than men with normal fertility, according to Nenkova et al study **[142].**

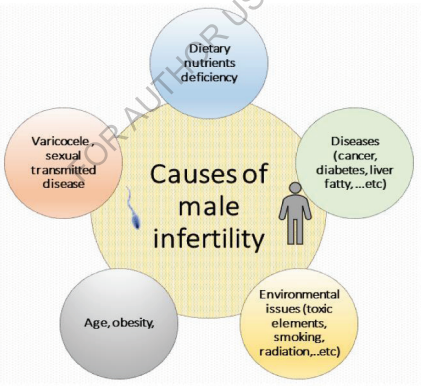


Figure 2. The main causes of human male infertility associated with numerous issues and diseases (cancer, diabetes, liver fatty, varicocele and sexually transmitted diseases), age, obesity, environmental and lifestyle issues (toxic elements, smoking, radiation), and dietary nutrient deficiency.

Elevated temperatures

Some studies have shown that elevated intrascrotal temperatures decrease sperm motility and count while increasing sperm DNA damage

[143,144]. It has been proposed that elevated intrascrotal temperatures may also contribute to low sperm quality. For instance, Mieusset al. et al. examined scrotal temperatures, testicular volumes, and sperm qualities in non-azoospermic, infertile men compared to those who were fertile. 30% of infertile men had scrotal temperatures that were +0.5°C higher than those of fertile men when measured through scrotal skin contact **[145].** Carlsen et al. followed 27 healthy males with monthly semen samples and noted the occurrence of any febrile episode over 16 months **[146].** They found a correlation between hyperthermia and poor semen quality. The findings show a 35% fall in sperm concentration, a 7% loss in normal morphology, and a 20% increase in immotile sperm after fever episodes. Moreover, these parameters got worse as the duration of the febrile episodes got longer. Tiemessen et al. investigated the relationship between underwear style and intrascrotal temperatures for one year and tight underwear for six months with semen samples analyzed every two weeks. They proposed that scrotal hyperthermia caused by tight-fitting underwear may result in decreased sperm parameters **[147].**

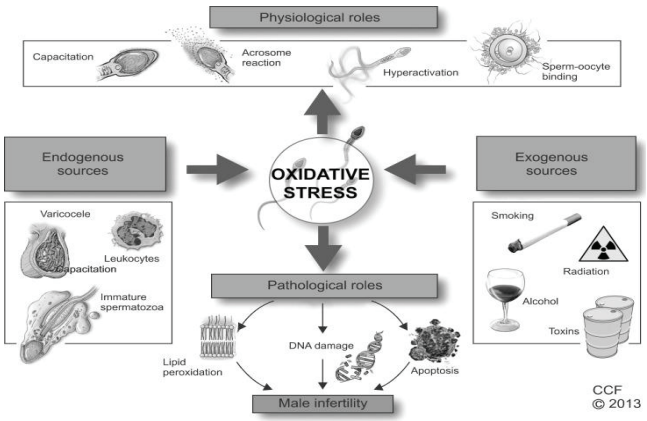


Figure 3. Oxidative stress in male reproduction.

Pathological Roles of Reactive oxygen species

Pathological abnormalities develop when the highly strong ROS overpowers the antioxidant defense mechanisms and upsets the delicate balance between ROS and antioxidants. Biomolecules such as lipids, proteins, nucleic acids, and carbohydrates are significantly damaged by these defects, depending on the type, quantity, and duration of the ROS insult **[148].**

Lipid peroxidation

The fluidity of membrane layers and the alterations that take place during capacitation in the female reproductive tract are caused by lipids **[149].** In terms of lipid composition, the plasma membrane of mammalian spermatozoa differs significantly from that of mammalian somatic cells. High concentrations of PUFA-containing lipids can be found in the plasma membrane. These lipids have double bonds that are unconjugated and are divided by methylene groups. The methyl carbon-hydrogen connection is weakened by the presence of a double bond close to a methylene group, rendering hydrogen very vulnerable to abstraction and oxidative damage. When the amount of ROS in the cell is large, ROS will target PUFA and set off a series of chemical processes known as LPO **[150].** DHA, which has makes up around 50% of the fatty acids in human spermatozoa. Spermatogenesis and membrane fluidity are thought to be significantly influenced by DHA **[151].** During the LPO cascade in the sperm, over 60% of the fatty acid is lost from the membrane, which affects how well it works by lowering its fluidity, increasing its non-specific permeability to ions, and inactivating membrane-bound receptors and enzymes. Knowing how LPO works, which can be boiled down to only three fundamental processes: initiation, propagation, and termination

[152]. Free radicals are created during initiation as a result of the abstraction of hydrogen atoms linked to carbon-carbon double bonds. These free radicals interact with fatty acid chains to create lipid radicals, which subsequently combine with oxygen to create peroxyl radicals. An autocatalytic chain reaction is triggered by these peroxyl radicals, which can remove hydrogen from lipid molecules, especially when copper and iron are present. Lipid peroxides are finally produced when the radicals combine with hydrogen **[153].** The propagation stage is characterized by this reaction. By acting on other lipids, these radicals cause the hydroperoxide to degrade, producing deadly aldehydes. During the third phase of termination, peroxyl and alkyl radicals are regenerated in a cycle until they interact with another radical to create a stable end product called malondialdehyde (MDA). As a result, MDA is utilized in biochemical arrays to track the severity of spermatozoa peroxidative damage **[149,154].** Low-density lipoproteins also produce 4-hydroxynonenal, another consequence of LPO. Since they are hydrophilic, hydroxynonenals can severely disrupt cell function at the genomic and proteomic levels **[155].**

DNA damage

The ability of sperm from an ejaculate to fertilize an egg is often assessed using semen parameters like concentration, motility, and morphology. This gives a broad picture of sperm quality, but it says nothing about DNA, which is one of the most crucial factors in the success of reproduction. DNA breaks that are single- or double-stranded may be the reason why infertile men and fertile men have different reproductive potential **[156].**

Human spermatozoa have highly ordered and compacted chromatin. This is then wound into a solenoid and packaged into nucleosomes. The sperm chromatin goes through some changes during the spermiogenesis process, including the replacement of histones with transition proteins and then protamines. The protamines compact DNA strands to create the toroid, which is the fundamental component of sperm chromatin packing. Sperm chromatin is particularly resistant to DNA damage because of the DNA compaction and structure that helps protect it from oxidative damage **[157].** DNA is more susceptible to OS in specific situations where there is insufficient compaction and incomplete protamination of sperm chromatin, leading to base-free regions, deletions, frame-shift mutations, DNA cross-links, and chromosomal rearrangements. Human spermatozoa from the testicles, epididymis, and ejaculate have all been found to have damaged DNA **[158].** Single- and double-stranded DNA breaks can be found. Double-strand breaks can result from exposure to 4-hydroxyl-2-nominal, a prominent LPO product, while single-strand breaks are a direct outcome of oxidative damage to sperm DNA **[159].**The two main DNA adducts observed in human sperm DNA are 8-hydroxy-2-deoxyguanosine and two ethenonucleosides (1, N6-ethenoadenosine and 1, N6-ethenoguanosine), both of which have been identified as important biomarkers of DNA damage brought on by OS **[160,161].** Not with standing these findings, DNA damage during intrauterine insemination and in vitro fertilization (IVF) is not a cause for worry because the simultaneous LPO damage by ROS prevents conception. Yet, if normal natural selection is bypassed during intracytoplasmic sperm injection (ICSI), sperm with high quantities of DNA damage have the chance to fertilize the oocyte **[150].** Spermatozoa can self-repair DNA damage to some extent, allowing them to once again fertilize the oocyte and continue their development **[162].** In actuality, the oocyte is also able to

repair sperm DNA that has been damaged. The embryo may not develop or implant in the uterus and may naturally abort if the oocyte repair system is unable to repair Genetic damage. In some situations, the egg may be able to successfully repair sperm DNA strand breaks before the start of the first cleavage division, resulting in the production of healthy children. According to a survey, paternal origin accounts for 80% of structural chromosomal abnormalities in people **[160].** Apoptosis, low fertility, frequent miscarriages, and morbidity in progeny are all influenced by DNA damage **[163].**

Apoptosis

Apoptosis is another idea relating to sperm DNA damage and poor conception. Apoptosis, commonly referred to as programmed cell death, is a physiological phenomenon that involves cellular morphological and biochemical alterations that result in cells dying predictably **[150].** To control the ratio of germ cells to Sertoli cells during early development, apoptosis is crucial to the germ line's ontogeny. By inhibiting the overproduction of germ cells from seminiferous tubules in response to ROS, apoptosis plays a crucial function in adulthood in the selective destruction of the premeiotic spermatogonia during the first round of spermatogenesis **[152].** Human ejaculate expresses some apoptotic markers that start apoptosis during this process, some of which include Fas, phosphatidylserine (PS), Bcl-Xl, and p53. The Sertoli cells found on the surface of germ cells release Fas, a type I membrane protein that is a member of the tumor necrosis factor-nerve growth factor receptor family **[164].** The same study found that men with aberrant sperm parameters had up to 50% Fas-positive spermatozoa, which gives more support to this theory **[164].** Moreover, this apoptotic pathway causes the inner and outer mitochondrial membranes to release the signaling molecule

cytochrome C, which in turn activates caspases such as caspases 3 and 9 **[165].** Earlier work described the use of annexin-V labeling to examine the externalization of PS-a sign for early apoptosis. It was found that mature spermatozoa from infertile patients with elevated ROS levels had considerably higher levels of apoptosis than mature spermatozoa from the control group **[166].**

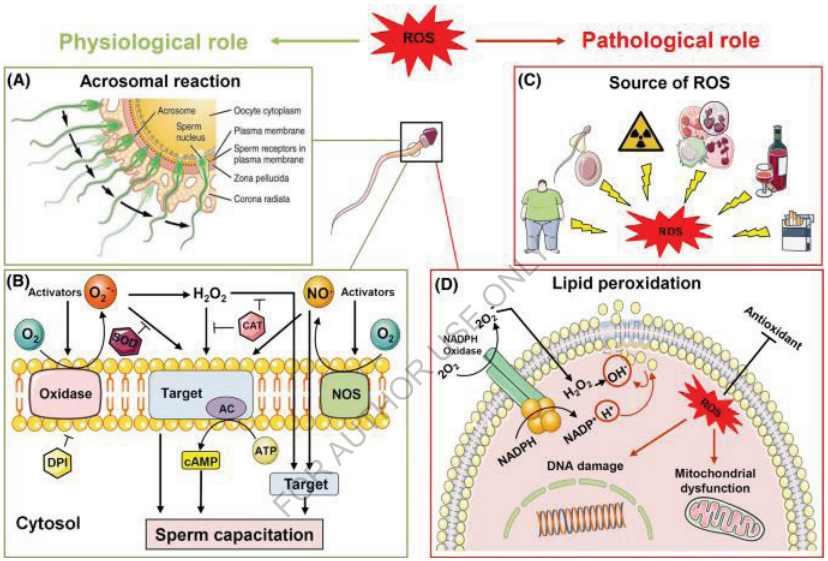


Figure 4. Oxidative stress and male infertility: pathophysiology and physiological role of antioxidant.

Antioxidants

Antioxidants can prevent or reverse the effects of ROS on specific pathways in sperm capacitation **[167].** Human seminal plasma contains antioxidants that scavenge for ROS as a protection and defensive mechanism **[168].** It is interesting that spermatozoa lack many antioxidant enzymes and rely on the antioxidant systems found in the semen for protection **[169].**Either enzymatic or non-enzymatic antioxidants are the fundamental compounds. Superoxide dismutase (SOD), catalase, and peroxidase are enzymes in the enzymatic group that catalyze the removal of ROS **[169].**Any form of ROS created by metabolic activity is scavenged and rendered inactive by the non-enzymatic groups, which are low molecular weight molecules **[170,171].** The most typical examples include glutathione, uric acid, and coenzyme Q10 **[172].** The non-enzymatic group can be further divided into hydrophilic and hydrophobic groups. The former is mostly found in seminal plasma, extracellular fluid, and blood serum **[172].**The spermatozoa have trouble preventing lipid peroxidation from damaging their tail and membrane, according to an earlier study **[169].** Accordingly, a recent investigation on the water- and fat-soluble antioxidants revealed that hydrophilic antioxidants are the primary defensive mechanism **[172].** This suggests additional research should be done on the function of hydrophobic antioxidants and whether adding them to a diet could be useful in reducing the risk of harm from too much ROS.Oxidative stress has been linked to deficiencies in both enzymatic and non-enzymatic antioxidants. Several antioxidant supplements added together in vitro have been found to improve sperm parameters. This is because antioxidants can specifically target external ROS from sources like leukocytes. Unfortunately, this does not apply to the DNA damage caused by ROS **[173].** Antioxidants added in vitro

cannot target the endogenous ROS, which is mostly created by the mitochondria **[174].** Systemic antioxidant therapy and lifestyle modifications may be able to make a difference in this case since they could serve as an earlier intervention to stop DNA damage.In general, antioxidants are substances and processes that eliminate, scavenge, reduce the production of ROS, or work against their effects. Biological and chemical antioxidants that target ROS and LPO are currently being researched. Superoxide dismutase (SOD) and its two isozymes, as well as catalase, are well-known biological antioxidants. Whereas catalase transforms H202 to 02 and H20, SOD spontaneously dismutases O2 to create 02 and H2O2.The role of SOD as an antioxidant in reproductive biology has been the subject of numerous studies published in the literature. Spermatozoa are shielded by SOD from spontaneous 02 toxicity and LPO **[175].** In addition to removing O2 produced by NADPH oxidase in neutrophils, SOD and catalase may be crucial in reducing LPO and protecting spermatozoa during genitourinary inflammation. The antioxidant enzyme GSH peroxidase, which contains selenium and uses GSH as an electron donor, eliminates peroxyl (ROOrn) radicals from a variety of peroxides, including H2O2. In rat sperm mitochondria, a selenium-associated polypeptide, most likely GSH peroxidase, has been found; it is crucial to this peroxyl scavenging process and, ultimately, to preserving sperm motility **[176].** Because spermatozoa include a large number of mitochondria, these antioxidant processes are crucial for maintaining sperm motility, the rate of hyperactivation, and the capacity to undergo the acrosome reaction during sperm preparation techniques, particularly in the absence of seminal plasma. By providing the thiol groups necessary for "chain-breaking" antioxidant activity, albumin, which is employed in sperm-washing operations, is expected to behave as an antioxidant. Spermatozoa will be better able to withstand oxidative

insults if their GSH/GSSG ratio is high. It appears that further research is needed to fully understand how these GSH enzymes and the mechanisms that go along with them connect to biological antioxidants in infertility.

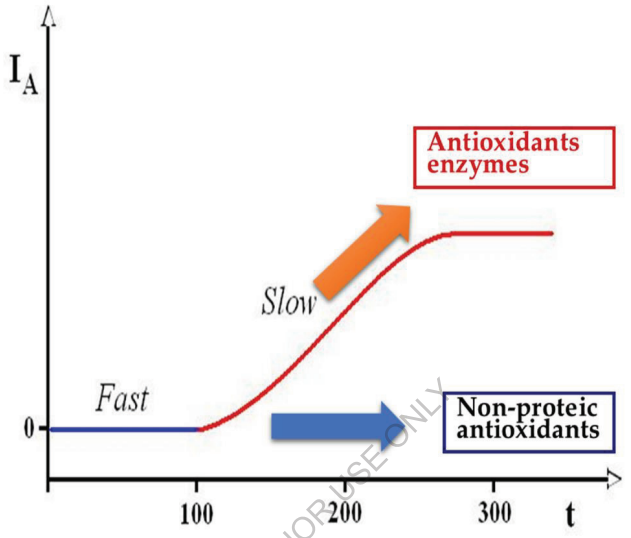


Figure 5. The components of "total" antioxidant capacity of seminal plasma, i.e., "Fast" (low-molecular-weight chain-breaking antioxidants) and "Slow" (antioxidant enzymes, e.g., SOD). In ordinate, "IA" represents the absorbance at 734 nm; in abscissa, "t" represents the time expressed in seconds. ABTS radical species appear after the initial period (latency phase) with the enrollment of "Fast" component, then gradually increase until a plateau related to the "Slow" component.

Measurement of OSS

A dynamic indicator of oxidative stress can be the rate of ROS production and generation utilizing luminol as a probe. The exceedingly brief half-life of these free radicals, however, restricts the clinical evaluation of this ROS production. The detection of an oxidized component that persists in body fluids (such as TBA-reactive compounds, GSH/GSSG balance, and the levels of unmodified tocopherol or ascorbate) may be used in future procedures for the evaluation of OSS. Although measuring TBA-MDA

activity as a sign of LPO has raised questions regarding its specificity, interference, and reliability, this test is still one of the most effective ways to determine oxidative damage to sperm **[175].** For the complete evaluation of OSS in infertility, this TBA-MDA measurement will eventually need to be paired with other assays that indicate ROS generation and antioxidant protection. Detection of IL-8, for instance, in conjunction with SOD or other antioxidants in infertile individuals with leukocytospermia, will suggest a positive OSS in this population **[13]** and should be treated accordingly. Before, during, and after clinical trials, it would be crucial to evaluate OSS in either male semen or female vaginal secretions. This would show whether someone with low OSS is less likely to experience infertility. A predictive value could be determined if there is a positive association between OSS and the trial's outcome.Over the past decade, research has gathered more and more evidence that excessive ROS production causes aberrant semen parameters and increased sperm destruction. The foundation of the clinical assessment of male infertility continues to be standard semen analysis. According to studies, 30% to 80% of infertile people without specific risk factors have sperm damage caused by ROS **[177-179].** As a result, it would be realistic to anticipate that all infertile males would be checked for elevated ROS levels. However, the regular assessment of subfertile men does not yet involve the measurement of the amounts and causes of excess ROS generation in semen **[180,182].** Simple inconvenience, cost-effective and efficient assays, and, perhaps most crucially, the absence of a generally acknowledged method of analysis are a few of the causes. Notwithstanding its significance, all three of these factors contribute to the general limits when ROS is measured as part of a male infertility examination.

Indication of sperm oxidative stress from routine semen analysis

Clinicians have been able to diagnose OS with a fair degree of accuracy thanks to routine semen analyses. Men with OS are more likely to see a decrease in any of the semen parameters (count, motility, and morphology). The most suitable biomarker for OS in a routine semen assay is probably asthenozoospermia. Impaired viscosity is a reasonable substitute for OS **[177]** due to the hyperviscosity of seminal plasma's correlation with elevated levels of seminal plasma MDA and decreased seminal plasma antioxidant status **[182].** Moreover, higher seminal plasma viscosity and an increase in ROS generation are linked to semen infection with the bacterial pathogen Ureaplasma urealyticum **[183].** These infections may harm the prostate and seminal vesicles, changing the substrates necessary to maintain the proper viscosity of the semen. The evidence of OS and the abundance of round cells point to a leukocyte spermic origin **[185].** The main characteristics of abnormal spermatozoa that produce significant levels of ROS are abnormal sperm morphology associated with ERC and cytoplasmic droplets **[185].**

Evaluation of oxidative stress in a laboratory

OS is brought on by an imbalance between the generation of ROS and the intracellular/extracellular antioxidants found in seminal plasma, as was previously mentioned. By identifying and quantifying the degree of oxidation in the sperm cell membrane, direct analyses of OS determine the net oxidative outcome of this imbalance. The thiobarbituric acid assay, one of the oldest and most popular direct assays for evaluating sperm membrane oxidation, can be used to quantify MDA, one of the byproducts of membrane LPO. Increased levels of MDA have been linked to reduced sperm motility and sperm-oocyte fusion, according to

several publications **[180,186].**Most frequently, seminal ROS are measured using chemiluminescence techniques. A luminometer is used in conjunction with a chemiluminescent probe, such as luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma-Aldrich, St. Louis, MO, USA). Aliquots of liquefied semen are centrifuged at 300 g for 7 minutes. For a later determination of the total antioxidant levels, seminal plasma is aliquoted and stored at -20°C. The pellet is re-suspended in the same washing medium at a concentration of 2 106 sperm/mL after being rinsed with phosphate-buffered saline (PBS, pH 7.4). To measure the basal ROS levels, 400-L aliquots of the resultant cell suspensions containing sperm and leukocytes are used. By combining 10 mL of 5-mM luminol with 400 mL of PBS, a negative control is created. The combination is then combined with luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma), a probe that is produced as a 5-mM stock in dimethyl sulfoxide. To determine the ROS levels, all test tubes are placed into the luminometer and run for 15 minutes **[187].** At neutral pH, Luminol reacts with a wide range of ROS because it is so sensitive **[188].** Both internal and extracellular ROS can be measured using it. The luminometer transforms the light signal produced by the free radicals in the semen sample's combination with luminol into an electric signal (photon). Relative light units/s/106 sperm are used to measure the number of free radicals created. Typical ROS concentrations in washed sperm suspensions vary from 0.10 to 1.03 CPM per 20 106 sperm **[189].**The total antioxidant capacity (TAC) of the seminal plasma can also be measured indirectly using luminol. Afterward, TAC is measured against the vitamin E analog Trolox (a water-soluble tocopherol analog). The results are expressed as a ROS-TAC score, which provides a summary of all constituents' antioxidant capabilities, including vitamins, proteins, and lipids. This assay appears to be the most reliable technique for

determining how well sperm antioxidant defenses balance off reactive oxygen species (ROS) **[190].** The expense of carrying out this approach, however, continues to prevent its usage in clinical laboratories **[191].**Nitroblue tetrazolium is an assay that has grown in popularity due to its affordability and user-friendliness (NBT). The source(s) of ROS can be determined with this test. This method, which just requires a light microscope, may identify whether leukocytes or spermatozoa are the sources of ROS. NBT is transformed into the blue pigment diformazam when it reacts with the O2-. Found in spermatozoa or leukocytes. The amount of diformazam can be seen, measured, and connected to the level of intracellular ROS using a light microscope **[180,191].**ROS levels have been measured using both direct and indirect techniques. However, compared to a standard semen examination, all sperm OS assays are rather pricey and time-consuming. Because of this, many practitioners still do not test their patients for OS and instead provide treatment programs in the hopes of reducing OS and raising the overall quality of the semen. As can be observed, an OS test can clinically detect male factor infertility as well as distinguish between fertile and infertile men with accuracy. Moreover, these tests can assist in identifying subgroups of infertile patients with OS who may benefit from antioxidant treatment **[181].**

Free radicals and assisted reproductive techniques (ART)

There is currently no known cure for many diseases linked to male infertility, including microdeletions of the Y chromosome, sperm maturational arrest, meiotic problems, aneuploidies, faulty centromeres, and defects in oocyte activation. The treatment of male factor infertility has, however, improved due to developments in ART **[192].** Despite being linked to the most miscarriages, ICSI is currently the most popular

ART technique. Poor sperm selection that may have been harmed by free radicals during ART procedures is one possible explanation.Oocytes, embryos, cumulus cells, and immature spermatozoa are the primary sources of ROS production during ART **[193].** Sperm preparation strategies can be utilized to reduce ROS formation to improve and maintain sperm quality following ejaculation **[192].** Density gradient centrifugation, migration-sedimentation, glass wool filtering, and traditional swim-up are the most frequently utilized sperm preparation methods to maintain and improve sperm quality following ejaculation **[194].** The standard swim-up strategy is less effective than the first three preparatory methods at lowering free radical levels **[194].** Nevertheless, repetitive centrifugation damages spermatozoa mechanically and boosts ROS production **[195].** Antioxidants and other compounds are now being evaluated for their potential to reduce ROS production during the sperm preparation process.According to Aitken et al., men with excessive ROS levels in semen had conception rates that are seven times lower than those with low ROS levels **[196].** Moreover, elevated ROS levels are linked to stopped embryo growth and a lower pregnancy rate after IVF or ICSI. Our team discovered that there is a significant association between ROS levels in spermatozoa and the fertilization rate during IVF **[197].** Thus, assessing ROS levels in semen specimens before IVF may help predict the success of IVF and in advising specific patients with a male factor or idiopathic infertility.

Conclusion

The aetiology of male infertility is not completely understood, but the effect of mitochondrial dysfunction and oxidative stress on seminal fluid quality has been implicated. OS has been regarded as a significant contributory factor to male infertility for both better and healthier sperm growth and function and for processes that reduce fertility. OS can be the only recognized cause of unexplained infertility, providing another reason to evaluate it. Identifying appropriate and easy-to-determine biomarkers for such situations still requires future investigation.To ensure proper fertilization, ROS is crucial for normal sperm physiological functions such as capacitation, hyperactivation, acrosome responses, and signaling. Although though ROS play a crucial role in fertilization, their overproduction leads to oxidative stress and negatively impacts sperm function. Many endogenous and external factors are responsible for this increase. It is widely known that ROS contributes to a deterioration in the success of IUI and IVF as well as a decrease in sperm count and motility, protein changes, lipid peroxidation, and DNA fragmentation, among other things. The first corrective action to be taken when the natural equilibrium between ROS and antioxidants is upset should be a change in lifestyle, such as stopping smoking, reducing substance usage, and keeping a healthy and balanced diet. The patient's outcomes can then be enhanced by taking antioxidant supplements concurrently.Further studies are warranted to overcome these limitations, improve fertility potential and reduce the risk of genetic diseases and malignant tumors in newborns.

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