Oxidative Stress in Male Infertility: Diagnostic Techniques and Implications for Sperm Function

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ABSTRACT

Male infertility affects approximately 7% of men worldwide and presents significant diagnostic and therapeutic challenges. Oxidative stress (OS), characterized by an imbalance between reactive oxygen species (ROS) and antioxidants, plays a pivotal role in impairing sperm quality and function due to the high susceptibility of spermatozoa to oxidative damage. This review provides a comprehensive analysis of current methods used to evaluate OS in sperm, including lipid peroxidation assays, ROS measurement techniques, total antioxidant capacity assessments, sperm DNA fragmentation testing, and oxidation-reduction potential measurement. Each diagnostic method is examined for its advantages, limitations, and clinical relevance in the context of male infertility. Understanding these techniques is essential for selecting appropriate candidates for antioxidant therapy and establishing reliable diagnostic standards. The review also discusses the physiological and pathological roles of ROS in spermatozoa, the main sources of ROS, and the effects of OS on semen parameters. Finally, recommendations for future research directions and therapeutic approaches are presented, emphasizing the importance of integrating OS assessment into routine infertility evaluations.

Keywords: Oxidative stress; Male infertility; Reactive oxygen species; Oxidation reduction potential; Measurement techniques

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Introduction

Male infertility, a complex condition that poses diagnostic and treatment challenges, is a prevalent problem affecting approximately 7% of men globally (1). Understanding and managing oxidative stress (OS) in the male reproductive system is paramount, given this complexity. OS, characterized by an imbalance between reactive oxygen species (ROS) and antioxidants, can inflict severe damage on sperm, thereby impairing their quality and function (2). Human spermatozoa are particularly susceptible to ROS due to their high content of polyunsaturated fatty acids, highlighting the crucial importance of this biochemical phenomenon in the context of male fertility (3). Sperm quality and viability can be severely affected by OS, which is common in the male reproductive system. OS, often caused by ROS, can affect sperm DNA, membranes, and organelles. The high concentration of unsaturated fatty acids in human sperm makes them susceptible to OS and rapid ROS destruction (4).

Although OS in male infertility has received considerable scientific attention, there is still no explicit agreement on the best diagnostic techniques and follow-up treatment plans. This article attempts to shed light on this challenging topic by thoroughly analyzing the methods currently used to evaluate OS in sperm. Each technique, from lipid peroxidation (LPO) assays to ROS, is thoroughly examined for its advantages, disadvantages, and potential uses (5). A thorough understanding of these methods is necessary to facilitate the selection of candidates for antioxidant treatment and to establish reliable diagnostic standards. In addition, understanding this topic is critical for future investigations that seek to understand the intricate relationships between OS and male infertility.

This article concisely overviews current methods for assessing OS in spermatozoa. We begin with a detailed explanation of the causes of OS, followed by a discussion of the physiological protective mechanisms that maintain sperm health under these challenging conditions. The remainder of the article details the methods used to assess OS in sperm and examines their respective advantages and disadvantages. In addition, clinical and research applications for detecting OS in sperm are reviewed. Recommendations for future therapeutic approaches, research efforts, and current opportunities and challenges are also discussed. A comprehensive understanding of the OS sperm measurement techniques and their practical implications in laboratory and clinical settings are presented.

OS in Sperm: Effects on Sperm Physiology and Male Fertility

Oxygen is a vital component of life and can positively and negatively affect biological systems. As part of oxidative phosphorylation in mitochondria, which is also involved in the synthesis of ROS and RNA, oxygen is primarily used to produce adenosine-5-triphosphate (ATP) (6). At modest concentrations, ROS/Reactive Nitrogen Species (RNS) significantly influences several intracellular signaling pathways, immunological and mitogenic responses, and cellular homeostasis (7). On the other hand, elevated ROS levels can cause oxidative damage to proteins, lipids, and nucleic acids (DNA, RNA), adversely affecting cellular health. A sophisticated system of antioxidant molecules has evolved to maintain redox balance and prevent damage to biological systems (7). OS can be caused by several circumstances, including environmental variables, excessive physical activity, antioxidant deficiency, immune system dysfunction, and chronic disease (8). Recent studies (7, 9, 10) have established that OS mediates tissue damage and cell death, underscoring its detrimental impact on cellular health. It plays a pathological role in several diseases, including inflammation and aging, cardiovascular and neurodegenerative diseases, autoimmune diseases, cancer, and changes in the reproductive system.

The Physiological Role of ROS in Spermatozoa

From a biological perspective, ROS is thought to control several intracellular metabolic pathways by affecting the activation of various transcription factors (9). By inhibiting tyrosine

phosphatase, ROS causes cyclic adenosine monophosphate (cAMP) to be produced in sperm (11). Several transcription factors involved in intracellular signaling cascades for sperm physiology are activated due to this biological process. Indeed, several studies (11, 12) have shown that higher levels of ROS promote sperm capacitation and hyperactivation, acrosome response, motility and chemotaxis, and chromatin compaction in mature spermatozoa. In addition, ROS can enhance the ability of sperm to adhere to the zona pellucida, leading to sperm-egg fusion (11). Incidentally, antioxidant molecules can alter sperm maturation and thus affect normal sperm function. Evidence that catalase or superoxide dismutase (SOD) specifically inhibits sperm capacitation or acrosome response supports the hypothesis that ROS play a key role in sperm function(11).

The Pathological Role of ROS in Spermatozoa

In addition to ROS's physiological function, excessive ROS production and OS appear to adversely affect spermatozoa, altering their morphology and dynamic cellular properties and ultimately reducing their fertilization ability. Recent studies (2, 13, 14) have demonstrated that an imbalance in the redox state of seminal fluid can negatively impact sperm homeostasis, subsequently contributing to male infertility. As recently described in a study (15) that showed higher production of leukocytes in blood ROS, increased LPO in plasma, and decreased total antioxidant capacity (TAC) in plasma in oligo asthenozoospermia men compared with healthy subjects, alterations in redox status in blood and plasma were found in infertile men (15). Consistent with this, several data also suggest that sperm oxidation induced by ROS may cause cellular dysfunction affecting sperm concentration, total number, and motility. Due to the high content of polyunsaturated fatty acids such as docosahexaenoic acid, which has six double bonds per molecule, in their plasma membrane, sperm are particularly susceptible to ROS-induced oxidation (16). Indeed, ROS facilitates the removal of hydrogen from the hydrocarbon side chain of a fatty acid, resulting in a carbon-centred lipid radical (L) that reacts with oxygen to form a lipid peroxyl radical (LOO), which can then react with another fatty acid to continue the process. Conjugated dienes and hydroperoxides are formed due to internal molecular rearrangements (17, 18). In addition, LPO products can interact with proteins, DNA, and phospholipids to generate byproducts that contribute to cell failure. Protein oxidation can affect proteins' structural and functional properties, especially when LPO products interact with amino acids (19). In this regard, LPO products such as 4-hydroxy-2-nonenal (4HNE) have been found to have the ability to increase the production of ROS by interacting with proteins in the sperm mitochondrial electron transport chain (16). LPO is specifically associated with changes in membrane fluidity and permeability, inhibition of membrane-bound enzymes and receptors, and activation of the apoptotic cascade, confirming the role of OS in abnormalities of sperm motility and morphology (20). According to several studies (20-22), 4HNE appears to be the LPO product most strongly associated with cytotoxic effects on the sperm membrane, resulting in loss of membrane integrity, motility alterations, and impaired sperm-ovule interactions. The number of substrates for 4HNE attack, the degree of cellular differentiation, and antioxidant defence mechanisms have been shown to influence the effects of 4HNE (22). Through post-translational oxidative protein changes, ROS can also impair sperm function (23, 24). Three nitro-tyrosines (3NT), a marker of protein oxidation, and sperm motility and morphology have been shown to correlate significantly in oligoasthenoteratospermia (25). Symptoms of tyrosine nitration and S-glutathionylation of sperm protein have been noted in infertile males (23, 24). Consequently, higher levels of peroxynitrite were found in human asthenozoospermic sperm samples, and their harmful effects on sperm motility through the formation of three nitro-tyrosines were highlighted (26). According to various studies, not all sperm proteins are equally susceptible to lipid aldehydes or ROS (20). Metabolic enzymes involved in bioenergetic pathways necessary for sperm motility are among the primary targets of 4HNE (27). Numerous proteomics studies have focused on the sperm of infertile men. Proteins associated with bioenergetic pathways were downregulated in the altered spermatozoa of asthenozoospermic men (27, 28).

Notably, several studies supported the notion that OS impairs sperm function by altering bioenergetic pathways by discovering that asthenozoospermic males had altered levels of proteins involved in metabolic pathways such as glycolysis, pyruvate metabolism, TCA, or beta-oxidation (29, 30). Another known primary target of OS is nucleic acids. Both nuclear and mitochondrial DNA are susceptible to hydroxyl radicals (OH) attacks, which cause several signs of OS. When OH combines with guanine, it can form the crucial DNA marker for oxidative damage, 8-hydroxy-2'-deoxyguanosine (8-OH-G), which is detectable in several biological samples (9). Sperm lack the necessary antioxidant systems, making them highly susceptible to DNA damage (2, 16). Moreover, the lack of complete DNA repair mechanisms in sperm favors sperm DNA oxidation. Namely, when the enzyme 8-oxo guanine glycosylase (OGG1) can remove the 8OHdG residue from DNA, creating an abasic site, sperm lack a base excision repair pathway to insert a new base (16). According to several studies (16, 31), apoptosis promoted by DNA breaks and insufficient chromatin packing (ROS generation) has implications for sperm count. In patients with asthenozoospermia, an increase in ROS generation has been associated with higher mtDNA copy number and integrity (32). Thus, other studies confirmed the efficacy of redox status assessment in diagnosing and monitoring male infertility by demonstrating the essential relationship between abnormalities in semen parameters and NO and 8-OHdG levels (33).

Main Sources of ROS

Many external variables are thought to play a role in inflammation and redox status changes that support male infertility. According to Iommiello et al. (31) and Agarwal et al. (34), environmental pollution, lifestyle variables such as smoking, alcohol consumption, obesity, a varicocele, bacterial or viral infections, microbial mutations, or sexually transmitted diseases actively contribute. However, leukocytes, which produce 1,000 times more ROS than healthy sperm, and immature sperm are the leading causes of OS in seminal fluid (31, 34, 35).

Leukocytospermia

Leukocytospermia, defined as a peroxidase-positive leukocyte concentration > $1x10^6$ per mL of seminal fluid, has been found in approximately 10-20% of infertile men, consistent with recommendations from World Health Organization (WHO) (34, 36). The two major cell types in the ejaculate, granulocytes, and macrophages, are responsible for the production of ROS, which are primarily associated with glucose-6-phosphate dehydrogenase (G6PDH) activity and high NADPH concentrations, which in turn strongly stimulate NADPH oxidase, one of the significant ROS sources (2, 34). Recent evidence suggests that sperm leukocytes may increase the ability of sperm to produce ROS directly or by releasing soluble products into the sperm environment (36). The clinical significance of leukocytospermia and its contribution to sperm quality remains controversial.

Infertile men had higher sperm WBC levels than healthy controls, and leukocytospermia was significantly associated with changes in sperm quantity, motility, and shape (37). In addition, in vitro studies have shown that leukocytosis impairs sperm function and hamster oocyte penetration, which are important prognostic indicators of the success rate of assisted reproductive technologies (ART) (37). Further studies confirmed these findings and suggested that leukocytes catalyze sperm production ROS, resulting in decreased sperm quality and DNA damage (36, 38). Compared to patients without leukocytospermia or healthy participants, patients with leukocytospermia exhibited altered sperm concentration, motility, and morphology. In addition, in vitro studies have shown that ROS remained high in pure sperm suspensions from leukocytospermia patients even after the removal of leukocytes or phorbol myristate acetate (PMA)-induced activation of ROS. Similar results were obtained after the incubation of sperm with leukocytes (36). Moreover, leukocytes in sperm showed a significant correlation with OS even at low levels, suggesting that removing leukocytes in sperm could help reduce OS in samples used for ART (38, 39).

Immature spermatozoa

Cytoplasmic extrusion processes are altered in impaired spermatogenesis, and spermatozoa are

discharged with excess leftover cytoplasm (cytoplasmic droplets) (2). Increased production of ROS is associated with immature sperm via G6PDH and higher creatine phosphokinase (CK) levels (40). It is controversial whether CK has a clinically significant impact on sperm development and quality (40-42). According to some studies, oligozoospermic men have higher CK levels than healthy men, and there is a significant correlation between CK levels and sperm parameters (such as concentration, motility, and morphology), suggesting that this marker could be used to predict sperm quality in patients treated for male infertility (41). Other researchers detected no change in the amount of CK in cells with or without DNA fragmentation, indicating that immature sperm are not responsible for DNA damage (42). In this context, it was found that spermatozoa at different stages of maturation have differences in ROS, membrane lipid content, chromatin compaction, morphology, and motility. Male infertility may be caused in part by immature spermatozoa that induce oxidation in mature spermatozoa as they travel from the seminiferous tubules to the epididymis and exhibit increased ROS production and DNA damage (43).

Mitochondria

In sperm, mitochondria are another potential source of ROS. Indeed, the electron transport chain at the mitochondrial membrane can be altered by variables such as electromagnetic radiation, polyunsaturated fatty acids, or apoptotic agents, leading to excessive production of ROS. According to several studies, OS and sperm mitochondrial dysfunction may play a role in asthenozoospermia (44). In particular, dysfunction of mitochondrial electron transport at complexes I and III can induce sperm tail oxidation and production of ROS, leading to DNA damage and motility abnormalities (45). Mitochondrial dysfunction in sperm increases the formation of ROS, which is associated with decreased sperm quality and fertility potential. Notably, sperm motility and mitochondrial activity are significantly correlated (46).

OS Effects on Semen Parameters

In recent years, much research has been conducted on the possible relationship between ROS sperm production and semen parameters (32, 38, 47-50). Numerous studies have documented how ROS affects sperm morphology and motility. Studies conducted in vitro have shown that sperm dependence on lipid aldehydes decreases human sperm motility (38). Accordingly, in astheno- and oligoastheno-teratospermic men, seminal fluid LPO and TAC levels were significantly associated with sperm motility, morphology, and number (48, 51). Evidence of the beneficial effects of therapeutic antioxidant supplements on sperm quality in infertile men also supports the critical role of OS in sperm changes (48). Thus, therapeutic coenzyme Q10 therapy improved redox status, sperm DNA fragmentation, and sperm parameters (sperm concentration and motility) in idiopathic male infertility(52).

Interestingly, infertile men with vitamin D deficiency and oligoasthenozoospermia showed an increase in sperm motility and concentration after vitamin D therapy (53). In patients with oligoasthenozoospermia receiving intracytoplasmic sperm injection (ICSI), the beneficial effects of antioxidant treatment on sperm quality have been suggested as a valuable strategy to increase the successful conception rate. However, other researchers found no relationship between ROS and sperm motility. They noted that it is still unclear whether the decreased functional performance of sperm is caused by a decrease in sperm quantity or a direct ROS influence (54). In light of this, it is becoming increasingly clear how valuable a new blood diagnostic tool is for diagnosing and treating male infertility by assessing sperm morphological and/or functional problems. In this regard, it was discovered that blood levels SOD and glutathione reductase (GSH) were positively correlated with sperm motility and count.

In contrast, elevated malondialdehyde (MDA) levels were associated with altered sperm morphology (55). Accordingly, infertile males were shown to have lower plasma TAC levels and indicators of OS in their seminal fluid. In particular, plasma TAC showed a significant and positive correlation with seminal fluid TAC and semen characteristics (56), demonstrating that redox status in plasma reflects the redox status of sperm quality and seminal fluid environment. It was found that higher levels of MDA and nitric oxide (NO) in plasma and seminal fluid of

infertile men corresponded with semen parameters, confirming that blood redox status is related to semen parameters (57). However, reports on the relationship between OS in blood and seminal fluid remain sparse and controversial, possibly due to different approaches and methods. There was no relationship between the oxidative state of blood and seminal fluid, suggesting that the redox homeostasis of seminal fluid is unrelated to the systemic microenvironment and external variables (58).

Exploring Sperm Characteristics and Advanced Assessment of Sperm Function

Studies repeatedly establish a relationship between changes in semen parameters and increased levels of ROS, suggesting the damaging effect of OS on male fertility (59, 60). A research project led by Homa et al. (61) compared the ROS levels in semen across three different groups: Group 1 with normal semen parameters without leukocytospermia; Group 2 with abnormal semen parameters without leukocytospermia; and Group 3 with varying semen parameters with leukocytospermia. Group 3, as expected, demonstrated the highest ROS levels. Interestingly, Group 2 also exhibited significantly elevated ROS levels compared to Group 1, indicating a negative relationship between ROS and normal semen parameters. This discovery was further confirmed by Agarwal et al. (62) who investigated the connection between semen parameters and ROS levels in the semen of fertile and infertile men, revealing significantly elevated ROS levels in infertile men compared to their fertile counterparts. They noticed significant positive relationships between ROS levels and sperm concentration and motility in all participants, regardless of their fertility status.

Research conducted by Venkatesh et al. (63) studied the connection between sperm morphological abnormalities and seminal OS in fertile and infertile men. They found that the median ROS levels in infertile men were up to 124 times higher than in fertile men. Moreover, they highlighted that lifestyle changes, majorly influenced by increased stress from work and personal issues associated with a developed society, significantly contribute to morphological anomalies. Increases in harmful behaviors like drug abuse, smoking, and unhealthy eating, identified as potential OS triggers, can be attributed to these lifestyle shifts. Mitigating these behaviors might lessen OS (64, 65).

Furthermore, exposure to pollution, heat, toxins, heavy metals, and other environmental factors significantly contribute to the onset of OS. Activities that lead to increased heat exposure, such as using saunas and hot baths, prolonged driving, and extended periods of sedentary office work, should also be regulated. Work environments should ensure adequate ventilation and protect against harmful chemicals or vapours that might enhance OS (66, 67).

One study indicated that the proportion of sperm with cytoplasmic droplets was considerably higher in infertile men than in fertile men, likely due to the higher ROS levels in their sperm samples. Another study identified a strong positive correlation between acrosomal anomalies and MDA levels (68), bolstering the notion that OS adversely affects the acrosomal structure.

Many studies point to seminal OS as a significant contributor to sperm DNA fragmentation, suggesting that testing for sperm DNA fragmentation could be an essential diagnostic tool for male infertility (69, 70). In a study by Iommiello et al. (31), a statistically significant positive correlation was observed between OS [measured with oxiSperm® (Halotech DNA, SL, Madrid, Spain)] and sperm DNA fragmentation index [measured with sperm chromatin structure assay (SCSA®; SCSA Diagnostics, Volga, SD, United States)]. Another study found that infertile men had a significantly larger proportion of spermatozoa with fragmented DNA than fertile men and confirmed strong positive relationships between sperm DNA fragmentation and seminal MDA levels (50). More recent research assessed the effect of seminal ORP and sperm DNA fragmentation (as measured by sperm chromatin dispersion) on sperm morphological abnormalities in fertile and infertile men. This study found ORP and sperm DNA fragmentation to have substantial positive correlations with sperm head abnormalities and significant negative correlations with normal morphology in infertile men. Additionally, ORP and sperm DNA fragmentation were negatively associated with normal sperm morphology (P 0.001) (71).

The first step to managing and preventing male reproductive OS is identifying the root causes of the imbalance between ROS production and antioxidant levels (60). Some key management aspects include lifestyle changes that can significantly influence the management of OS. As previously mentioned, increased stress from work and personal issues, primarily associated with a developed society, contributes to the emergence of harmful behaviors such as drug abuse, smoking, and unhealthy eating, recognized as potential OS triggers. Therefore, reducing these behaviors should help in managing OS (4, 65). Additionally, exposure to environmental hazards like pollution, heat, toxins, heavy metals, etc., significantly contributes to the onset of OS. Activities resulting in increased heat exposure should be controlled, and workplaces should provide adequate ventilation and protection from harmful chemicals or vapours that might escalate OS (66, 67).

The role of antioxidants in managing OS is critical (72, 73). Antioxidants work to eliminate or limit the production of ROS, thereby stopping the oxidative chain reaction (74). Preventive antioxidants, like lactoferrin and transferrin, prevent ROS formation (75), while scavenging antioxidants, such as vitamins C and E, neutralize ROS (76). Antioxidants can be categorized into enzymatic or non-enzymatic types. Enzymatic antioxidants include natural antioxidants like GSH, SOD, and catalase. Non-enzymatic antioxidants include vitamins C, E, and B, carnitines, cysteines, carotenoids, pentoxifylline, taurine, metals, hypotaurine, and albumin. These non-enzymatic antioxidants can be obtained by consuming certain foods or supplements (4, 77, 78).

Several studies have explored the effect of antioxidant supplementation on sperm parameters and OS measurements in seminal plasma (79, 80). A literature review by Gharagozloo and Aitken (81) identified 20 studies demonstrating the effects of antioxidant therapy on OS measurements in human spermatozoa. In 19 of the 20 analyzed trials, a significant decrease in OS or DNA damage was observed following antioxidant treatment. A significant improvement in sperm motility was reported, particularly in men with asthenospermia (81). According to another comprehensive review by Majzoub and Agarwal (82), vitamin E, vitamin C, N-acetyl-cysteine, selenium, and zinc effectively treat OS-related male infertility.

The surgical intervention also plays a role in managing male reproductive OS. Varicocele is characterized by an abnormal enlargement and dilation of the pampiniform venous plexus around the spermatic cord. These dilated veins are surgically occluded in subfertile men and male patients experiencing testicular discomfort. This procedure has been observed to decrease seminal ROS levels, thereby protecting sperm from oxidative damage (67). Surgical repair also improves other vital indicators of infertility, such as sperm parameters and conception rates (4, 83). Recent data also suggest the potential of surgical therapy in increasing the success of assisted reproductive technologies, thereby aiding in managing male reproductive OS (84, 85).

Diagnostic Techniques for Assessing Oxidative Stress in Male Infertility

Male infertility is a complex condition contributing to approximately half of all infertility cases worldwide. Among the various factors implicated in male reproductive dysfunction, OS has emerged as a significant contributor. Oxidative stress results from an imbalance between ROS production and the body's antioxidant defenses. Excessive ROS can impair sperm function and structure by inducing lipid peroxidation, DNA fragmentation, and protein modifications. Consequently, evaluating oxidative stress parameters has become essential in diagnosing and managing male infertility. This paper discusses the commonly used diagnostic techniques for assessing oxidative damage in sperm, highlighting their methodologies, clinical relevance, strengths, and limitations.

Lipid Peroxidation (LPO) Assays

Lipid peroxidation is a primary consequence of oxidative stress in sperm cells. Sperm membranes are rich in polyunsaturated fatty acids (PUFAs), making them highly susceptible to ROS-induced oxidation. This process leads to the formation of toxic aldehyde byproducts, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal

(4-HNE), which can disrupt membrane integrity and impair sperm function (86, 87).

Measurement Methods

Thiobarbituric Acid Reactive Substances (TBARS) Assay: The TBARS assay quantifies MDA levels by reacting it with thiobarbituric acid under acidic conditions and high temperature to form a colored complex. This complex can be measured using spectrophotometry at 532 nm (88).

High-Performance Liquid Chromatography (HPLC): HPLC allows for the separation and precise quantification of lipid peroxidation products like MDA and 4-HNE. It offers higher specificity and sensitivity compared to spectrophotometric methods (89).

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA kits designed for specific aldehyde adducts enable the detection of lipid peroxidation products with high specificity and are suitable for processing multiple samples simultaneously (90).

Clinical Relevance

Elevated levels of lipid peroxidation products are strongly associated with decreased sperm motility and abnormal morphology. The peroxidation of PUFAs alters membrane fluidity and permeability, impairing essential sperm functions such as capacitation and the acrosome reaction. Hosseinzadeh et al. demonstrated a significant correlation between increased MDA levels and reduced sperm motility and viability in infertile men. Assessing lipid peroxidation is thus critical for understanding oxidative damage, particularly in cases of idiopathic male infertility where conventional semen parameters may appear normal (48).

Strengths and Limitations

Strengths: LPO assays are relatively simple, cost-effective, and can be performed with standard laboratory equipment. They provide direct evidence of oxidative damage to sperm membranes.

Limitations: The TBARS assay lacks specificity, as thiobarbituric acid can react with other substances besides MDA, potentially leading to overestimation. Sample handling is crucial, as MDA is unstable and can degrade if not processed promptly (2).

Measurement of Reactive Oxygen Species (ROS) Levels

Reactive oxygen species play a dual role in sperm physiology. While low levels are necessary for normal sperm functions, excessive ROS can lead to oxidative stress, damaging sperm DNA, proteins, and lipids (11).

Measurement Methods

Chemiluminescence Assays: Utilize probes like luminol or lucigenin that emit light upon reacting with ROS. The emitted light intensity, measured using a luminometer, correlates with ROS concentration. This method is highly sensitive and allows for real-time monitoring (91).

Flow Cytometry: Involves staining sperm with fluorescent probes such as dichlorofluorescein diacetate (DCFH-DA), which fluoresce upon oxidation. Flow cytometry enables the analysis of ROS levels within individual sperm cells (92).

Colorimetric Assays: Employ color-changing dyes that react with ROS, measured using a spectrophotometer. These are less sensitive and less commonly used due to limitations in specificity (93).

Clinical Relevance

Elevated ROS levels are a hallmark of male infertility. Agarwal et al. reported significantly higher ROS concentrations in the semen of infertile men compared to fertile controls, implicating oxidative stress in sperm dysfunction. Measuring ROS levels aids clinicians in diagnosing oxidative stress-related infertility and monitoring antioxidant therapy efficacy (4).

Strengths and Limitations

Strengths: Chemiluminescence assays offer high sensitivity and specificity. Flow cytometry provides

detailed analysis at the single-cell level.

Limitations: These methods require specialized equipment and technical expertise. ROS are highly reactive and have short half-lives, making accurate measurement challenging. Pre-analytical variables like sample handling can affect results (94, 95).

Total Antioxidant Capacity (TAC) Measurement

Total antioxidant capacity reflects the collective ability of all antioxidants present in seminal plasma to neutralize ROS. TAC assessments provide insight into the antioxidant defense system's overall effectiveness in counteracting oxidative stress (96).

Measurement Methods

Ferric Reducing Antioxidant Power (FRAP) Assay: Measures the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions by antioxidants, resulting in a colored ferrous-tripyridyltriazine complex detectable at 593 nm (97).

Trolox Equivalent Antioxidant Capacity (TEAC) Assay: Assesses the capacity of antioxidants to scavenge the stable ABTS^+ radical cation compared to the standard antioxidant Trolox (98).

Oxygen Radical Absorbance Capacity (ORAC) Assay: Evaluates the inhibition of peroxyl radical-induced oxidation by antioxidants using a fluorescent probe, providing a measure of antioxidant efficacy (99).

Clinical Relevance

Reduced TAC levels have been observed in men with poor semen quality. Benedetti et al. found a positive correlation between TAC and sperm parameters such as motility and morphology. Assessing TAC helps identify individuals who may benefit from antioxidant supplementation to restore redox balance and improve fertility outcomes (56).

Strengths and Limitations

Strengths: TAC assays are relatively easy to perform and provide a comprehensive assessment of antioxidant status.

Limitations: They do not identify specific antioxidants or their individual contributions. Results can be influenced by assay conditions and are not standardized across different laboratories (100).

Sperm DNA Fragmentation Testing

DNA integrity is crucial for the transmission of genetic information. Oxidative stress can cause sperm DNA fragmentation, leading to compromised fertility potential, poor embryo development, and increased miscarriage rates (101).

Measurement Methods

Sperm Chromatin Structure Assay (SCSA): Utilizes flow cytometry to detect DNA denaturation in sperm nuclei after acid treatment. Acridine orange staining differentiates between normal double-stranded DNA (green fluorescence) and denatured single-stranded DNA (red fluorescence) (102).

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Assay: Labels DNA strand breaks by incorporating fluorescently tagged dUTP at free 3'-OH ends using the enzyme terminal deoxynucleotidyl transferase. Labeled sperm are then quantified using fluorescence microscopy or flow cytometry (103).

Sperm Chromatin Dispersion (SCD) Test: Involves treating sperm to remove nuclear proteins, leading to the formation of halos of dispersed DNA loops in cells with intact DNA. Sperm with fragmented DNA show minimal or no halo formation (104).

Clinical Relevance

High sperm DNA fragmentation levels are associated with lower pregnancy rates and higher incidences of miscarriage, especially in assisted reproductive technologies (ART) like IVF and ICSI. Iommiello et al. reported significantly higher DNA fragmentation indices in infertile men. Testing for sperm DNA fragmentation is recommended for couples with recurrent IVF failure or unexplained infertility, as it can influence the choice of ART procedures and inform clinical decisions (31).

Strengths and Limitations

Strengths: These assays provide specific information about sperm DNA integrity, which is not assessed by standard semen analysis.

Limitations: Techniques can be time-consuming, require specialized equipment, and lack standardization across laboratories. Interpretation of results may vary, necessitating careful consideration of clinical context(105).

Oxidation-Reduction Potential (ORP) Measurement

Oxidation-reduction potential represents the overall redox balance in a biological system, encompassing both oxidant and antioxidant activity. Measuring ORP provides a holistic view of the oxidative status in seminal fluid(106).

Measurement Methods

Electrochemical Sensors: Devices like the MiOXSYS system measure ORP using a galvanostatic technique. A small semen sample is placed on a disposable sensor that quantifies electron transfer between oxidants and reductants, providing results in millivolts (mV)(107).

Clinical Relevance

Higher ORP values indicate a shift towards oxidative stress. Majzoub et al. demonstrated that elevated ORP levels correlate with decreased sperm concentration, motility, and increased DNA fragmentation. ORP measurement offers a rapid, comprehensive assessment of oxidative stress, aiding in the diagnosis and guiding antioxidant therapy(71).

Strengths and Limitations

Strengths: ORP measurement is quick, requires minimal sample preparation, and provides an integrated assessment of oxidative and reductive activities.

Limitations: It does not specify the individual contributions of oxidants or antioxidants. External factors such as temperature and semen viscosity can influence readings. Further validation and standardization are needed for widespread clinical adoption(108).

Summary

Accurate assessment of oxidative stress is vital for diagnosing and managing male infertility. The discussed diagnostic techniques—LPO assays, ROS measurement, TAC assessment, sperm DNA fragmentation tests, and ORP measurement—each offer unique insights into oxidative damage and its impact on sperm quality. Combining these methods can enhance diagnostic accuracy, allowing for personalized therapeutic interventions such as targeted antioxidant supplementation. By addressing oxidative stress, clinicians can improve reproductive outcomes and offer better prognostic information to affected couples. Future research should focus on standardizing these assays, establishing reference ranges, and integrating oxidative stress evaluation into routine infertility assessments.

Conclusions

Oxidative stress is a critical factor contributing to male infertility, impacting sperm physiology and function through mechanisms such as lipid peroxidation, DNA fragmentation, and protein modifications. The diagnostic techniques discussed offer valuable insights into the oxidative damage affecting sperm quality. By accurately assessing OS through methods like lipid peroxidation assays, ROS measurements, total antioxidant capacity assessments, sperm DNA fragmentation tests, and oxidation-reduction potential measurements, clinicians can better diagnose and manage male infertility. Combining these techniques enhances diagnostic accuracy and allows for personalized therapeutic interventions, such as targeted antioxidant supplementation. Future research should focus on standardizing these assays, establishing reference ranges, and integrating oxidative stress evaluation into routine infertility assessments to improve reproductive outcomes for affected couples.

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