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REVIEW

OXIDATIVE STRESS AND MALE INFERTILITY: MECHANISMS, BIOMARKERS, AND THERAPEUTIC IMPLICATIONS: A COMPREHENSIVE REVIEW

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ABSTRACT

Male infertility accounts for approximately 40–50% of all infertility cases worldwide, with oxidative stress (OS) identified as a pivotal etiological contributor in up to 80% of infertile men. This review examines the molecular underpinnings of OS-mediated spermatotoxicity, its clinical manifestations, and emerging diagnostic and therapeutic strategies. A systematic narrative review was conducted utilizing peer-reviewed literature sourced from PubMed, Scopus, Web of Science and Google Scholar databases, encompassing publications from 1995 to 2024. Studies addressing reactive oxygen species (ROS) biology, spermatozoa redox physiology, antioxidant defense mechanisms, and clinical interventions were critically appraised and synthesized. Compelling evidence implicates elevated seminal ROS—primarily superoxide anion (O_2•$^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH)—in the pathogenesis of sperm DNA fragmentation, lipid peroxidation of the plasma membrane, and impaired mitochondrial membrane potential. These insults collectively compromise sperm motility, morphology, and fertilization competence. Seminal antioxidant capacity, as measured by total antioxidant capacity (TAC) and specific enzymatic activity (superoxide dismutase, catalase, glutathione peroxidase), is significantly attenuated in infertile cohorts. Oxidative stress represents a central, mechanistically validated axis of male reproductive dysfunction. Its reliable quantification and targeted pharmacological mitigation offer viable clinical avenues for improving assisted reproductive technology (ART) outcomes and natural fertility. Further large-scale randomized trials are warranted to standardize antioxidant supplementation protocols.

Keywords: Oxidative stress; Male infertility; Reactive oxygen species; Sperm DNA fragmentation; Antioxidants; Seminal plasma; Spermatogenesis

INTRODUCTION

Infertility, broadly defined as the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse, afflicts approximately 15% of couples globally, representing a significant public health burden with substantial psychosocial and economic ramifications [1]. Historically attributed predominantly to female reproductive insufficiency, contemporary epidemiological data reveal that the male factor is implicated in nearly half of all infertility cases, either as an exclusive or contributing etiology [2]. Within the spectrum of male reproductive dysfunction, oxidative stress has emerged as one of the most consequential and pervasive pathophysiological mechanisms, identified in excess of 30–80% of infertile male patients across diverse clinical settings [3].

Spermatozoa are intrinsically vulnerable to oxidative damage by virtue of their unique cellular architecture. The characteristic abundance of polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA, 22:6n-3), within the sperm plasma membrane renders these cells exquisitely susceptible to lipid peroxidation. Furthermore, the compaction of DNA within the spermatozoon head—mediated by protamine replacement of histones—and the near-total elimination of cytoplasm during spermiogenesis severely curtail the cell's endogenous antioxidant and DNA repair

capacity [4]. These anatomical and biochemical constraints conspire to render spermatozoa uniquely defenseless against redox imbalance.

Reactive oxygen species (ROS) are chemically reactive molecules derived from molecular oxygen, encompassing both radical (superoxide anion, hydroxyl radical) and non-radical (hydrogen peroxide, singlet oxygen) species. Under physiological conditions, low-level ROS production is indispensable for normal sperm function, mediating capacitation, the acrosome reaction, and zona pellucida binding via redox-sensitive signal transduction cascades [5]. However, when ROS generation exceeds the neutralizing capacity of the seminal antioxidant system—comprising enzymatic scavengers and non-enzymatic small molecules—a state of oxidative stress ensues, with deleterious consequences for virtually all aspects of sperm structure and function.

This review synthesizes the current mechanistic and clinical understanding of oxidative stress in the context of male fertility, examining the cellular sources of excessive ROS, the molecular pathways by which they impair sperm function, contemporary approaches to assessing the oxidative burden in clinical andrology, and the evidence base for antioxidant-directed therapies.

Reactive Oxygen Species: Sources, Biochemistry, and Physiological Roles

Chemical Classification and Reactivity: Reactive oxygen species constitute a heterogeneous class of oxygen-derived molecules distinguished by the presence of unpaired electrons in their outer orbital shells, conferring exceptional reactivity with biological macromolecules. The superoxide anion ($O_2^{\bullet -}$) is the primary ROS generated in aerobic metabolism, produced via the univalent reduction of molecular oxygen. It undergoes spontaneous or enzymatically catalyzed (via superoxide dismutase, SOD) dismutation to hydrogen peroxide (H_2O_2), a relatively stable and membrane-permeable oxidant that serves as a crucial signaling intermediate. In the presence of transition metals, particularly ferrous iron (Fe^{2+}), H_2O_2 undergoes Fenton-type reactions to yield the hydroxyl radical (•OH), the most potent and indiscriminate biological oxidant known, capable of initiating chain reactions of lipid peroxidation and directly cleaving nucleic acid backbones [6,7].

Cellular Sources within the Reproductive Tract: Within the male reproductive system, ROS originate from both endogenous and exogenous sources. Endogenously, immature spermatozoa retaining residual cytoplasm constitute a principal cellular source, generating superoxide via an NADPH oxidase-dependent mechanism and releasing it into the surrounding seminal milieu [4, 6]. Leukocytes—predominantly polymorphonuclear neutrophils and macrophages infiltrating the seminal plasma in the context of genitourinary tract infections or subclinical inflammation—represent another major endogenous ROS source, capable of generating oxidant bursts orders of magnitude exceeding those of spermatozoa themselves [8].

Exogenous contributors to seminal OS include environmental toxicants (pesticides, heavy metals, and endocrine-disrupting chemicals), ionizing and non-ionizing radiation, and lifestyle variables including tobacco smoking, excessive ethanol consumption, and sedentary behavior with associated obesity. Scrotal hyperthermia, arising from varicocele, occupational exposure, or behavioral factors, further augments testicular ROS production by impairing mitochondrial electron transport chain efficiency [9]. Varicocele, the most surgically correctable cause of male infertility, has been consistently associated with elevated seminal ROS levels and reduced antioxidant enzyme activities, suggesting that local venous hypertension, hypoxia, and reflux of adrenal metabolites collectively engender a state of chronic testicular oxidative stress [10].

Physiological ROS Signaling in Spermatozoa: It is essential to recognize that ROS play indispensable physiological roles in male gamete function. Sperm capacitation—the process of hyperactivation and acquisition of fertilization competence occurring in the female reproductive tract—is critically dependent on H_2O_2-mediated activation of protein kinase A (PKA) and subsequent phosphorylation of tyrosine residues on flagellar proteins [11]. The acrosome reaction, triggered by zona pellucida contact and essential for gamete fusion, is regulated by redox-sensitive calcium flux and phospholipase C activation. These observations underscore the concept of a physiological 'redox window,' wherein basal ROS concentrations are necessary for normal reproductive function, while supraphysiological levels are spermatotoxic. The balance between ROS generation and antioxidant neutralization defines the reproductive competence of the ejaculate.

Mechanisms of Oxidative Spermato-toxicity

Lipid Peroxidation and Membrane Integrity: The sperm plasma membrane is distinguished by an exceptionally high PUFA content, with DHA comprising up to 55% of total fatty acids in the principal piece of the flagellum [12]. This lipid composition is functionally critical for membrane fluidity, receptor mobility, and the lateral phase separations required for acrosomal exocytosis, but simultaneously renders the membrane profoundly susceptible to lipid peroxidation—a self-propagating radical chain reaction initiated by ROS abstraction of bis-allylic hydrogen atoms. The principal lipid peroxidation byproduct, malondialdehyde (MDA), and the more reactive 4-hydroxynonenal (4-HNE), form adducts with membrane proteins and DNA, amplifying cellular dysfunction. Elevated seminal MDA concentrations correlate inversely with sperm motility and morphology parameters across numerous clinical studies [13], and the degree of lipid peroxidation has been proposed as a surrogate marker of overall seminal oxidative burden [14].

Sperm DNA Fragmentation: Perhaps the most clinically consequential consequence of oxidative stress in male reproduction is DNA strand breakage within the spermatozoon. Unlike somatic cells, mature spermatozoa possess only vestigial capacity for DNA repair owing to the elimination of cytoplasm and the transcriptional silencing entailed by chromatin condensation. Hydroxyl radicals and other ROS directly induce single- and double-strand breaks in the sperm DNA backbone, as well as base modifications—most notably the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a premutagenic oxidized nucleoside now widely utilized as a biomarker of oxidative DNA damage [15] (Shen & Ong, 2000). Elevated sperm DNA fragmentation index (DFI), as measured by the sperm chromatin structure assay (SCSA), TUNEL assay, or comet assay, is associated with reduced fertilization rates, impaired embryonic development, increased miscarriage risk, and adverse offspring health outcomes, including childhood cancer, independent of conventional semen parameters [16, 17].

Mitochondrial Dysfunction: The mitochondrial sheath of the sperm midpiece is the principal site of ATP synthesis via oxidative phosphorylation, providing the energy substrate for flagellar axonemal dynein ATPase activity and progressive motility. Oxidative stress disrupts mitochondrial membrane potential ($\Delta\Psi m$) by peroxidizing cardiolipin—the mitochondria-specific phospholipid essential for maintaining the electrochemical gradient—and by damaging mitochondrial DNA (mtDNA), which, unlike nuclear DNA, lacks protective histones and possesses limited repair mechanisms. Mitochondrial dysfunction further augments ROS production in a self-reinforcing cycle, as impaired electron transport chains increase electron leakage and superoxide generation. The consequent impairment of ATP synthesis translates directly into reduced sperm motility, and mitochondrial membrane depolarization has been linked to the initiation of apoptotic cascades within the spermatozoon [18].

Apoptosis and Proteome Alterations: Beyond the specific molecular targets described above, excessive ROS activate apoptotic signaling pathways in spermatogenic cells and mature spermatozoa. Activation of the intrinsic mitochondrial apoptotic pathway, mediated by cytochrome c release and caspase-3 activation, has been documented in association with seminal OS. ROS also induce post-translational modifications of the sperm proteome—including carbonylation of critical flagellar and acrosomal proteins—that may impair sperm-egg recognition and binding even in the absence of conventional semen parameter abnormalities, potentially explaining the phenomenon of unexplained infertility in normozoospermic men [19].

The Seminal Antioxidant Defense System

Enzymatic Antioxidants: The male reproductive tract has evolved a multilayered antioxidant defense system to maintain redox homeostasis. Enzymatic components include superoxide dismutase (SOD), which catalyzes the dismutation of $O_2\bullet^-$ to H_2O_2 and oxygen, thereby preventing the accumulation of the more reactive hydroxyl radical. Catalase subsequently decomposes H_2O_2 to water and oxygen. The glutathione peroxidase (GPx) family—particularly GPx5, expressed in the epididymis, and the phospholipid hydroperoxide glutathione peroxidase (PHGPx/GPx4), abundant within the mitochondrial capsule—reduce both soluble and membrane-bound hydroperoxides at the expense of reduced glutathione (GSH). Thioredoxin/peroxiredoxin systems and glutaredoxins provide additional layers of redox regulation, particularly relevant to the disulfide bond formation required for sperm chromatin condensation [20, 21].

Non-Enzymatic Antioxidants: Seminal plasma is uniquely enriched with non-enzymatic antioxidants, including ascorbic acid (vitamin C), α-tocopherol (vitamin E), reduced glutathione (GSH), ubiquinol (coenzyme Q10), zinc, selenium, carnitines, and uric acid. These molecules act as sacrificial electron donors, intercepting and neutralizing ROS prior to their interaction with critical biological macromolecules. Ascorbic acid, present at concentrations approximately eight-fold higher in seminal plasma than in blood plasma, is particularly effective against aqueous-phase ROS, while α-tocopherol, embedded within lipid bilayers, provides the primary chain-breaking defense against propagating lipid peroxidation [21, 22]. Seminal zinc, secreted predominantly by the prostate gland, exerts antioxidant effects both by displacing redox-active transition metals from critical binding sites and by supporting the structural integrity of SOD.

Oxidative Stress Index and Clinical Assessment

The functional antioxidant status of the semen is increasingly characterized by the oxidative stress index (OSI), computed as the ratio of total oxidant status (TOS) to total antioxidant capacity (TAC). Elevated OSI values have demonstrated superior discriminative power for male infertility compared with individual ROS measurements or antioxidant assays in isolation [23]. Clinical assessment of seminal oxidative burden encompasses measurement of ROS levels via chemiluminescence or colorimetric assays, MDA/4-HNE quantification, 8-OHdG immunoassay in sperm DNA, sperm DFI, and individual antioxidant enzyme activities. The MiOXSYS system, a rapid point-of-care electrochemical assay measuring the oxidation-reduction potential (ORP) of the semen, represents a clinically practical advance, yielding results within minutes from unprocessed semen samples [24].

Clinical Associations and Etiological Contexts

Oxidative stress has been documented as a mechanistic common pathway in a diverse array of clinical conditions associated with male infertility. Varicocele—present in 35–40% of infertile men—is the most extensively characterized OS-associated andrological condition, with seminal ROS levels and lipid peroxidation biomarkers demonstrably elevated in affected individuals and significantly reduced following surgical or radiological repair, with concomitant improvements in conventional semen parameters and spontaneous pregnancy rates [25]. Male accessory gland infections (orchitis, epididymitis, prostatitis), whether acute or chronic, generate profound leukocyte-mediated oxidative insults, with seminal leukocyte concentrations correlating directly with ROS levels in clinical series.

Systemic conditions including type 2 diabetes mellitus, obesity, and the metabolic syndrome impose a state of systemic and local oxidative stress that measurably impairs spermatogenesis and post-testicular sperm maturation. Diabetic men exhibit elevated seminal MDA, reduced SOD and GPx activities, and increased sperm DFI, collectively indicative of OS-mediated male reproductive toxicity [26, 27]. Emerging evidence also implicates psychological stress—mediated through glucocorticoid receptor activation and hypothalamic-pituitary-adrenal axis dysregulation—in the suppression of antioxidant gene expression and the augmentation of ROS generation within the testis [28].

Exposure to environmental and occupational chemical hazards warrants particular clinical attention. Persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and bisphenol A (BPA), impair Sertoli and Leydig cell function and induce OS both directly and via endocrine disruption. Tobacco cigarette smoke delivers a complex mixture of ROS and depletes seminal antioxidants, with dose-dependent adverse effects on sperm motility, morphology, and DNA integrity conclusively established in meta-analytic evidence [29].

Diagnostic Approaches and Biomarker Development

The field of male reproductive medicine is witnessing a paradigm shift from the exclusive reliance on conventional semen analysis—encompassing sperm concentration, motility, and morphology as defined by WHO 2021 criteria—toward a more comprehensive, biochemically nuanced evaluation that incorporates OS biomarkers and functional sperm assessments. This transition is motivated by the recognition that approximately 15% of infertile men present with idiopathic infertility despite normal semen parameters, while many men with abnormal parameters retain fertility potential, suggesting that conventional analyses incompletely capture the functional reproductive capacity of the ejaculate [30].

Current biomarker development trajectories include seminal plasma proteomics and metabolomics analyses, which have identified panels of ROS-sensitive proteins and low-molecular-weight metabolites capable of discriminating fertile from infertile men with high sensitivity and specificity. MicroRNA (miRNA) profiling of seminal plasma exosomes has revealed ROS-regulated miRNA signatures that modulate post-transcriptional gene expression in recipient cells. Sperm mitochondrial membrane potential measurement via flow cytometry, and high-content image analysis of sperm chromatin integrity, are progressively transitioning from research tools to clinical laboratory capabilities. The clinical implementation of an integrated oxidative stress profile—incorporating ORP measurement, sperm DFI, and mitochondrial function assessment alongside conventional parameters—has been advocated as the standard of care for the comprehensive evaluation of male reproductive potential [2].

Therapeutic Strategies Targeting Oxidative Stress

Antioxidant Supplementation

Oral antioxidant supplementation represents the most widely investigated and clinically accessible pharmacological strategy for mitigating OS-mediated male infertility. Individually studied agents include vitamin C (ascorbic acid), vitamin E (α-tocopherol), zinc, selenium, coenzyme Q10, lycopene, folate, carnitines (L-carnitine and acetyl-L-carnitine), and N-acetyl cysteine (NAC). A Cochrane systematic review by Showell et al. encompassing 90 randomized controlled trials (n = 10,303) concluded that antioxidant supplementation may significantly increase live birth rates and clinical pregnancy rates compared with placebo, though the authors noted considerable heterogeneity in study design, supplementation regimens, outcome definitions, and patient selection criteria, limiting definitive conclusions [31].

The emerging consensus favors combination antioxidant formulations over monotherapy, based on the rationale that the biological antioxidant network operates synergistically—with vitamin C regenerating vitamin E from its radical form, selenium serving as an essential GPx cofactor, and zinc stabilizing SOD. Coenzyme Q10, functioning both as an electron carrier in the mitochondrial respiratory chain and as a lipid-phase antioxidant, has demonstrated consistent benefit in improving sperm motility and concentration, with one meta-analysis reporting significant improvements in total motility (weighted mean difference +9.37%) and DFI reduction following CoQ10 supplementation [32]. NAC, a glutathione precursor, has shown promise in men with varicocele-associated infertility, improving seminal TAC and sperm kinematics in controlled trials [33].

Surgical and Lifestyle Interventions: Varicocelectomy—whether performed via open, laparoscopic, or microsurgical subinguinal approaches—consistently reduces seminal OS biomarkers and improves sperm DFI, with natural pregnancy rates of 30–50% reported in observational series [25]. The integration of pre- and post-surgical antioxidant supplementation may potentiate the reproductive benefits of varicocele repair, an approach supported by preliminary evidence from several prospective trials. Optimization of lifestyle determinants of OS—smoking cessation, weight reduction, moderation of alcohol intake, avoidance of anabolic steroids and gonadotoxic medications, and heat exposure reduction—represents a foundational and evidence-based component of male fertility management, with measurable improvements in semen parameters and OS biomarkers documented following behavioral modification [34].

ART-Specific Applications

In the context of assisted reproductive technology, OS exerts clinically relevant effects on gamete quality and embryonic development. Sperm preparation techniques for IVF and ICSI inherently generate iatrogenic ROS through centrifugation, temperature fluctuation, and exposure to atmospheric oxygen. The supplementation of sperm preparation and culture media with antioxidants—including reduced glutathione, melatonin, vitamin C, and pentoxifylline—has been explored as a strategy to mitigate processing-induced OS, with several studies reporting improved embryo quality and clinical pregnancy rates. Cryopreservation of spermatozoa is particularly associated with OS-mediated damage, as freeze-thaw cycles disrupt mitochondrial integrity and antioxidant enzyme activity; antioxidant-supplemented cryoprotectant media represent an active area of translational research [3].

Emerging Research Directions and Translational Perspectives

The interface between oxidative stress, epigenetics, and paternal inheritance of reproductive phenotypes constitutes an area of intense contemporary investigation. Oxidative modifications of sperm DNA—including 8-OHdG formation and strand breaks—may escape repair in the fertilized oocyte, persisting as somatic mutations in embryonic and fetal tissues and potentially conferring transgenerational health consequences [35]. Furthermore, ROS-mediated alterations in sperm epigenetic programming, encompassing aberrant DNA methylation patterns at imprinted loci and modifications of sperm-borne non-coding RNAs, may influence embryonic gene expression and offspring phenotypes independently of DNA sequence changes, a concept increasingly supported by experimental evidence from murine models.

Nanotechnology-based antioxidant delivery systems—including nanoparticle-encapsulated curcumin, resveratrol-loaded liposomes, and selenium nanoparticles—are under preclinical investigation for targeted intratesticular and intraseminal antioxidant delivery, aiming to overcome the pharmacokinetic limitations of conventional oral supplementation. The application of high-throughput multi-omics platforms (genomics, transcriptomics, proteomics, metabolomics) to seminal samples from well-phenotyped infertile cohorts holds promise for the identification of redox-sensitive biological signatures that could serve as precision diagnostic and therapeutic stratification tools. Finally, the gut microbiome-reproductive axis has emerged as a novel potential modulator of systemic and local OS, with probiotic supplementation demonstrating preliminary evidence of seminal antioxidant enhancement in early clinical investigations.

CONCLUSIONS

Oxidative stress occupies a central and mechanistically validated position in the pathophysiology of male infertility, operating across a continuum from subtle functional impairment of normozoospermic men to severe spermatotoxicity in oligoasthenoteratozoospermia. The intrinsic vulnerability of spermatozoa to ROS-mediated damage—rooted in their membrane lipid composition, compacted chromatin, and attenuated antioxidant and repair capacity—renders the seminal redox environment a critical determinant of male reproductive outcome. Advances in the clinical quantification of OS burden, including ORP measurement, sperm DFI assessment, and multi-parametric seminal oxidative profiling, are progressively enabling the integration of oxidative evaluation into routine andrological practice.

Therapeutic strategies targeting OS—encompassing lifestyle modification, surgical correction of anatomical contributors such as varicocele, and targeted antioxidant supplementation regimens—collectively represent a biologically rational and clinically actionable approach to male infertility management. Nevertheless, the field is constrained by the heterogeneity and methodological limitations of existing clinical trials, the absence of standardized OS assay protocols, and a paucity of adequately powered randomized controlled trials employing clinically meaningful reproductive endpoints such as live birth rate. Future progress necessitates the establishment of validated, clinically standardized OS biomarker panels, the conduct of large multicenter randomized trials with harmonized intervention protocols, and the elucidation of gene-environment interactions that modulate individual susceptibility to OS-mediated reproductive impairment. The emerging insights into OS-driven epigenetic reprogramming and transgenerational effects further underscore the imperative of addressing male seminal oxidative stress not merely as a determinant of immediate fertility, but as a modifiable contributor to offspring health across generations.

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