

Review

Deciphering the Role of Oxidative Stress in Male Infertility: Insights from Reactive Oxygen Species to Antioxidant Therapeutics

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Abstract

Male infertility represents a major health concern, accounting for approximately 50% of all infertility cases in couples. This condition arises from multiple etiologies, with oxidative stress gaining increasing attention in recent studies. During the final stages of sperm maturation, the majority of the cytoplasm is discarded, leaving sperm with a diminished antioxidant defense system, which makes them highly susceptible to the detrimental effects of reactive oxygen species (ROS). ROS can be generated from both intrinsic and extrinsic sources. Intrinsically, ROS are primarily produced by mitochondrial activity, while extrinsic factors include alcohol consumption, smoking, circadian rhythm disruption, gut microbiota imbalance, and leukocyte infiltration. Excessive ROS production leads to DNA damage, apoptosis, and epigenetic modifications in sperm, ultimately impairing sperm motility and contributing to infertility. This review provides a comprehensive examination of ROS sources and examines the mechanisms by which ROS induce sperm damage. Furthermore, it explores the therapeutic potential of antioxidants in mitigating oxidative stress and improving sperm quality.

Keywords: male infertility; oxidative stress; reactive oxygen species; sperm function; antioxidants

1. Introduction

Male infertility is defined as the inability to achieve pregnancy in a female partner after one year or more of regular unprotected intercourse, attributed to male factors [1]. Approximately 50% of infertility cases in couples are due to male infertility [2]. The causes of male infertility are varied, including: (1) spermatogenic dysfunction, such as oligoasthenoteratozoospermia, (2) genetic disorders, such as Y chromosome microdeletions and androgen insensitivity syndrome, (3) varicocele, (4) ejaculatory disorders, and (5) environmental factors, such as exposure to high temperatures and heavy metals [3–5]. Semen analysis is essential for the evaluation of male infertility, with research showing significantly elevated levels of reactive oxygen species (ROS) in the sperm from infertile men, suggesting that oxidative stress may play a role in the pathogenesis of male infertility [6]. ROS have been implicated in the peroxidative damage to sperm plasma membranes in infertile men [7]. Such damage impairs sperm motility and membrane fusion capabilities, both of which are critical for fertilization [8].

In 1943, MacLeod [9] first demonstrated that human sperm could generate ROS, specifically hydrogen peroxide. Subsequent research identified the superoxide anion as the primary ROS produced by human sperm, which is rapidly

converted to hydrogen peroxide by the enzyme superoxide dismutase (SOD) within the cell [10]. In addition to hydrogen peroxide and superoxide anions, other ROS, such as hydroxyl radicals and nitric oxide radicals, also play significant roles in sperm function [11]. Low levels of ROS are essential for normal sperm activities, including fertilization capability (acrosome reaction, hyperactivation, capacitation, and chemotaxis) and sperm motility [12]. However, as shown in Fig. 1, excessive ROS levels can induce to increased sperm apoptosis, thereby elevating the risk of male infertility [13]. Sperm are particularly sensitive to oxidative stress, as they lose most of their cytoplasm during the later stages of differentiation, resulting in a significant reduction in [14]. This deficiency in the antioxidant system not only heightens oxidative stress but also leaves sperm highly vulnerable to ROS-induced damage, including mitochondrial and nuclear DNA damage, DNA fragmentation, telomere shortening, and Y chromosome microdeletions [15], which further exacerbate the risk of male infertility [16]. Additionally, sperm cell membranes are rich in polyunsaturated fatty acids (PUFAs), making them highly susceptible to ROS-induced damage [17]. External factors, such as aging, air pollution, smoking, high-temperature environments, and alcohol consumption, further contribute to oxidative stress, thereby increasing the risk of male infertility [18–21].



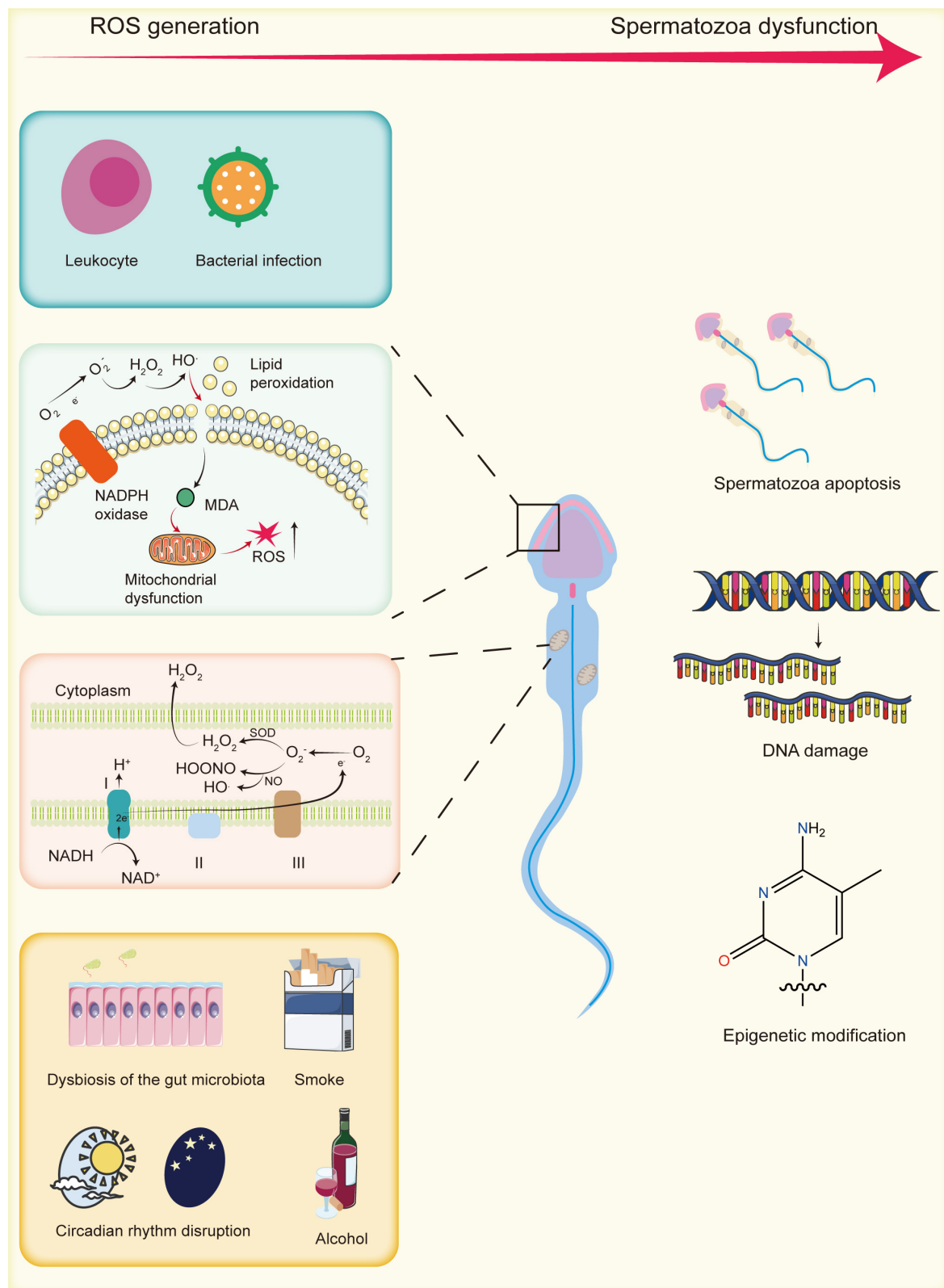


Fig. 1. The generation of reactive oxygen species (ROS) and their impact on sperm. Endogenous ROS primarily originate from mitochondria, lipid peroxidation, and white blood cells. Exogenous sources include gut microbiota alterations, smoking, circadian rhythm disruptions, and alcohol consumption. Excessive ROS can cause sperm DNA damage, epigenetic modifications, and apoptosis, thereby contributing to male infertility. MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; NADH, nicotinamide adenine dinucleotide; SOD, superoxide dismutase. Created by using Adobe Illustrator (version 28.7.1, Adobe Systems Incorporated, San Jose, CA, USA).

This review provides a comprehensive analysis of the sources of ROS in sperm, addressing both endogenous and exogenous origins. Endogenous ROS include those produced by mitochondria and through lipid peroxidation, while exogenous sources stem from factors such as gut microbiota, inflammation, and lifestyle habits (e.g., smoking, circadian rhythm disruption, and alcohol consumption). The review also explores how ROS contribute to reduced sperm motility by inducing DNA damage, inducing apoptosis, and alterations in histone modifications and DNA methylation. Furthermore, it examines the potential role of antioxidants in enhancing sperm motility, offering valuable insights into oxidative stress, male infertility, and sperm vitality, while advocating for the application of antioxidants in male infertility treatment.

2. Sources of Oxidative Stress: Intrinsic Factors

Oxidative stress arises from an imbalance between pro-oxidants and antioxidants, favoring oxidizing agents. In the reproductive system, oxidative stress occurs when free radical production surpasses the body's antioxidant defense capacity. Research has demonstrated that the levels of superoxide anions and peroxynitrite in the semen of infertile men are significantly elevated compared to fertile men, with a positive correlation between oxidative stress levels and sperm DNA fragmentation [22]. Sperm DNA fragmentation is linked to various adverse clinical outcomes, including reduced fertility and higher miscarriage rates [23]. Therefore, ROS levels are closely associated with fertilization rates and sperm physiological functions [24]. The sources of oxidative stress in sperm can be categorized into endogenous and exogenous origins. Endogenous sources involve the oxidative stress caused by an excess of ROS generated from intracellular metabolic activities and immune responses. The sources primarily include the mitochondrial electron transport chain, lipid peroxidation, and leukocytes.

2.1 Mitochondrial Contribution to ROS Generation and Sperm Oxidative Stress

Mitochondria, in addition their role in adenosine triphosphate (ATP) production through oxidative phosphorylation, participate in various intracellular physiological processes, including ROS generation [25]. Research has indicated that human sperm motility depends more on glycolysis than on mitochondrial ATP production [26]. However, damage to the mitochondrial structure or the mitochondrial genome significantly impacts sperm motility. Further study has shown that sperm mitochondria is closely linked to ROS production [27]. During cellular respiration, the mitochondrial electron transport chain (ETC), located on the inner mitochondrial membrane, plays a key role in energy production. As electrons traverse the ETC, they can leak and interact with oxygen molecules to form superoxide anions,

primarily occurring at Complex I and Complex III. ROS generated by Complex I are the main contributors to oxidative damage in human sperm [28]. Additionally, flavoenzymes in the mitochondrial electron transport chain, such as electron-transferring flavoprotein, have also been implicated in ROS production [29]. Changes in environmental conditions can also induce mitochondrial ROS production. Sperm experience significant oxidative stress during freezing and thawing, leading to a decline in sperm quality [30]. Studies suggest that cryopreservation results in osmotic changes, with mitochondria being more susceptible to damage from osmotic shifts than the sperm plasma membrane, further triggering ROS production [31,32]. Due to their charged nature, superoxide anions have limited ability to cross mitochondrial membranes. Within mitochondria, SOD converts superoxide anions into hydrogen peroxide [33]. Hydrogen peroxide can oxidize the thiol groups at the active site of glyceraldehyde-3-phosphate dehydrogenase (GAPDS), a key enzyme in the glycolytic pathway, explaining the observed decline in ATP production in sperm cells as ROS levels rise [34]. Moreover, superoxide anions can react with nitrogen oxides to form peroxynitrite, which can dissociate into nitrogen dioxide and hydroxyl radicals [35].

2.2 Lipid Peroxidation and Oxidative Stress

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a membrane-bound enzyme, is present on the sperm membrane. Under the influence of NADPH oxidase, NADPH acts as an electron donor, transferring its electrons to oxygen, which leads to the generation of superoxide anions and other ROS, including hydrogen peroxide and hydroxyl radicals [36]. These ROS further oxidize the lipids in the sperm plasma membrane, initiating a cascade of lipid peroxidation reactions [37]. The sperm plasma membrane is rich in PUFAs, which contain multiple carbon-carbon double bonds, offering numerous sites for radical oxidation. Additionally, the bis-allylic methylene positions in PUFAs have low C-H bond dissociation energies, making them particularly susceptible to oxidation. ROS produced by NADPH oxidase oxidize PUFAs, generating lipid radicals, which then react with oxygen to form peroxyl or alkoxyl radicals. These radicals stabilize by abstracting hydrogen atoms from adjacent carbon atoms, perpetuating the chain reaction of lipid peroxidation. This process results in the formation of various reactive aldehydes, such as 4-hydroxynonenal and malondialdehyde [38]. These aldehydes can bind to mitochondrial proteins, altering their conformation, leading to electron leakage and further ROS production [37].

2.3 Inflammation and Oxidative Stress

In addition to ROS generated by sperm, semen ROS levels are also influenced by the presence of leukocytes.

An increase in leukocyte concentration in semen raises nitrite levels, thereby diminishing the semen's antioxidant capacity [39]. WHO guidelines define leukocytospermia as a leukocyte $\geq 1 \times 10^6$ cells/mL. However, even when leukocyte counts fall below this threshold, ROS levels in semen may still parallel to those observed in leukocytospermia [40]. Leukocyte-derived ROS production is associated with inflammation and infection. Under such conditions, leukocytes activate the myeloperoxidase system, catalyzing the reaction between hydrogen peroxide and halides to form hypochlorous acid, a potent oxidant with bactericidal properties [41]. Aitken and West's study [42] demonstrated that ROS levels rise significantly when leukocyte concentrations exceed 1×10^6 per 10^7 sperm. As this study lacked an effective method for quantifying leukocytes, Kessopoulou *et al.* [43] further validated the relationship between leukocyte concentration and semen ROS levels, utilizing antibody-coated magnetic beads to isolate leukocytes. Their results confirmed that ROS levels in semen are predominantly originate from leukocytes, with leukocyte-derived ROS being at least 100 times higher than those produced by sperm [43]. This indicates that oxidative stress in semen is primarily attributable to leukocytes rather than sperm.

3. Origins of Oxidative Stress: External Influences

In addition to the intrinsic ROS production, various external environmental factors, such as shifts in the microbiota, microbial infections, tobacco use, disturbances in circadian rhythms, and alcohol intake, can contribute to elevated ROS levels. These external factors generate free radicals, collectively termed extrinsic ROS. Together, they contribute to extrinsic oxidative stress, where these harmful environmental influences enhance free radical production, exceeding the capacity of the body's antioxidant defense systems and resulting in oxidative damage. Research indicates demonstrated that male infertility arises from a combination of environmental influences and genetic factors. Therefore, understanding the relationship between environmental influences and ROS is essential for advancing therapeutic strategies for male infertility.

3.1 Microbiota and Oxidative Stress

Recent research has increasingly focused on the microbiota's role in human health. Evidence suggests that men with male infertility exhibit significant imbalances in the composition and functionality of the microbiota in both the gastrointestinal and reproductive tracts [44]. The gut, acting as a crucial conduit between the body and the external environment at both the oral and anal openings, serves as an "endocrine organ" influencing various physiological functions. Notably, research by Tremellen *et al.* [45] has demonstrated that compromised gut mucosal barrier integrity can lead to the translocation of gut microbiota into

systemic circulation, which may reduce testosterone levels in men of reproductive age. Modulating the gut microbiota with oligosaccharides has been shown to improve lipid metabolism, which in turn ameliorates sperm quality and the testicular microenvironment. Concurrently, an enhancement in the antioxidant capacity within the testes and blood has been observed [46]. These observations highlight the gut microbiota's ability to modulate the redox balance in both the blood and the testicular microenvironment, a process known as the gut-testis axis [47].

Gut microbes and their metabolites, such as lipopolysaccharide (LPS), can enter the systemic circulation as microbial-associated molecular patterns (MAMPs) and subsequently reach the testes *via* the testicular arteries. In the testes, they are recognized by Toll-like receptors (TLRs) on sperm, triggering the production of pro-inflammatory cytokines, including TNF- α and IL-1 β (Fig. 2) [48]. This inflammatory response, mediated by the activation of the xanthine oxidase system, can lead to testicular damage through the enhanced production of ROS [49]. Additionally, LPS has been shown to activate the mitochondrial transcription factor A (TFAM), causing its translocation from the sperm head to the midpiece, and stimulating the expression of cytochrome c oxidase subunits, key components of the mitochondrial electron transport chain. This upregulation results in increased mitochondrial oxidative phosphorylation and a corresponding rise in mitochondrial ROS production [50]. Furthermore, the gut microbiota can regulate lipid droplet accumulation and facilitate the conversion of PUFAs to stearic acid, thereby reducing PUFA levels [51]. PUFAs are known to promote the expression of SOD, which mitigates ROS levels [52]. A meta-analysis has shown that elevated ROS can act as a molecular initiators, contributing to the disruption of the blood-testis barrier and the dysregulation of the gut microbiota at the cellular level [53].

In addition to the influence of the gut microbiota, approximately 15% of male infertility cases are associated with microbial infections within the urogenital system [54]. Clinically, the presence of more than 1000 CFU/mL of semen in aerobic culture conditions is considered indicative of bacterial prostatitis. Gram-negative urethral pathogens have been identified as agents that diminish sperm motility and viability, compromise the integrity of the sperm plasma membrane, and escalate intracellular ROS levels [55–57]. Alongside the elevation of ROS *via* LPS, an increase in leukocyte counts has been observed in the semen of patients with bacterial prostatitis [58], further contributing to ROS production in the semen.

3.2 The Impact of Lifestyle on ROS and Male Infertility

3.2.1 The Impact of Smoking on Oxidative Stress

Although the precise effect of smoking on male fertility remains uncertain, research indicates that smoking impairs sperm motility and antioxidant capacity [59]. A meta-

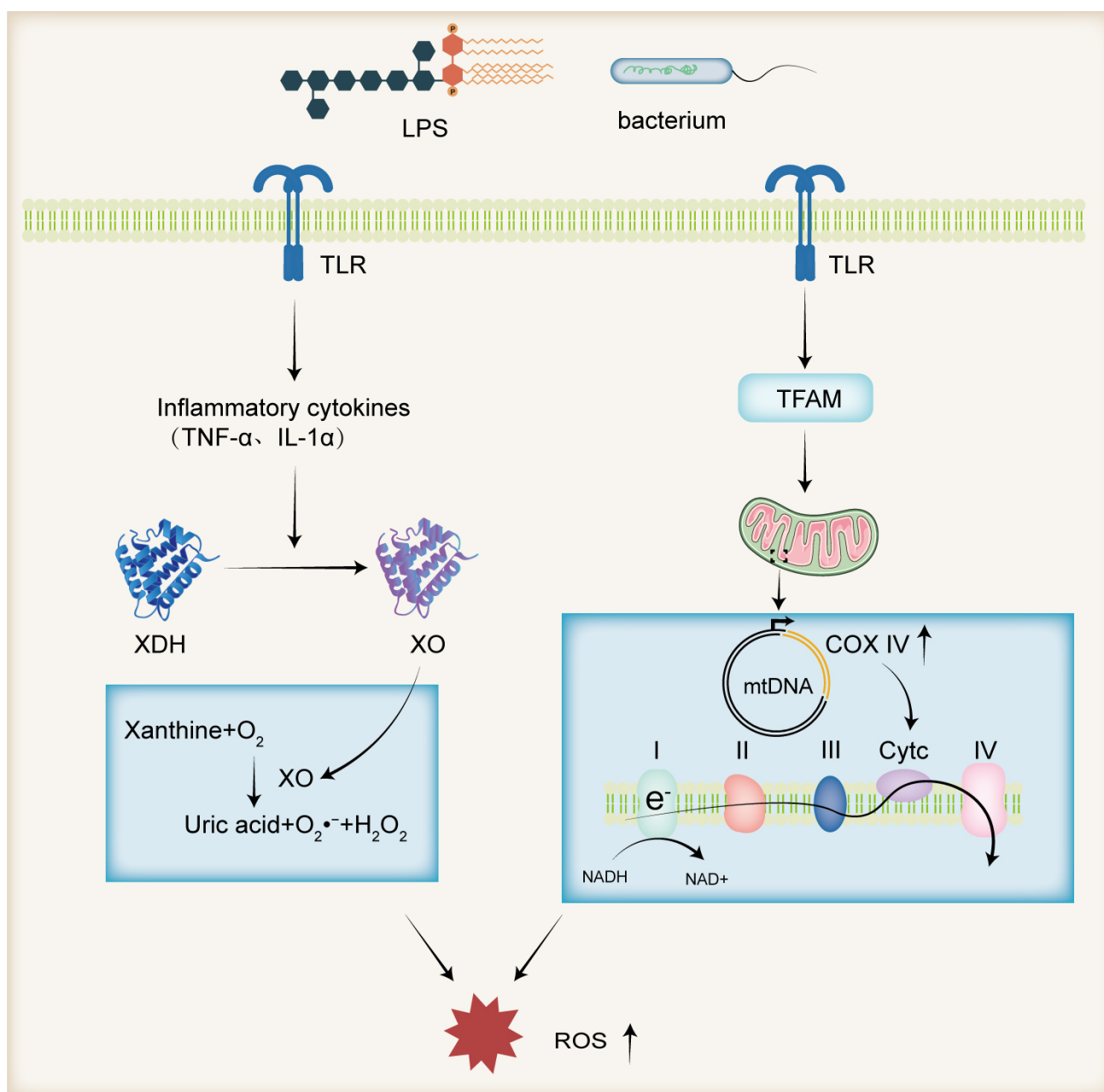


Fig. 2. Gut Microbiota and ROS Production. Gut microorganisms and their metabolite LPS function as MAMPs, which are recognized by TLRs on the surface of sperm. This recognition induces the production of pro-inflammatory cytokines such as TNF- α and IL-1 α , which can activate the xanthine oxidase system, thereby enhancing ROS production. Additionally, LPS activates TFAM, which increases the expression of cytochrome c oxidase subunit IV (COX IV), thereby enhancing mitochondrial oxidative phosphorylation and raising ROS levels. LPS, lipopolysaccharide; MAMPs, microbial-associated molecular patterns; TLRs, Toll-like receptors; TFAM, mitochondrial transcription factor A; COX IV, cytochrome c oxidase subunit IV; XDH, xanthine dehydrogenase; XO, xanthine oxidase; Cyt c, cytochrome c. Created by using Adobe Illustrator (version 28.7.1, Adobe Systems Incorporated, San Jose, CA, USA).

analysis of 20 studies involving 5865 participants revealed that smoking adversely affects semen parameters, notably reducing sperm count and motility, with the correlation intensifying as smoking intensity increases [60]. Additionally, even smokeless cigarettes exhibit a dose-dependent decline in sperm parameters [61]. Cigarettes contain a variety of toxic chemicals, including significant amounts of reactive free radicals and ROS, which damage lipids,

proteins, and nucleic acids, contributing to infertility [62]. Moreover, secondhand smoke exposure has been shown to elevate ROS levels in tissues, resulting in DNA and methylation damage [63]. Increased oxidative stress from smoking may also correlate with reduced seminal plasma zinc levels. Zinc, a coenzyme for numerous enzymes, plays a critical role in antimicrobial activities and inhibition of cellular ROS production [64]. Kumosani *et al.* [65] found

that smoking reduces zinc levels in seminal plasma, heightening oxidative stress and negatively affecting sperm density and motility. Furthermore, Arabi and Moshtaghi [66] demonstrated that, in addition to directly increasing ROS production, sperm from smokers exhibit increased sensitivity to peroxides, rendering them more vulnerable to oxidative damage.

3.2.2 Circadian Disruption and its Impact on Oxidative Stress

Sleep deprivation has been shown to negatively affect male fertility. In an experiment involving rats subjected to sleep deprivation for either 4 days or 7 days, Choi *et al.* [67] found a significant decrease in sperm motility in the group deprived of sleep for 7 days, although this association was not seen in the other groups. However, all groups exhibited a significant decline in testosterone levels [67], which has been reported to lead to sexual dysfunction [68]. These observations underscore the close relationship between sleep and male fertility. Sleep plays a crucial role in ROS elimination, with ROS being produced during the day and cleared during sleep. Insufficient sleep significantly elevates ROS levels in the body, triggering oxidative stress [69]. In a Wistar rat model, peripubertal sleep restriction (21 days, 18 h/day) significantly increased lipid peroxidation, glutathione levels, and total radical-trapping antioxidant potential, decreased neutrophil migration and epithelial compartment size, and ultimately impaired epididymal development and sperm motility [70]. To explore the relationship between sleep patterns and male fertility, Lu *et al.* [71] conducted on a Mendelian randomization study, highlighting the potential influence of chronotype on testosterone secretion, a key hormone in male reproductive health. In contrast, no substantial causal associations were found between other sleep-related factors, such as sleep duration or insomnia, and male fertility potential [71]. This distinction suggests that circadian preferences play a more critical role in fertility than other sleep characteristics. It is worth noting that circadian rhythm disruptions have also been reported to be associated with decreased female fertility [72]. These findings indicate that circadian rhythm imbalance, rather than sleep duration, is likely the primary factor affecting male fertility *e* lipid peroxidation and oxidative stress.

3.2.3 Alcohol Intake and Oxidative Stress

Case report demonstrates that long-term severe alcohol intake (approximately 165 g of alcohol per day, over a period of 10 years) significantly impacts male fertility and ultimately leads to azoospermia [73]. Akang *et al.* [74] demonstrated that alcohol-treated mice exhibited substantially elevated levels of testicular malondialdehyde and DNA fragmentation, along with reduced SOD activity and glutathione content, indicating that alcohol induces infertility by increasing oxidative stress. Alcohol consumption can also cause mitochondrial DNA damage, disrupting mi-

tochondrial function and exacerbating mitochondrial ROS production [75]. This effect may arise from alcohol's ability to alter the structure of mitochondrial ribosomes, impairing mitochondrial protein synthesis [76] and affecting the translation of oxidative phosphorylation-related proteins. As a result, mitochondrial DNA undergoes oxidative modifications, antioxidant defenses are depleted, and glutathione levels are reduced, ultimately leading to cell apoptosis [77]. Additionally, chronic alcohol consumption induces the expression of cytochrome P450 enzymes, particularly CYP2E1, which increases NADPH oxidase activity in cells, thereby enhancing ROS production [78].

3.2.4 Diet-Induced ROS and its Impact on Male Fertility

Over the past few decades, obesity resulting from high-fat and high-calorie diets has been shown to significantly impair male fertility [79]. Research indicates that the increase in adipose tissue promotes insulin resistance, which, in turn, reduces sperm uptake and metabolism of glucose [80]. Disruption of glucose homeostasis leads to an increase in leptin concentrations in seminal plasma, which impairs sperm quality by promoting the generation of ROS [81]. Furthermore, excessive adiposity has been reported to enhance the activity of aromatase, which facilitates the conversion of testosterone to estradiol, thereby reducing testosterone levels. Lower testosterone levels have been associated with mitochondrial dysfunction in Leydig cells [82]. Mitochondrial dysfunction leads to oxidative damage to mitochondrial DNA and further promotes ROS production [82]. Additionally, high-energy diet-induced pre-diabetic Wistar rat model demonstrated a reduction in testicular antioxidant capacity and mitochondrial DNA copy numbers [83]. This study also demonstrated that high-calorie diets reduce the expression of testicular PGC-1 α and SIRT3 [83]. PGC-1 α is essential for the induction of ROS detoxifying enzymes under oxidative stress conditions; it increases the expression of GPx1 and SOD2, thereby inhibiting ROS production [84]. SIRT3 has also been shown to deacetylate respiratory enzymes to regulate mitochondrial function, with its loss leading to increased ROS generation [85]. The knockout of SIRT3 has been demonstrated to alleviate the antioxidant effects of PGC-1 α , resulting in increased ROS levels, indicating that SIRT3 functions as a downstream target gene of PGC-1 α , mediating its regulation of cellular ROS production and mitochondrial function [86].

3.2.5 Exercise-Induced ROS and its Effect on Sperm Quality

An increasing body of evidence suggests that a sedentary lifestyle predisposes men to hypogonadism and reduced sperm motility [87], whereas physically active men exhibit a more favorable anabolic hormonal environment and healthier semen quality [88]. Moderate physical exercise has been shown to alleviate inflammation and DNA damage in sperm [89]. For instance, a study involving 159 infertile men and 143 fathers revealed that a lack of

physical activity and excess body fat relative to age-related norms were positively correlated with infertility [90]. Men engaging in outdoor activities (≥ 1.5 hours per week) and weightlifting (≥ 2 hours per week) had sperm concentrations that were 42% and 25% higher, respectively, while men who cycled for ≥ 1.5 hours per week had sperm concentrations 34% lower than non-cycling counterparts, suggesting that different types of exercise can have varying impacts on sperm quality [91]. Moreover, a study on 16 weeks of high-intensity cycling training demonstrated a significant increase in ROS and malondialdehyde (MDA) levels in the semen, with elevated levels persisting even 30 days post-recovery. Simultaneously, semen levels of SOD, catalase, and Total antioxidant capacity (TAC) remained lower even after the 30-day recovery period [92]. These findings suggest that cycling, particularly high-intensity cycling, has a detrimental effect on sperm, possibly through increased ROS production and reduced antioxidant capacity.

3.3 Environmental Exposures and their Impact on ROS-Induced Male Fertility Disruption

One of the major sources of environmental ROS is the heat stress effect on the reproductive organs [93]. Study on cryptorchid mice has shown that exposure of the testes to abdominal temperature results in an increase in ROS production [94]. Research by Ikeda *et al.* [95] demonstrated that after exposing testicular cells to 43 °C for 1 hour, followed by a 23-hour incubation at 32.5 °C, intracellular peroxide levels were elevated, and H₂O₂ could attenuate heat stress-induced apoptosis. These findings indicate that heat stress can elevate testicular oxidative stress, leading to apoptosis of testicular cells. The increase in environmental temperature raises the metabolic rate of the testes, which consequently increases the oxygen demand of the tissue, resulting in higher ROS production [96]. Furthermore, the study by Paul *et al.* [97] demonstrated that heat-stressed mouse testes exhibited enhanced HIF1A expression, which in turn promoted the upregulation of antioxidant enzymes, such as heme oxygenase 1, to counteract the increased oxidative stress.

Airborne pollutants have also been shown to affect sperm membrane integrity, increasing the generation of free radicals and promoting oxidative stress, which negatively impacts sperm parameters [98]. Phthalates, commonly found in food packaging materials and personal care products, have been reported to induce oxidative stress and lead to sperm DNA damage [99]. Pant *et al.* [100] observed that the levels of di(2-ethylhexyl) phthalate in semen were negatively correlated with sperm quality and positively correlated with mitochondrial depolarization, increased ROS production, lipid peroxidation, and DNA fragmentation. This suggests that phthalates may deteriorate semen quality through ROS, lipid peroxidation, and mitochondrial dysfunction [100]. Another environmental pollutant, heavy metals, has been implicated in oxidative stress. Previous research has demonstrated that mice injected in-

traperitoneally with lead acetate exhibit a significant increase in peroxidative potential within testicular tissue, accompanied by reduced testicular weight, an increased incidence of abnormal sperm, and a decreased total sperm count [101]. Additionally, the concentrations of lead and cadmium in seminal plasma have been significantly correlated with biomarkers of oxidative DNA damage, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), indicating that these metals exacerbate oxidative stress in sperm and contribute to sperm DNA damage [102].

4. Mechanisms of Oxidative Stress-Induced Sperm Damage

4.1 Oxidative Stress and DNA Damage

ROS originating from sperm cells or the external environment can disrupt the redox balance within sperm cells, resulting in increased oxidative stress and subsequent DNA damage. Sperm DNA damage can be categorized into two types based on its location: intratesticular and post-testicular. Intratesticular DNA damage occurs during the chromatin remodeling and apoptosis processes in sperm maturation, serving as a primary factor affecting sperm DNA integrity. Post-testicular DNA damage occurs as sperm traverse the reproductive tract. Both types of damage are associated with increased oxidative stress-induced damage to nuclear and mitochondrial DNA in sperm. Oxidative stress-induced sperm DNA damage encompasses DNA fragmentation, mitochondrial DNA damage, telomere shortening, Y chromosome deletions, and epigenetic abnormalities [15]. These alterations lead to genomic instability, replication errors, and transcriptional arrest [103]. ROS-induced DNA damage also exacerbates sperm apoptosis, reducing male fertility [104].

A significant correlation exists between ROS levels and sperm chromosomal telomere DNA length. Under severe oxidative stress (ROS >35 RLU/s/million sperm), telomere DNA undergoes attack by oxygen radicals, resulting in telomere shortening [105]. This is attributed to the guanine-rich repeat sequences at the 3' end of telomere DNA, which have a low redox potential, making them highly susceptible to radical attack [106]. These guanine repeat sequences are essential for telomerase-mediated telomere extension. ROS-induced damage to the telomere DNA prevents its repair and elongation [107]. Noblanc *et al.* [108] demonstrated that oxidative damage to sperm DNA predominantly occurs in the less condensed regions at the periphery of the cell nucleus. Under oxidative stress, guanine bases are attacked, leading to the oxidation of the carbon at position 8, forming 8-oxoguanine and 8-OHdG [109]. In sperm from infertile men, 8-OHdG levels are significantly elevated compared to the normal population [110]. 8-OHdG can pair with adenine, causing G to T base substitutions during DNA replication [111]. In sperm nuclei and mitochondria, 8-OHdG is excised and released extracellularly by 8-oxoguanine DNA glycosylase 1 (OGG1),

facilitating DNA damage repair [112]. Additionally, it has been reported that mice with triple knockout of OGG1, MTH1, and MUTYH exhibit a marked increase in G to T mutation frequency, with 99% of mutations attributed to G to T transversions caused by 8-oxoG [113]. The activity of 8-oxoguanine DNA glycosylase 1 is inhibited by cadmium (II) ions, consistent with previous findings on cadmium's effects on male fertility [114]. Sperm cells have limited DNA repair capabilities due to the absence of purine nucleoside endonuclease 1 (APEX1) and X-ray repair cross-complementing protein 1 (XRCC1), essential for downstream base excision repair pathways. Although APEX1 and XRCC1 are present in oocytes, enabling sperm DNA repair within the oocyte, this repair capacity depends on oocyte quality and the type of sperm DNA damage [115]. Incomplete pairing caused by 8-OHdG can lead to the formation of abasic sites, disrupting the ribose-phosphate backbone of DNA and resulting in DNA fragmentation [116]. DNA fragmentation is significantly higher in infertile men compared to fertile men [6], with the DNA fragmentation index showing a significant negative correlation with sperm motility. A higher DNA fragmentation index is associated with lower clinical pregnancy rates [117]. DNA fragmentation, commonly used in clinical practice alongside other sperm parameters, serves as a key assessment of male fertility [118].

4.2 Oxidative Stress-Induced Apoptosis in Sperm Cells

Programmed cell death of germ cells is vital for normal development. Apoptosis eliminates abnormal or excess cells, enabling growth factors to promote the growth of neighboring cells and maintaining the stability of germ cell populations [119]. However, dysregulated apoptosis can result in sperm cell death, exacerbating male infertility [120].

Oxidative stress induces apoptosis through multiple pathways. As illustrated in Fig. 3, excessive ROS cause DNA damage, which triggers the phosphorylation of p53. Due to the limited DNA repair capacity in sperm cells, damaged DNA remains unrepaired, leading to further phosphorylation and activation of p53. This, in turn, activates the pro-apoptotic protein Bax, initiating the mitochondrial-dependent (intrinsic) apoptosis pathway [121]. In this pathway, cytochrome C within the mitochondria plays a pivotal role in initiating intrinsic apoptosis [122]. Emokpa and Chima [123] observed that the total antioxidant capacity of semen in infertile men declines significantly with age, while apoptotic markers such as cytochrome C and caspase-3 show a positive correlation with increasing age. In response to apoptotic signals such as ROS, pro-apoptotic proteins from the Bcl-2 family, including Bax and Bak, translocate from the interior of the mitochondria to the outer mitochondrial membrane. These proteins oligomerize or interact with the mitochondrial permeability transition pore to form channels [124]. These channels allow small molecules

like cytochrome C to pass through the inner mitochondrial membrane into the cytoplasm, where cytochrome C binds to and activates Apaf1. This, in turn, activates caspase-9 [125]. Activated caspase-9 triggers a cascade of downstream caspases, including caspase-3, -6, and -7, which degrade intracellular proteins and ultimately lead to apoptosis [126].

ROS can also promote apoptosis through the MAPK pathway. Under normal physiological conditions, ASK1 is inhibited by its binding to Trx, preventing stress-induced apoptosis [127]. However, in the presence of ROS, Trx undergoes oxidation, leading to the release of ASK1. As a member of the MAPK family, ASK1 phosphorylates downstream c-Jun N-terminal kinase (JNK) and p38 MAPK [128]. JNK induces apoptosis by activating pro-apoptotic Bcl-2 family members, Bax and Bak [129]. p38 MAPK regulates apoptosis by modulating the balance between anti-apoptotic (Bcl-2, Bcl-xL, Mcl-1) and pro-apoptotic (Bak, Bax, Bad, Bid) Bcl-2 family members [130], and also by activating caspase-3, thereby promoting cell death [131]. In infertile men, elevated levels of BAX and significantly reduced levels of BCL2 have been reported in semen [132].

In addition to the mitochondrial-dependent pathway, ROS also contribute to the extrinsic apoptosis pathway, which is mediated by the activation of death receptors such as Fas. Upon binding with FasL, Fas trimerizes and recruits the adaptor protein FADD through its death domain [133]. FADD then recruits initiator caspase-8, forming the death-inducing signaling complex (DISC). DISC directly cleaves and activates caspase-3 to initiate apoptosis [134], and also cleaves the pro-apoptotic Bcl-2 family protein BID, thereby activating the mitochondrial-dependent apoptosis pathway [135].

4.3 Epigenetic Modifications in Male Germ Cells: Impact of Oxidative Stress on Histone and DNA Methylation

Epigenetic changes involve modifications in gene activity without altering the underlying DNA sequence, thereby affecting cellular functions and developmental processes [136]. Among these, histone modifications represent a key epigenetic regulatory mechanism, which can be influenced by oxidative stress [137,138]. In male germ cells, genomic DNA is organized into nucleosomes, which are octameric complexes composed of histones H2A, H2B, H3, and H4 [139]. Various post-translational modifications (PTMs) on histones influence the stability of the nucleosome octamer, driving chromatin conformation changes critical for spermatogenesis [140]. Methylation, a prominent PTM, typically occurs on lysine and arginine residues, with key sites including H3K4, H3K9, and H3K27 [141]. ROS have been shown to regulate histone methylation by activating the expression of SET7, a histone H3K4 methyltransferase [142]. The NADPH oxidase (NOX) family, a primary source of intracellular ROS, catalyzes the production of superoxide anions. Zhou *et al.* [143] demonstrated that the NOX inducer, succinate, enhances H3K4

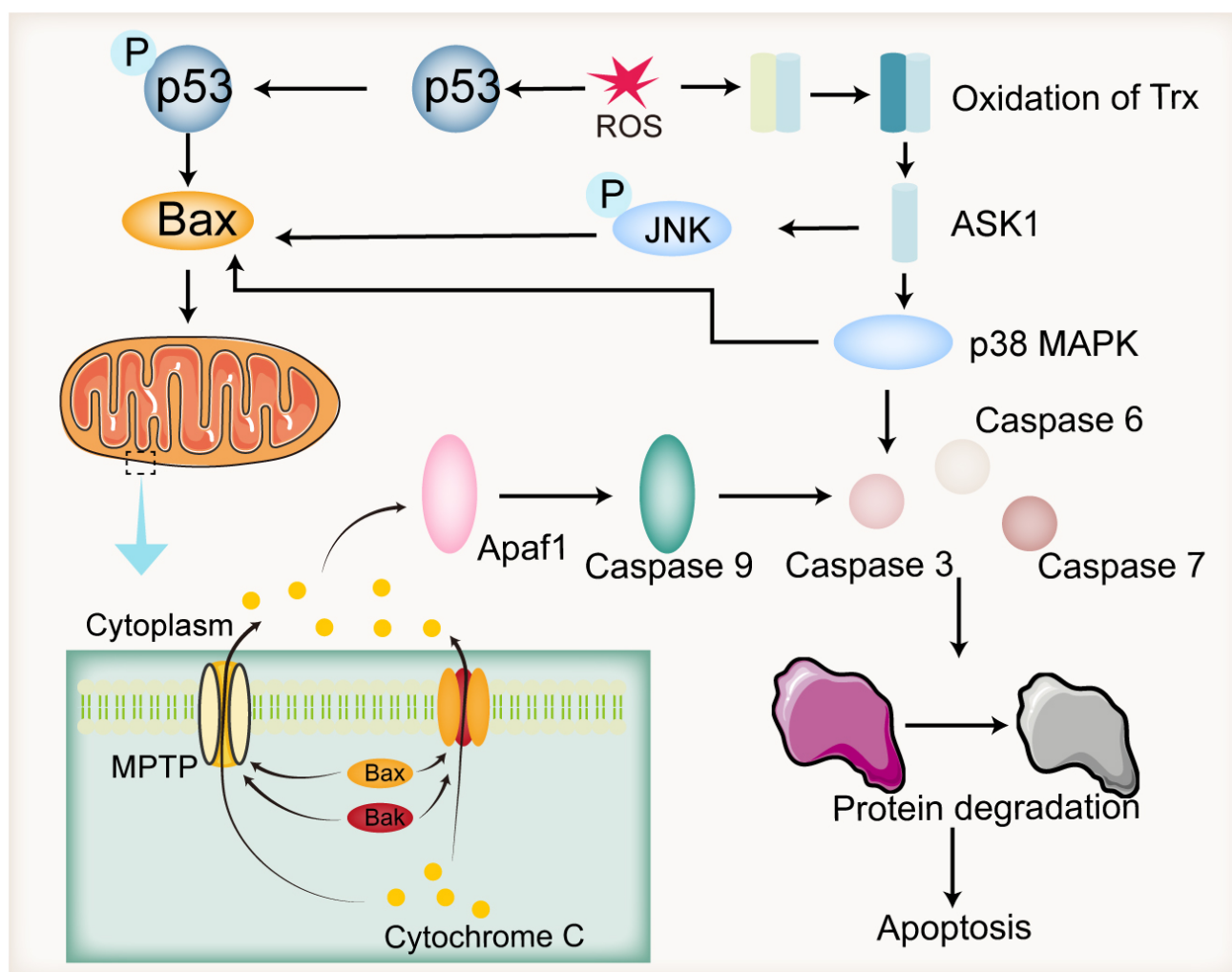


Fig. 3. Endogenous apoptotic pathways induced by ROS. ROS-induced DNA damage leads to the phosphorylation of p53, which activates Bax, resulting in the release of cytochrome C from mitochondria. The released cytochrome C binds to Apaf1, activating it, which subsequently triggers caspase 9 activation, followed by the activation of caspase 3, caspase 6, and caspase 7. These caspases further degrade intracellular proteins, leading to apoptosis. Additionally, ROS can also induce apoptosis by oxidizing Trx, releasing ASK1, and activating the JNK and p38 MAPK pathways. Apaf1, apoptotic protease activating factor 1; ASK1, apoptosis signal-regulating kinase 1; Trx, thioredoxin; MPTP: mitochondrial permeability transition pore; Bax, Bcl-2-associated X protein; Bak, Bcl-2 antagonist/killer; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase. Created by using Adobe Illustrator (version 28.7.1, Adobe Systems Incorporated, San Jose, CA, USA).

trimethylation and H3K9 dimethylation while decreasing H3K27 trimethylation. Recent studies indicate that histone modifications in human sperm cells are critical for transmitting the epigenetic information necessary for embryonic development. Aberrant methylation levels at H3 sites have been associated with impaired spermatogenesis and defective epigenetic reprogramming in sperm [144, 145]. Furthermore, transgenic mice overexpressing lysine demethylase 1A (KDM1A), a lysine-specific demethylase, exhibit reduced H3K4 and H3K9 methylation levels in sperm, with 25% of their offspring dying within 21 days after birth [146]. KDM1A generates H_2O_2 as a by-product of demethylation, which can further inhibit its enzymatic activity by forming disulfide bonds between

Cys600 and Cys618 residues, thus impairing demethylation [147]. These findings underscore the pivotal role of ROS in regulating histone methylation and demethylation by modulating the activities of methyltransferases and demethylases like KDM1A, ultimately impacting offspring development and survival. In addition to methylation, phosphorylation is another critical post-translational modification of histones. Brunner *et al.* [148] identified phosphorylation modifications at the T9 and T120 sites of H2B in mature mouse sperm, suggesting that these modifications may play a role in epigenetic traits underlying intergenerational inheritance. Metafora *et al.* [149] demonstrated that SV-IV, an immune-regulatory, anti-inflammatory, and sperm immune-protective protein derived from rat seminal vesicle

epithelium, can compete with histone H1 as a substrate for protein kinase C phosphorylation. Additionally, the phosphorylation of SV-IV enhances the activity of Glutathione peroxidase (GPX) and horseradish peroxidase, indicating that histone modifications may be involved in the regulation of ROS. Phosphorylation of histone H3 plays a crucial role in the G2/M transition of the cell cycle and serves as an important marker for chromosome condensation. ROS can activate ERK and promote increased phosphorylation of histone H3 [150]. The phosphorylation of histone H3 is a prerequisite for the ubiquitination and degradation of excess histones, suggesting that ROS may facilitate the removal and degradation of intracellular histone H3 through phosphorylation, thereby regulating sperm DNA packaging [151]. Phosphorylation of H2AX is an early marker of the DNA damage response and aids in the recruitment of DNA repair factors [152]. Li *et al.* [153] demonstrated that hydrogen peroxide can induce phosphorylation of H2AX in human sperm cells in a time- and dose-dependent manner. Histone acetylation is also a significant post-translational modification. Salehi *et al.* [154] found that frozen sperm cultured with Beltsville extender exhibited elevated levels of ROS and H3K9 acetylation, along with reduced sperm parameters, suggesting that ROS may promote H3K9 acetylation, thereby decreasing sperm motility.

DNA methylation is another key epigenetic mechanism that plays a critical role in regulating gene expression. Abnormal sperm DNA methylation patterns have been frequently observed in patients with male infertility [155,156]. Research by Tunc and Tremellen [157] demonstrated a significant inverse correlation between ROS levels and sperm DNA methylation, with antioxidant supplementation shown to restore normal methylation patterns. This suggests that ROS disrupt with the normal DNA methylation process in sperm [157]. DNA methylation primarily occurs at CpG islands located in the 5' region of genes, catalyzed by the DNA methyltransferase (DNMT) family, which includes DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, resulting in the formation of 5-methylcytosine [158]. Different DNMT enzymes operate at distinct stages of DNA methylation. While DNMT3B knockout has minimal impact on sperm, DNMT1 knockout leads to abnormal DNA methylation in mature sperm [159]. DNMT1 contains cysteine residues, making it highly susceptible to oxidative damage. ROS can oxidize DNMT1, impairing its methylation activity and leading to DNA methylation defects [160]. Furthermore, inactivation of DNMT1 has been shown to activate the p53 pathway, triggering apoptosis [161].

These studies underscore the pivotal role of ROS in modulating both histone and DNA methylation. ROS not only upregulate the expression of histone methyltransferases but also suppress the activity of histone demethylases. Through these mechanisms, ROS orchestrate the epigenetic landscape of sperm cells, influencing spermatogenesis and the epigenetic programming necessary for offspring development and survival. Additionally, ROS can

inactivate DNMT1, a key member of the DNA methyltransferase family, by facilitating the formation of intramolecular disulfide bonds. This inactivation reduces DNA methylation, triggers apoptosis, and ultimately impairs sperm motility and fertility.

4.4 Role of Ferroptosis and ROS in Male Reproductive Function and their Regulatory Mechanisms

Under normal physiological conditions, spermatogenesis is an iron-dependent process, and disruption of iron metabolism can impair spermatogenesis and lead to reproductive dysfunction [162]. When ferritin is degraded under the guidance of the Nuclear receptor coactivator 4 (NCOA4) receptor, iron is released into the labile iron pool (LIP), which then triggers ferroptosis in testicular cells [163]. Ferroptosis is a form of cell death induced by iron-dependent oxidative stress, characterized by excessive intracellular iron accumulation and lipid peroxidation [164]. The iron in the LIP participates in the Fenton reaction, catalyzing the conversion of hydrogen peroxide into hydroxyl radicals, which in turn lead to the peroxidation of PUFAs on the cell membrane, causing membrane rupture and dysfunction [165]. Furthermore, iron-dependent oxidases, such as NADPH oxidase, can further promote ROS production, thus exacerbating ferroptosis [166]. Elevated iron and ROS levels have been observed in the semen of asthenozoospermic patients, indicating that ferroptosis contributes to sperm dysfunction [167]. Additionally, increased levels of ferroptosis, iron ions, and ROS have been detected in the semen of infertile smokers, with higher iron and ROS levels correlating with decreased sperm motility [168]. Research by Zhao *et al.* [169] demonstrated that ferroptosis inhibitors and deferoxamine could suppress ferroptosis and restore sperm concentration and motility in Busulfan-treated mice. These findings suggest that maintaining cellular iron balance is crucial for spermatogenesis, as excessive iron can induce ferroptosis and cause male reproductive disorders.

As a key regulator of ferroptosis, GPX4 plays a central role in alleviating lipid peroxidation and protecting cells from oxidative damage. Under the action of GPX4 and its cofactor glutathione (GSH), oxidized PUFAs are reduced to hydroxy phospholipids, thereby protecting the cell membrane [170]. In infertile men diagnosed with oligospermia, GPX4 expression in sperm is significantly reduced [171], and GPX4 knockout mice show a significant decrease in sperm count and exhibit infertility [172]. The activity of GPX4 depends on selenium and GSH [173], and selenium supplementation has been shown to enhance GPX4 activity and promote germ cell proliferation [167]. GSH, an important antioxidant, is synthesized with the involvement of system Xc-, which exchanges extracellular glutamate for cystine. Cystine is then reduced to cysteine inside the cell, which is crucial for GSH synthesis [174]. This is consistent with the study by Hamashima *et al.* [175], which showed that system Xc- knockout mice had an increased number of immature spermatogenic cells in the cauda epididymis.

Furthermore, increased GSH levels have been shown to improve sperm quality and testicular histomorphology in diabetic mice [176].

5. Antioxidant Therapy: A Key Approach to Mitigating Oxidative Stress in Male Infertility

Increased oxidative stress is a major cause of decreased sperm motility. Consequently, the use of antioxidants is considered one of the primary effective treatments for male infertility [177]. Antioxidant therapy has been shown to significantly reduce ROS level and improve sperm motility [178]. Recent reports also suggest that certain plant extracts can enhance sperm activity through their antioxidant properties [179]. The effects of antioxidants on sperm are summarized in Table 1 (Ref. [180–202]).

5.1 Small Molecule Antioxidants

Glutathione, a thiol-containing tripeptide, is a potent antioxidant activity. Research has shown that reduced levels of glutathione in seminal plasma are associated with impaired sperm viability and motility [203]. Chemotherapeutic agents such as bleomycin, etoposide, and cisplatin, commonly used in the treatment of testicular cancer, can induce the ROS production [204]. The use of glutathione has been reported to significantly mitigate bleomycin, etoposide, and cisplatin-induced sperm DNA fragmentation and morphological abnormalities in rats [180]. N-Acetylcysteine (NAC), a precursor of glutathione, effectively scavenges ROS effectively as an antioxidant. *In vitro* study has demonstrated that NAC inhibits apoptosis in human testicular germ cells [181]. Clinical trials have shown that NAC significantly improves sperm oxidative stress indices and motility in infertile men [182]. Vitamin C neutralizes hydroxyl radicals, superoxide anions, and hydrogen peroxide, reducing oxidative stress. The concentration of vitamin C in seminal plasma is more than ten times higher than in blood plasma, indicating its critical antioxidant role in semen [205]. A marked decrease in vitamin C levels and total antioxidant capacity has been observed in patients with asthenozoospermia, indicating a significant positive correlation between lower vitamin C levels and male infertility [206]. However, clinical outcomes regarding vitamin C supplementation remain mixed. Rolf *et al.* [207] reported that high doses of vitamin C and E did not improve sperm concentration and motility in patients with asthenozoospermia. Nonetheless, recent study suggests that a combination of zinc, vitamin C, and vitamin E can effectively reduce DNA fragmentation, improve sperm motility, and decrease oxidative stress in infertile patients [183]. Further investigation is needed to establish the therapeutic efficacy of vitamin C in male infertility. Additionally, low concentrations (100 mmol/L) of the antioxidant carnitine have been shown to improve sperm motility and viability *in vitro*. However, this effect appears to be mediated through the regulation

of specific gene expressions (*Vasa*, *Dazl*, *Acr*, and *Prm1*) rather than direct ROS modulation [184,185]. Edaravone, a drug with notable antioxidant and neuroprotective properties, functions primarily through free radical scavenging. Recent study has shown that edaravone reduces the proportion of immature sperm in busulfan-induced azoospermia and improves azoospermia by scavenging free radical and regulating autophagy in testicular tissue [186].

5.2 Plant Extracts

Numerous plant extracts, rich in antioxidant compounds, present promising solutions for addressing male infertility. For example, celery, which is rich in phenols and coumarins, has been shown to significantly increase sperm count in mice treated with celery extract [187]. Similarly, Safarnavadeh and Rastegarpanah [189] demonstrated that Satureja Khuzestanica essential oil, extracted from Satureja Khuzestanica Jamzad, contains up to 90.8% carvacrol, which effectively enhances sperm quality and fertility by acting as an antioxidant [208]. This suggests that the active components in carvacrol can significantly improve sperm quality by reducing oxidative stress, though further clinical study is required to substantiate its efficacy. In addition to carvacrol, other plants also exhibit notable antioxidant properties. Zarei *et al.* [188] demonstrated that *Cornus mas* extract significantly prevented the reduction of total antioxidant capacity induced by methotrexate treatment in mice. Additionally, it effectively mitigated methotrexate-induced sperm DNA damage and the decline in sperm maturation [188]. Using a diabetic mouse model, Khaki demonstrated that both individual and combined use of ginger and cinnamon significantly improve serum antioxidant levels (TAC, SOD, GPX, and catalase) while markedly enhancing sperm vitality and motility [190]. Curcumin, extracted from the rhizomes of *Curcuma longa*, possesses potent antioxidant properties, with its antioxidant activity being 300 times stronger than that of vitamin E [209]. A study by Alizadeh *et al.* [193] demonstrated that daily supplementation with 80 mg of curcumin nanoparticles significantly improved total antioxidant capacity, reduced MDA levels, and enhanced sperm count, concentration, and motility in infertile men. Research by Hamden *et al.* [191] showed that the combined use of 1α , 25-dihydroxyvitamin D₃ and *Afuga i*va methanol extract effectively prevented the decrease in testicular antioxidant enzymes (SOD, CAT, GPx) and non-enzymatic antioxidants (copper, magnesium, and iron) induced by diabetes, thereby protecting the reproductive system from oxidative stress and cytotoxicity, and maintaining sperm count and motility. Han *et al.* [192] reported that green tea powder and black tea powder were able to reduce body weight gain in high-fat diet-fed mice, while enhancing testicular SOD and GSH levels, reducing MDA levels, alleviating oxidative damage in the testes, and improving testicular index and testosterone levels. Grami *et al.* [194] found that water extracts of *Eruca sativa* contained high lev-

Table 1. Antioxidant compounds and their beneficial impacts on sperm viability and function.

Antioxidant	Effects	References
Glutathione	Reduce sperm DNA fragmentation and morphological anomalies while enhancing sperm count restoration.	[180]
N-Acetylcysteine	Diminish apoptosis in reproductive cells to bolster sperm vitality.	[181,182]
vitamins C	Ameliorate the integrity of sperm DNA by reducing fragmentation and concurrently improving sperm vigor.	[183]
Carnitine	Enhance sperm kinematics and energetic potential.	[184,185]
Edaravone	Reduce the prevalence of immature spermatozoa.	[186]
Aqueous extract of celery	Enhance sperm numerical density.	[187]
Cornus mas fruit extract	Elevate spermatozoal antioxidant defenses alongside sperm quantity.	[188]
Satureja Khuzestanica essential oil	Upgrade sperm quality and augment fecundity indices.	[189]
Ginger and cinnamon	Optimize sperm viability through enhanced motility.	[190]
1 α , 25-dihydroxyvitamin D ₃ and <i>Afuga iva</i> methanol extract	Prevent diabetes-induced decline in testicular antioxidants, protecting the reproductive system and maintaining sperm count and motility.	[191]
Tea powder	Increase testicular antioxidants, reduces MDA, alleviates oxidative damage, and improves testicular index.	[192]
Curcumin nanomicelle	Improve antioxidant capacity, reduces MDA, and boosts sperm count, concentration, and motility in infertile men.	[193]
Eruca sativa aqueous extracts	Reduces testicular MDA levels, increases thiol levels, and enhances the activity of SOD, CAT, and GPx, preventing testicular damage in rats, and improving sperm density, motility, and viability.	[194]
Ethanol extract of <i>Ionidium suffruticosum</i> (L.) Ging leaves	Increase testicular SOD and CAT levels, promotes spermatogenesis, increases sperm count, and reduces sperm aggregation in rats.	[195]
Pomegranate juice	Restore the decline in testicular SOD, CAT, GPx, GST, GR, and GSH levels, and improves the degeneration of germ cells and Leydig cells, as well as sperm morphological.	[196]
Lycopene	Enhance total sperm antioxidant capacity, increases ejaculate volume, total sperm count, sperm concentration, and total motility.	[197]
Aqueous leaf extract of <i>Moringa oleifera</i>	Reduce sperm reactive oxygen species levels, improve sperm DNA fragmentation, and maintain sperm function.	[198]
Camellia sinensis	Enhance the CAT and SOD enzyme activities and glutathione levels in rat sperm, thereby protecting against oxidative stress-induced sperm damage.	[199]
Aspalathin linearis	Increase the SOD enzyme level, reduce the MDA level, and enhance sperm concentration in rats.	[200]
<i>Lactobacillus rhamnosus</i> CECT8361, <i>Bifidobacterium longum</i> CECT7347	Mitigate DNA fragmentation to reinforce sperm motility.	[201]
FamiLact	Enhance sperm concentration and motility, attenuate sperm morphological abnormalities, oxidative stress, and DNA fragmentation indices.	[202]

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione S-Transferase; GR, glutathione reductase; GSH, glutathione.

els of antioxidant phenolic compounds that could reverse bisphenol A-induced oxidative stress-related reproductive toxicity, including reducing testicular MDA levels, increasing thiol levels, and enhancing the activity of SOD, CAT, and GPx, thereby preventing testicular oxidative damage in rats and improving sperm density, motility, and viability. The ethanol extract of *Ionidium suffruticosum* (L.) Ging leaves was shown to increase testicular SOD and CAT levels, promote spermatogenesis, increase sperm count, and reduce sperm aggregation in rats [195]. Pomegranate juice significantly restored the decline in testicular SOD, CAT, GPx, GST, GR, and GSH levels caused by carbon tetrachloride (CCl₄) injection, improving the degeneration of germ cells and Leydig cells as well as sperm morphological abnormalities induced by CCl₄ injection [196]. Nouri *et al.* [197] reported that lycopene supplementation significantly enhanced total antioxidant capacity and improved sperm concentration and motility in infertile men with oligozoospermia. *Moringa Oleifera*, a tree widely distributed across tropical and subtropical regions, has been reported to possess significant antioxidant activity [210]. Water extracts of *Moringa Oleifera* have been shown to mitigate oxidative stress in cryptorchid rats, evidenced by reduced MDA levels and increased SOD activity [211]. Furthermore, research by Moichela *et al.* [198] demonstrated that *Moringa Oleifera* leaf water extracts improve sperm DNA fragmentation by reducing ROS production, thereby preserving essential sperm functions. Tea leaves, derived from the leaves and buds of the *Camellia sinensis*, are rich in catechin polyphenols and have been reported to exhibit ROS-scavenging properties [212]. Awoniyi *et al.*'s study [199] highlighted that tea consumption enhances CAT, SOD activity, and glutathione levels in rat sperm, providing protective effects against oxidative stress-induced sperm damage. Similarly, Omolaoye *et al.*'s research [200] indicated that fermented *Aspalathus linearis* significantly increased SOD enzyme levels in Wistar rat sperm, reduced MDA levels, and improved sperm concentration.

5.3 Probiotic Therapy for Male Infertility

Probiotics can enhance testicular function and promote sperm regeneration through antioxidant effects and modulation of the gut microbiota. Valcarce *et al.*'s research [213] demonstrated that the antioxidant probiotic strains *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 improved sperm quality and offspring survival rates in zebrafish. Subsequent human trials confirmed these effects, revealing that the intake of these strains reduced hydrogen peroxide levels in sperm cells by approximately 3.5-fold, decreased DNA fragmentation, and enhanced sperm motility [201]. This finding supported by Abbasi *et al.* [202], who administered the probiotic supplement FamiLact (containing *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium*

longum, and *Streptococcus thermophilus*) to male subjects with idiopathic infertility. Significant increases in sperm concentration and motility were observed, along with reductions in abnormal sperm morphology, oxidative stress, and DNA fragmentation [202]. Further study indicated that FamiLact administration in patients post-varicocelelectomy resulted in improved sperm concentration and a higher proportion of normal sperm [214]. Additionally, a study on male Japanese quails supplemented with *Bifidobacterium longum* and mannan oligosaccharides found that probiotic and prebiotic supplementation significantly upregulated the expression of SOD and catalase in testicular cells, thereby enhancing male fertility in juvenile quails [215].

6. Conclusions

Male infertility affects approximately half of infertile couples, with elevated ROS levels in sperm indicating that oxidative stress may play a pivotal role in this condition. ROS can arise from both endogenous and exogenous sources. Mitochondria serve as the primary endogenous source of ROS within sperm, where electron leakage from the mitochondrial electron transport chain generates superoxide anions, thereby exacerbating oxidative stress. This heightened oxidative stress promotes lipid peroxidation, and its by-products of this process can damage mitochondrial proteins, further enhancing electron leakage. Exogenous factors including gut microbiota dysbiosis, circadian rhythm disruptions, smoking, leukocyte-derived ROS, and alcohol consumption, also contribute to excessive ROS production. These external sources increase oxidative stress in sperm, resulting in DNA damage and fragmentation the risk of male infertility. Additionally, oxidative stress activates both mitochondria-dependent and death receptor-dependent apoptotic pathways, leading to sperm apoptosis and further contributing to infertility. Moreover, oxidative stress can also induce epigenetic modifications by modulating the expression of histone methyltransferases and demethylases, leading to altered histone methylation patterns, which may impact offspring development. It also disrupts sperm DNA methylation by impairing DNA methyltransferase activity, thereby reducing sperm motility.

Antioxidants, including small molecule antioxidants, plant extracts, and probiotics, have shown considerable potential in alleviating oxidative stress in sperm and enhancing motility. However, the beneficial effects of these treatments have primarily been demonstrated at the cellular and animal levels, and further clinical studies are necessary to validate their therapeutic efficacy in male infertility. In addition to histone and DNA methylation, epigenetic regulation involves various mechanisms, such as miRNA expression and histone acetylation, which also influence sperm function. Future research should focus on the interplay between ROS, epigenetic modifications, and sperm motility to enhance our understanding of oxidative stress and its impact on male fertility. Abnormal sperm function is a key

contributor to male infertility, with sperm abnormalities are often linked to reproductive tract disorders, including varicocele, vas deferens obstruction, cryptorchidism, and orchitis. Future studies should explore the relationship between oxidative stress and reproductive tract disorders to further advance the clinical application of antioxidants in treating male infertility.

Author Contributions

YZ, HZ, HY, PH, JZ, and YL contributed to the literature review and manuscript writing. YZ and HZ also critically reviewed and revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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