

Review

Oxidative Stress, Antioxidants, Gut Microbiota and Male Fertility

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Key Words

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Abstract

It is imperative to comprehend the multifactorial causes of male infertility and to identify effective treatment methods, to enhance male reproductive health, and to develop more personalised and effective therapeutic interventions. This review discusses the multifactorial aspects contributing to male infertility, focusing on oxidative stress (OS), sperm quality, gut microbiota, and the potential role of adaptogens. A comprehensive literature search was conducted across several major databases, including the Cochrane Library, Medline, Embase, SciSearch, PubMed, Web of Science, Scopus, and Google Scholar. The findings from the studies included in the databases highlight the significant role of oxidative stress in male infertility, with reactive oxygen species (ROS) contributing to sperm DNA fragmentation and impairment of spermatogenesis. The review further elucidates the influence of both endogenous and exogenous sources of ROS, including lifestyle factors and environmental exposures, on male reproductive health. Emerging research also highlights the involvement of key molecular pathways, such as Nrf2, AMPK/PGC-1 α , and NF- κ B, in regulating OS within the male reproductive system. Additionally, the review outlines the relationship between endothelial dysfunction, cardiovascular health, and male infertility, identifying OS as a common underlying factor. In addition to the OS, the gut microbiota has been identified as a pivotal factor in male fertility, influencing inflammation and hormonal regulation. This review underscores the potential merits of a synergistic strategy that integrates dietary interventions, antioxidants, gut microbiota modulation, and adaptogens to enhance fertility outcomes. Adaptogens, recognised for their capacity to assist the body in coping with stress and re-establishing equilibrium, may confer protective effects against OS and improve reproductive health. The review under discussion emphasises the importance of a holistic approach to male infertility, integrating molecular, clinical, and lifestyle factors to optimise reproductive health.

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Introduction

In approximately 50% of cases of infertility in couples, male factors are either the sole or contributing cause, thus highlighting their significant role in the aetiology of infertility [1, 2]. This emphasises the necessity to prioritise male reproductive health in both diagnostic assessments and therapeutic strategies to address the issue of infertility effectively [3]. A global prevalence of erectile dysfunction (ED) is reported, reflecting the widespread impact of this mechanism [3]. It is estimated that over 72 million people worldwide are affected by this condition [4]. The rising prevalence of ED underscores the pressing need to address the underlying OS through targeted interventions, including antioxidant therapy and lifestyle modifications, to mitigate its effects on endothelial function and male reproductive health [3, 5].

The incidence of marital infertility is estimated at approximately 15%, with male factor infertility accounting for approximately 50% of these cases [6, 7, 8]. Male factor infertility is characterised by such factors as poor sperm quality, low sperm count, and impaired sperm motility, which significantly reduce the chances of successful fertilisation [9]. Various biological, environmental, and lifestyle factors can cause these problems. In approximately 30-50% of cases of male infertility, the underlying causes remain unexplained and are classified as idiopathic [10]. This signifies that, despite extensive testing and medical evaluation, no specific cause of infertility can be identified. Idiopathic infertility can be particularly challenging for healthcare providers, as it makes it difficult to develop targeted treatments. The absence of definitive diagnostic markers often necessitates a more comprehensive approach to treatment, emphasising the enhancement of general reproductive health as opposed to the specific targeting of identifiable conditions [11].

Evidence presented in the literature has highlighted that oxidative stress (OS) is emerging as a critical factor in male infertility, driven by the overproduction of reactive oxygen species (ROS) – toxic byproducts of oxygen metabolism [12]. While ROS are harmful in excessive amounts, at physiological levels they play a central role in regulating intracellular signalling pathways essential for reproductive processes, according to the findings reported by [13]. These include sperm maturation, hyperactivation, capacitation, acrosome reaction, and successful fertilisation [14]. ROS levels in cells are tightly regulated by a balance between their production and neutralisation by antioxidant defence mechanisms. Perturbation of this balance by increased ROS generation or decreased antioxidant activity leads to redox dysfunction, significantly impairing cellular functions [15].

Previous studies suggest that there is a significant relationship between male infertility and a multitude of factors, including hormonal imbalances, genetic abnormalities, varicocele, infections, age, obesity, smoking, alcohol consumption, environmental toxins, and exposure to heat or radiation [16, 17]. Notwithstanding, idiopathic male infertility is the most prevalent category, as its aetiology is frequently unexplained despite rigorous diagnostic procedures and the absence of identifiable underlying factors [18]. The unexplained nature of idiopathic male infertility may be attributable to intricate genetic and molecular factors, microbial infections in the genital tract, or environmental interactions that remain to be fully elucidated, as evidenced by the observations reported by Wang et al. [19]. Recent research has explored several potential mechanisms, including subtle genetic mutations, environmental exposures to toxins (such as endocrine-disrupting chemicals), or immune-related issues that may affect sperm function or quality without being immediately apparent [20, 21]. Despite the challenges posed by idiopathic male infertility, advancements in assisted reproductive technologies, such as *in vitro* fertilisation and intracytoplasmic sperm injection, have offered couples renewed hope. These technologies can circumvent many problems associated with male infertility by enabling sperm with minimal motility or abnormal morphology to directly fertilise an egg, as shown in the experiments by Zheng et al. [22] and Ribeiro et al. [23].

It has previously been demonstrated that spermatozoa are uniquely susceptible to ROS owing to their limited capacity for repair and the high content of polyunsaturated fatty acids (PUFA) in their plasma membranes [24]. The oxidation of these lipids has been shown to

compromise membrane integrity, resulting in ATP leakage and impaired flagellar motility [25]. In addition to this, it has further been demonstrated that OS can cause direct DNA damage in spermatozoa, which results in the disruption of chromatin integrity and the consequent reduction in fertilisation potential [13, 26]. Moreover, OS has been observed to affect the intracellular metabolism of carbohydrates, proteins, and nucleic acids [25], exacerbating its deleterious effects on sperm functionality. In the context of male infertility, the antioxidant defence mechanisms present in semen have been shown to be significantly impaired. In the context of infertile males exhibiting elevated levels of ROS, a decline in antioxidant capacity has been observed, leading to a range of sperm dysfunctions [13]. In conjunction with diminished enzymatic scavenging systems, the combination of excessive ROS production by immature germ cells and leukocytes may lead to oxidative imbalance. This imbalance can result in substantial oxidative damage to crucial biomolecules, such as lipids, proteins, and DNA, thereby reducing sperm viability and fertility potential [15].

In the context of the growing interest in the topic of male infertility, particular attention has been paid to the analysis of sperm quality in men worldwide, leading to concerns regarding reproductive health [27]. This decline has been attributed to environmental pollutants and lifestyle factors, such as poor diet and lack of exercise [28]. Recent decades have seen a marked decrease in the molecular mechanisms involving increased reactive oxygen species overload and sperm DNA damage, as shown in the experiments by various research teams [26, 29, 30], and impaired mitochondrial function, which collectively reduce fertility potential [31]. The relationship between lifestyle factors and sperm quality remains under-explored in the scientific literature, leaving critical gaps in our understanding of how specific behaviours and environmental exposures interact to influence male fertility [28]. While some studies address individual factors like diet, exercise, and smoking, there is a lack of comprehensive understanding of their combined impact, underlying mechanisms, and long-term consequences [32].

This underscores the necessity for more integrative and multidisciplinary research to comprehensively elucidate the intricate interrelationships between lifestyle factors and reproductive health and develop effective interventions and public health strategies. The present study hypothesises that a synergistic approach involving the gut microbiota and nutritional interventions for male fertility is crucial due to the increasing prevalence of male infertility and the complex multifactorial nature of its causes [33]. Given the significant influence of male fertility on such factors as redox imbalance, hormonal imbalances, and systemic inflammation, it is crucial to understand how these factors interact with gut health and nutrition. This understanding is essential for developing more effective treatments, as previously demonstrated by some authors [34]. Conventional approaches tend to address these factors individually. However, combining probiotics, prebiotics, antioxidants, amino acids, omega-3 fatty acids, and adaptogens holds promise for tackling multiple underlying causes concurrently.

Previous studies suggest a significant relationship between the gut microbiota composition and overall health, including reproductive function, which has become a subject of mounting research interest [35]. The gut microbiome plays a significant role in regulating inflammation, metabolic processes, and hormone levels, which are critical to male fertility [36]. Consequently, it is imperative to comprehend how dietary and microbiota-based interventions may augment fertility outcomes. This presents novel prospects for both the prevention and treatment of male reproductive health, adopting a more holistic and sustainable approach. Given the increasing prevalence of lifestyle-related infertility, the present research domain is not only timely but also harbours considerable potential in enhancing the quality of life for individuals grappling with infertility, as evidenced in a recent study [37].

The innovative character of the present studies is evidenced by their exploration of the multifactorial nature of male infertility, with attention not only to well-established factors, including OS and sperm quality, but also to emerging considerations such as the gut microbiota and synergistic dietary interactions. Integrating molecular pathways, such as Nrf2, AMPK/PGC-1 α , and NF- κ B, with novel approaches that combine antioxidants,

probiotics, and adaptogens, provides a holistic and novel framework for addressing male reproductive health. This multidisciplinary approach offers new insights into the prevention and treatment of male infertility, paving the way for more personalised and effective therapeutic strategies.

The objective of this research was threefold: first, to identify reliable biomarkers of OS in seminal fluid; second, to evaluate the efficacy of antioxidant therapies in mitigating OS and restoring fertility, particularly about gut microbiota parameters; and third, to explore the synergistic interplay between gut microbiota modulation and dietary interventions in addressing lifestyle factors, environmental exposures, and oxidative damage to develop comprehensive preventive strategies. A comprehensive literature review was conducted, encompassing several databases, including the Cochrane Library, Medline, Embase, SciSearch, and conference proceedings, along with additional sources, such as PubMed, Web of Science, Scopus, and Google Scholar.

The search criteria encompassed studies that focused on male infertility, OS, sperm quality, the role of gut microbiota, and the relationship between lifestyle factors and male reproductive health. Peer-reviewed scientific journals, including clinical trials, systematic reviews, and meta-analyses, were considered. Search terms included “male infertility”, “oxidative stress”, “sperm quality”, “gut microbiota”, and “lifestyle factors and male reproductive health”. Studies published between 2014 and 2024 were included to ensure data relevance. Research on molecular mechanisms, dietary interventions, and microbiome-related topics was also included. To enhance the reliability of the results, studies with high methodological quality, such as randomised controlled trials, were given preference. In the course of the present review, some articles were excluded based on specific criteria, including publications not available in full text, not peer-reviewed (conference abstracts, articles from non-peer-reviewed journals), focusing on populations, interventions, or outcomes outside the scope of the study, or based solely on expert opinion without empirical data.

Our review addresses critical gaps in the existing literature by providing a comprehensive and integrated analysis of male reproductive health, focusing on the molecular mechanisms and lifestyle factors that influence sperm quality. A vital gap we address is the interplay between oxidative stress and antioxidant defence mechanisms in male infertility, particularly the role of redox imbalance in sperm DNA fragmentation. By examining key molecular pathways such as Nrf2, AMPK/PGC-1 α , and NF- κ B, we can better understand how these pathways regulate sperm function and contribute to infertility. While previous studies have examined individual components, our review synthesises these mechanisms into a cohesive framework, providing a more holistic view of the factors involved in male infertility.

In addition, our work provides new perspectives on the therapeutic potential of phytochemicals, micronutrients, and gut microbiota modulation in improving male fertility. While phytomedicinal therapeutics such as ginsenosides and anthocyanins have been studied in isolation, our review highlights the synergistic effects of combining these interventions with dietary approaches to optimise sperm quality. We also explore the under-researched relationship between gut microbiota and male fertility, particularly how gut health may influence reproductive outcomes through mechanisms such as the gut-brain axis and immune system regulation. By highlighting these emerging areas, we provide a new avenue for future research and intervention strategies, filling essential gaps and advancing the understanding of male reproductive health.

The present study analysed synergistic approaches to improving male fertility by integrating gut microbiota modulation and dietary interventions, investigating the combined effects of probiotics, prebiotics, antioxidants, amino acids, omega-3 fatty acids, and adaptogens, to identify effective strategies for the improvement of reproductive health.

Markers for male infertility

As sperm quality has declined worldwide in recent decades, global trends and environmental factors in male fertility represent a critical area of research [38]. A complex interplay of factors, including pollution, endocrine-disrupting chemicals, climate change, and lifestyle changes, has been implicated in this phenomenon [39]. As male fertility is a key indicator of reproductive health and a broader marker of population health and environmental sustainability, it is vital to understand these influences. Developing a comprehensive framework for studying and managing these trends is essential for designing interventions that can reduce risks, improve reproductive outcomes, and inform global health policy, a perspective extensively discussed by Knapke et al. [40].

In the context of the growing interest in male infertility, particular attention has been paid to the analysis of such conditions as oligospermia and asthenozoospermia, such markers as sperm concentration, motility analysis (progressive motility), and semen analysis that are essential to assess sperm quality [41, 42, 43]. These dependencies are shown in Fig. 1. Hormonal markers, such as testosterone, follicle-stimulating hormone (FSH), and luteinising hormone (LH), are essential in assessing hormonal imbalances, as shown by Oduwole et al. [44]. In cases of azoospermia, genetic testing (e.g., Y chromosome microdeletions) and scrotal ultrasound are commonly used to assess anatomical blockages, as shown in the experiments conducted by Cioppi et al. [45]. In addition, reactive oxygen species overload markers, such as ROS and antioxidant levels in seminal plasma, can provide insights into sperm quality. In the context of teratozoospermia, the analysis of sperm morphology (per Kruger's criteria) is imperative for evaluating aberrant sperm morphologies [46, 47, 48].

Research in this area has suggested that scrotal and Doppler ultrasound are used to assess varicocele, and semen analysis is performed to determine sperm count and quality [49]. For such conditions as hypo- and hypergonadism, key markers include serum testosterone, FSH, LH, oestradiol, and inhibin B, along with semen analysis to assess sperm production [50]. Kumanov's study [50] indicates that inhibin B is a more accurate marker of fertility status than FSH and LH. Furthermore, inhibin B levels in infertile patients may provide valuable insights into spermatogenesis, potentially serving as a more direct indicator of spermatogenic function than FSH [50].

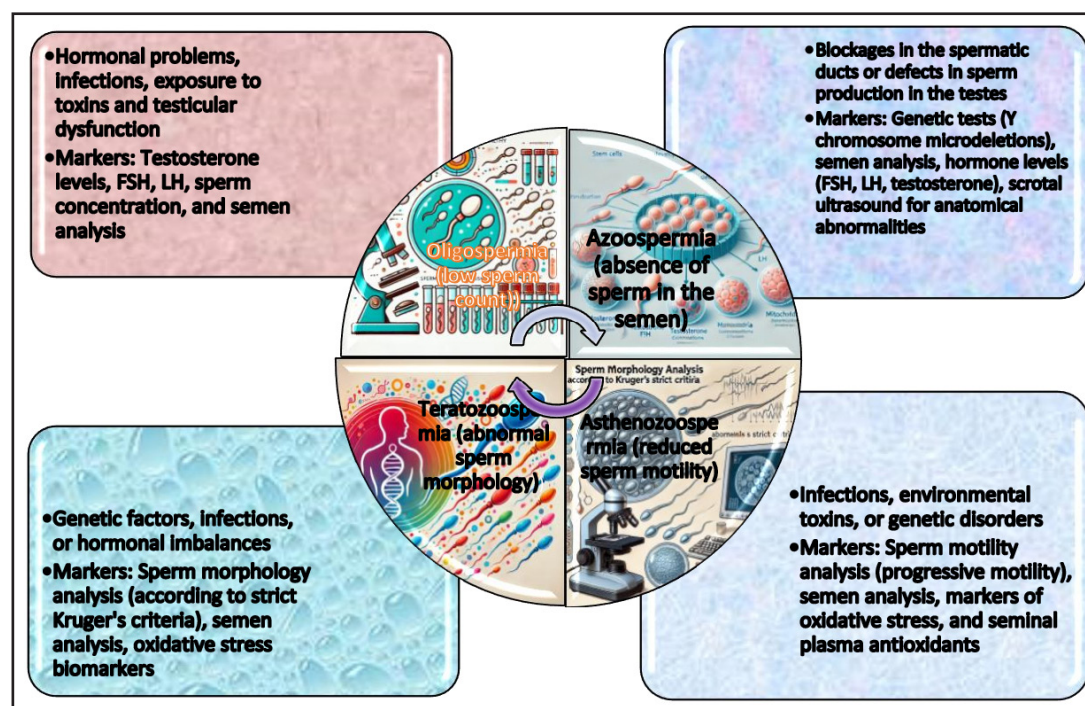


Fig. 1. Sperm quality assessment in such conditions as oligospermia, asthenozoospermia, teratozoospermia, and azoospermia relies on four key markers: sperm concentration, progressive motility, semen analysis, and hormonal evaluation. Abbreviations: FSH – Follicle-Stimulating Hormone; LH – Luteinizing Hormone.

Findings from prior investigations have indicated that genetic testing (e.g., karyotyping and Y chromosome microdeletion testing) is essential for identifying genetic disorders, such as Klinefelter's syndrome, which may result in infertility [51]. In the context of infection and inflammation, such as epididymitis and prostatitis, prostate-specific antigen (PSA), inflammatory cytokines (IL-6, TNF- α), and semen culture have been identified as valuable diagnostic markers [52]. The assessment of environmental and lifestyle factors can be facilitated by the measurement of biomarkers of OS (e.g., malondialdehyde, an end product of lipid peroxidation processes), the analysis of sperm quality, and the testing for toxin exposure (e.g., pesticide or plastic levels), as demonstrated previously [20, 21, 25]. These markers provide crucial insights into the causes of male infertility and help to determine appropriate interventions (Fig. 2).

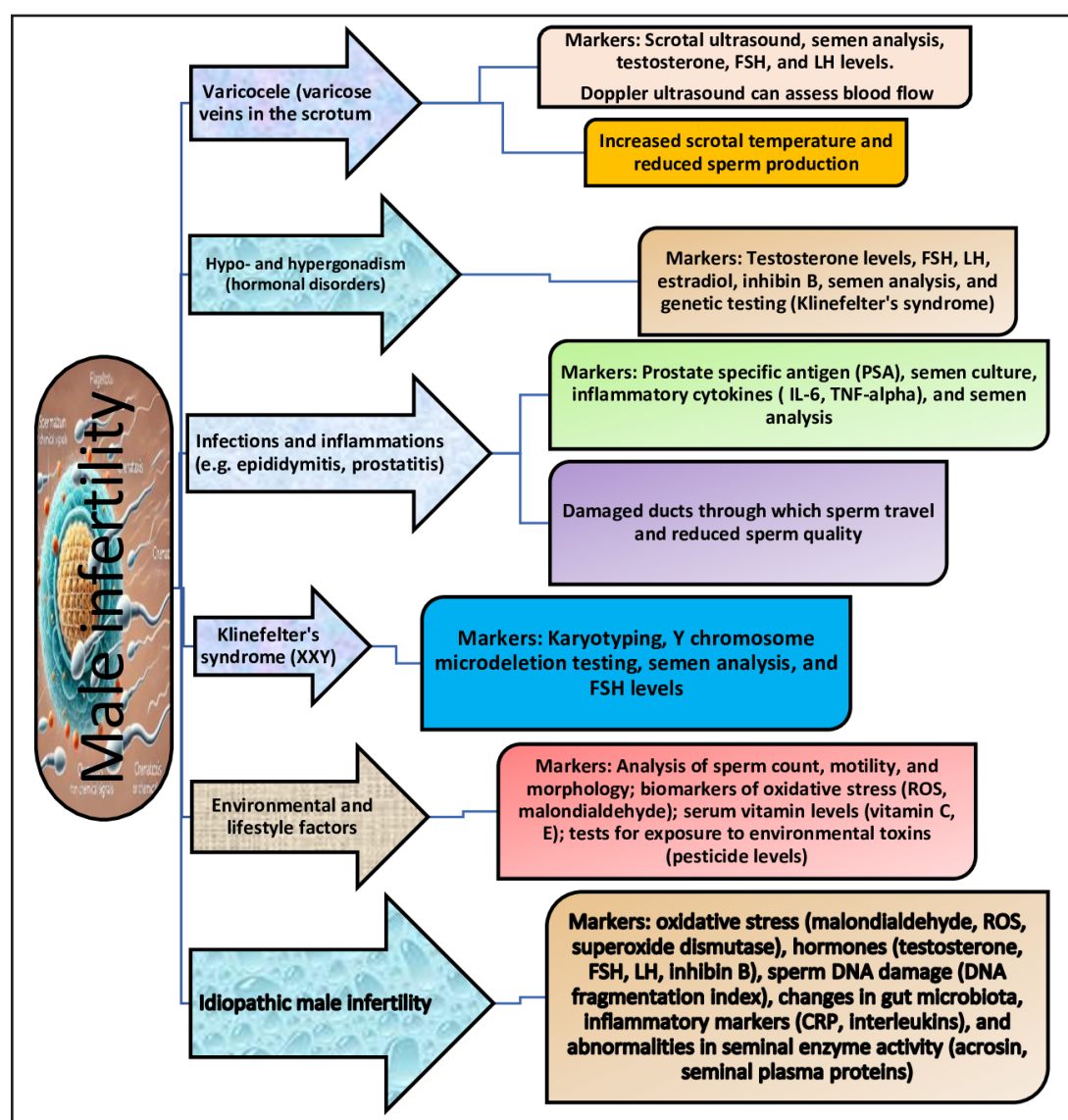


Fig. 2. Common pathological conditions associated with male infertility, conditions and markers. Abbreviations: L-6 - interleukin-6; TNF-alpha - tumour necrosis factor-alpha; FSH - follicle stimulating hormone; ROS - reactive oxygen species; LH - luteinising hormone; CRP - C-reactive protein.

Relationship between lifestyle and sperm quality

The subsequent section delves into lifestyle factors and male reproductive health to further clarify these observations. It was previously understood that sperm quality is a critical determinant of male fertility, and lifestyle factors play a significant role in influencing fertility; numerous studies have demonstrated the profound impact of diet, physical activity, substance use, and environmental exposures on various parameters of sperm health, such as motility, concentration, morphology, and DNA integrity [53, 54]. Understanding these factors provides actionable insights to improve male reproductive health. These dependencies are shown in Fig. 3.

It has been established that psychological stress and lifestyle factors are strictly related. Indeed, chronic psychological and emotional stress leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases the production of cortisol [56, 57]. While cortisol is essential for the stress response, it can also increase ROS production. This imbalance leads to oxidative damage to lipids, proteins, and DNA, affecting various cellular functions [58]. In addition, unhealthy lifestyle choices, such as smoking, alcohol consumption, and poor diet, contribute to the generation of ROS [11]. Smoking, for example, introduces free radicals into the body, which directly damage cellular structures, as shown by Messner and Bernhard [58].

Environmental pollutants, such as toxic metals and industrial toxins, have been demonstrated to increase OS by disrupting the body's antioxidant systems [7, 9]. Once absorbed into the body, these toxins have been shown to promote the production of ROS and interfere with the function of antioxidant enzymes [59]. Current findings demonstrate that elevated levels of arsenic, cadmium, and lead in the urine are significantly associated with increased OS markers, including 8-OHdG and 8-isoPGF2 α , which correlate with impaired semen quality. Mediation analysis by He et al. [59] revealed that OS markers partially mediate the relationship between exposure to selected heavy metals and reduced sperm motility and morphology, highlighting their potential role as key mediators of environmental toxins.

Another study conducted by Anyanwu and Orisakwe [60] highlights the mechanisms by which toxic metals contribute to male reproductive toxicity, including ion mimicry, disruption of cell signalling pathways, oxidative imbalance, alterations in gene expression, epigenetic regulation, apoptosis, blood-testis barrier disruption, inflammation processes, and endocrine disruption. Their findings also highlight the central role of non-coding RNAs (ncRNAs) in mediating paternal intergenerational epigenetic inheritance, underscoring their functional importance and potential as novel biomarkers in the context of male reproductive toxicity.

Toxic metals contribute to male infertility through a variety of molecular mechanisms. First, ion mimicry occurs when these metals disrupt the balance of essential metal ions in the body, as shown previously [61], interfering with protein folding, enzyme activity, and ion channel regulation critical for spermatogenesis. In addition, toxic metals affect key cell signalling pathways, such as MAPK, Nrf2, NF- κ B, and PI3K, by altering kinase activation, receptor binding, and activation of downstream transcription factors, thereby leading to impaired cellular responses vital for reproductive function [62, 63]. In addition, these metals induce oxidative burden by generating ROS that cause lipid peroxidation, DNA damage, and protein oxidation, ultimately triggering apoptosis and cellular senescence [29, 64].

Toxic metals also affect gene expression by interfering with transcription factor binding and epigenetic regulation, including changes in DNA methylation and histone acetylation, leading to altered gene expression profiles critical for reproductive cell function, as shown by [65]. In addition, metals activate mitochondrial pathways and unfolded protein response, inducing apoptotic cell death in germ cells and reducing sperm count and quality, as shown in the experiments carried out by Park and Pang [66]. In addition, toxic metals disrupt the blood-testis barrier by affecting the integrity of tight junctions and adhesion molecules, allowing harmful substances to enter the testes and impair spermatogenesis [67]. Inflammatory pathways, including activation of NF- κ B and release of pro-inflammatory

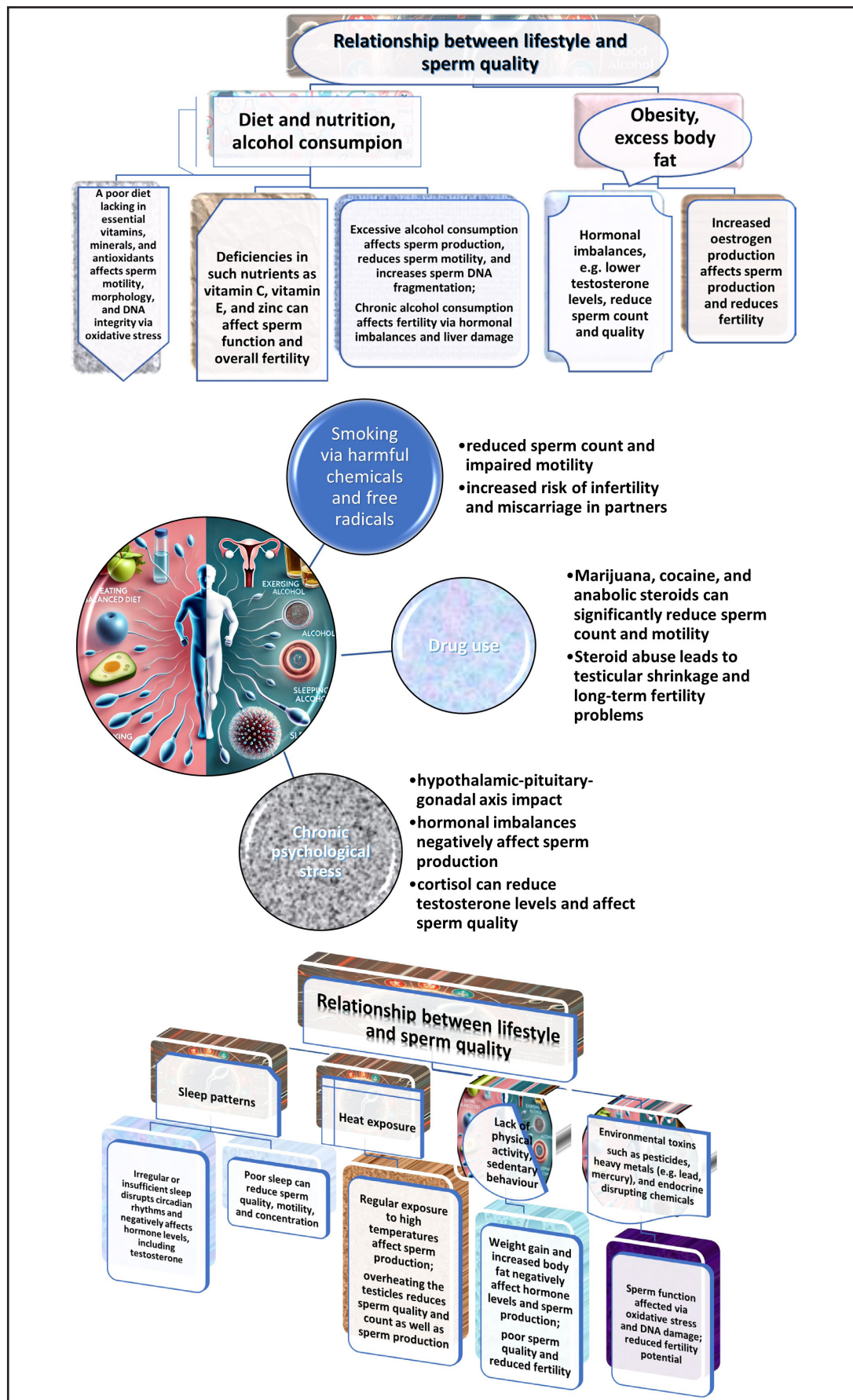


Fig. 3. Factors influencing the relationship between lifestyle and sperm quality and their negative effects.

cytokines, further exacerbate testicular damage and reduce sperm quality, as shown by Hasan et al. [68]. Thus, toxic metals interfere with endocrine regulation by disrupting the hypothalamic-pituitary-gonadal axis, affecting the synthesis, release, and receptor binding of key reproductive hormones, further compromising male fertility.

Research conducted in this area focuses on the interaction between toxic metals (aluminium, boron, cadmium, arsenic), genetic polymorphisms [MTHFRv.C677T (rs1801133) (chromosome-1) and IL-4v.C589T (rs2243250) (chromosome-5)], and oxidative damage (lipoperoxidation level estimated via MDA concentration), which was analysed in men from central Poland. A study conducted by Baszyński et al. [69] highlights that concentrations of toxic metals (aluminium, boron, cadmium, arsenic), lipid peroxidation (malondialdehyde, MDA levels), and genetic polymorphisms (MTHFRv.C677T and IL-4v.C589T) should be measured. The results suggest that, while toxic metals and reactive oxygen species overload influence male reproductive potential, genetic polymorphisms indirectly influence these processes without directly causing fertility disorders. The research also suggests that, while MTHFR and IL-4 polymorphisms indirectly influence metal concentrations and OS biomarkers, cadmium exposure significantly increases the risk of infertility by predisposing men to reproductive disorders. These findings highlight the importance of identifying and mitigating environmental stressors to improve diagnostic accuracy, reduce idiopathic infertility, and develop targeted therapeutic strategies [69].

Another study carried out by Baszyński et al. [70] highlighted the significant impact of environmental factors on male reproductive potential through their influence on OS and antioxidant defence mechanisms, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) as well as the levels of chemical elements (sodium, barium, aluminium, and boron) and the level of malondialdehyde (MDA), a marker of oxidative imbalance. The results showed that environmental exposure to specific elements significantly modulates antioxidant defences, with higher GPx activity observed in infertile men, suggesting an enhanced oxidative response. Furthermore, Na, Ba, Al, and B were identified as key environmental modulators of male fertility in this study, highlighting the importance of mitigating ecological stressors to maintain reproductive health.

A growing body of work has demonstrated that nutrition is an essential factor in preventing male infertility, as it plays a vital role in regulating various physiological processes that impact reproductive health [71]. Many studies demonstrated that a balanced diet rich in antioxidants, vitamins, and essential nutrients supports optimal sperm quality, i.e., such antioxidants as vitamin C, vitamin E, zinc, and selenium combat oxidative imbalance, which is a major cause of DNA damage in sperm [29, 72]. Diets high in processed foods, trans fats, and sugars have been linked to reduced sperm motility and concentration [73]. Recent research suggests that the Mediterranean diet, which incorporates the traditional healthy lifestyles of people living around the Mediterranean Sea, has many health benefits, including improvement of male reproductive health by increasing the number and quality of sperm, because omega-3 fatty acids and a plant-based diet rich in polyphenols may have a protective effect on sperm quality, as previously indicated by Montano et al. [74].

The study highlights the evidence provided by the authors, showing that moderate physical activity positively affects sperm parameters by improving hormonal balance, reducing redox imbalance, and improving blood flow to the testes [75]. However, excessive physical activity, especially endurance exercise, can have an adverse effect due to increased ROS overload and hormonal imbalances, such as reduced testosterone levels, and can provide compelling evidence for the significance of this phenomenon [76]. Conversely, a sedentary lifestyle and obesity are associated with decreased sperm concentration and increased DNA fragmentation [77].

As Zhang et al. [78] demonstrated, the core action pathways emphasise stress and mental health in relation to sperm quality. Indeed, chronic stress has been shown to increase cortisol levels, which can in turn suppress testosterone production and impair spermatogenesis [79]. Furthermore, mental stress is associated with increased oxidative damage, which further exacerbates sperm damage [80]. However, mindfulness and stress management have shown

promise in mitigating these effects. The results reported by Kim et al. [81] and Potter et al. [82] demonstrate that sleep and circadian rhythms are significant factors since inadequate sleep quality and disrupted circadian rhythms harm reproductive hormones, consequently leading to diminished sperm quality. Furthermore, a study by Lateef and Akintubosun [83] indicated that sleep deprivation is associated with reduced testosterone levels and augmented oxidative damage in sperm cells.

Therefore, a healthy lifestyle is essential for maintaining optimal sperm quality and overall reproductive health. Addressing modifiable factors, such as diet, physical activity, substance use, and environmental exposures, offers a practical approach to reducing the risk of infertility and improving outcomes with assisted reproductive technologies. However, relevant studies were unable to establish any definitive positive or negative correlations between a vegetarian diet and semen quality, sex hormone levels, and infertility [84].

In their study, Karayiannis et al. [85] examined the association between adherence to the Mediterranean diet (MD) and semen quality in men from subfertile couples seeking fertility treatment. The findings indicated that higher adherence to the MD was significantly associated with improved semen parameters, including sperm concentration, total sperm count, and sperm motility. Specifically, men in the highest tertile of the MD score exhibited a significantly lower prevalence of abnormal sperm characteristics, such as lower sperm concentration and motility, than those in the lowest tertile. However, the study's cross-sectional design precludes the ability to establish causality between MD adherence and semen quality. Nevertheless, the results are consistent with previous research suggesting that dietary patterns rich in fruits, vegetables, legumes, and whole grains, characteristic of the MD, may positively influence semen quality [85]. Findings reported by another scientific team [86] imply a potentially beneficial effect of traditional Mediterranean dietary patterns on male reproductive potential.

Thus, preventing and treating ROS overload and antioxidant deficiency requires a multifaceted approach that includes lifestyle modifications, environmental management, and dietary interventions. Addressing the causes of ROS production and boosting the body's antioxidant capabilities are key steps towards reducing the harmful effects of OS. Targeting these mechanisms makes it possible to mitigate the detrimental effects of oxidative damage on male reproductive health and overall well-being.

Stress reaction to testicular hyperthermia

In the following section, the application of these insights to the realm of thermal and environmental stressors impacting sperm integrity is examined. Research in this field has indicated a critical interdependence between hyperthermia and temperature regulation in human health. Specifically, Durairajanayagam et al. [87] and Mieusset and Bujan [88] have demonstrated that testicular function is maintained at approximately 2°C lower than the body's core temperature. Furthermore, elevated scrotal temperatures resulting from such factors as physical inactivity, obesity, occupational heat exposure, and laptop use have been shown to exert several detrimental effects on testicular health [89]. These include germ cell apoptosis and autophagy, DNA damage, testicular atrophy, spermatogenic arrest, reduced inhibin B levels, and increased production of reactive oxygen species, all contributing to impaired male reproductive function. Specifically, elevated scrotal temperatures, which are defined as temperatures higher than the body's core temperature, can increase ROS production [90].

As demonstrated in studies conducted by Jenget al. [91, 92, 93], individuals occupationally exposed to polycyclic aromatic hydrocarbons exhibited increased bulky DNA adducts and 8-oxo-dGuo levels, suggesting DNA damage. Additionally, the study indicates that oxidative imbalance, possibly exacerbated by thermal stress, contributes to sperm DNA fragmentation. It is crucial to note that the presence of bulky DNA adducts does not necessarily indicate the existence of oxidative damage and fragmentation. This underscores the deleterious impact of thermal and environmental stressors on maintaining the integrity of spermatozoa.

Findings from prior investigations have indicated that the scrotum, being outside the body cavity, is highly sensitive to temperature changes and prolonged exposure to heat (through such activities as hot baths, saunas, and prolonged sitting), which can increase OS, especially in testicular cells [94]. It was shown that hyperthermia inhibits spermatogenesis (sperm production) and can also cause lipid peroxidation, which disrupts sperm membrane integrity and motility [95]. Paul et al. [90] investigated the stress response in mouse testes following a mild transient scrotal heat exposure (40°C or 42°C for 30 minutes). The results showed an increase in the expression of hypoxia-inducible factor 1 alpha (HIF1 α) mRNA and translocation of HIF1 α protein to the germ cell nucleus, indicating hypoxic stress. In addition, upregulation of haem oxygenase 1 (Hmox1) and antioxidant enzymes, including glutathione peroxidase 1 (GPX1) and glutathione S-transferase alpha (GSTA), confirmed the activation of redox dysfunction pathways. Germ cell death, as evidenced by increased expression of cleaved caspase-3 and decreased levels of caspase-activated DNase inhibitor (ICAD), was associated with DNA fragmentation observed by TUNEL staining. These findings confirm that transient mild testicular hyperthermia induces temperature-dependent germ cell death and a complex stress response involving hypoxia and redox dysfunction pathways [90, 94].

Next, elevated temperatures affect mitochondrial function in sperm cells, which is essential for ATP production, leading to the generation of greater amounts of ROS. In a study conducted by Kopalli et al. [96], it was demonstrated that *Panax ginseng* Meyer, more commonly known as Korean Red Ginseng (KRG), is a traditional herb that has been used to enhance libido and male fertility. The study evaluated the effects of an Rg3-enriched KRG extract (KGC04P) on heat stress-induced testicular damage in rats. The results significantly improved parameters, including sperm kinematics and testicular enzyme expression. The findings suggest that KGC04P can effectively prevent heat stress-induced damage, offering potential as a therapeutic agent for hyperthermia-related male infertility [96].

Physiological role of reactive oxygen species in spermatogenesis

A study conducted by Dutta et al. [97] demonstrated that ROS play a critical role in sperm capacitation, maturation, hyperactivation, acrosome reaction, and fertilisation. The authors elucidated that, in physiological conditions, ROS are indispensable for regulating cellular signalling and activating molecular mechanisms that support the proper function and reproductive capacity of sperm [8]. Spermatozoa generate ROS via two primary mechanisms: the plasma membrane and the mitochondria [98]. Aitken [99] demonstrated that reactive oxygen species overload is a critical factor in the life and function of mammalian spermatozoa, with ROS generated in three ways involving sperm mitochondria, cytosolic L-amino acid oxidases, and plasma membrane NADPH oxidases. These ROS have been shown to drive physiological changes during sperm capacitation by activating the cAMP/PKA phosphorylation cascade, upregulating tyrosine phosphorylation in the sperm tail, and inducing sterol oxidation, all of which are essential for sperm function [99].

Research conducted in this area has suggested that mitochondrial respiration is the primary biological source of ROS in normal physiological conditions [100]. However, mitochondrial dysfunction can significantly exacerbate electron leakage within the electron transport chain, leading to overproduction of ROS that can reach toxic levels, as shown by Koppers et al. [101]. This disrupts redox homeostasis and triggers oxidative damage. High levels of ROS can induce lipid peroxidation, resulting in the formation of reactive aldehydes that further propagate cellular damage. At the plasma membrane, ROS are generated via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system [102]. In mitochondria, ROS generation occurs predominantly through NAD-dependent redox reactions, driven by the high metabolic activity of these organelles. Thus, mitochondrial functioning plays a critical role in energy production for sperm motility, making them a significant site of ROS generation [103].

Studies conducted by Perl et al. [104] and Perl [105] emphasise the pivotal function of transaldolase (TAL) in signalling pathways that are indispensable for sperm function. In particular, it was highlighted that the pentose phosphate pathway plays a role in male sterility, and TAL is particularly important in this regard. Earlier research by the same authors had already demonstrated that TAL deficiency disrupts the balance of NAD(P)H and reactive oxygen intermediates, resulting in mitochondrial dysfunction in sperm cells. This structural and functional damage ultimately leads to male infertility [105]. TAL deficiency (TAL^{-/-}) in animals has been shown to disrupt the mitochondrial transmembrane potential by impairing the redox balance involving reactive oxygen intermediates, NADPH, NADH, and glutathione (GSH). This results in mitochondrial dysfunction, characterised by diminished Ca²⁺ flux, reduced mitochondrial ROS production, and intracellular acidosis, all contributing to the impairment of forward motility and fertility. Furthermore, compensatory changes in signalling molecules, such as the downregulation of carbonic anhydrase IV and the upregulation of CD38 and gamma-glutamyl transferase, further reflect the disruption of key metabolic and signalling pathways. Microarray analysis has revealed the predominant impact of TAL deficiency on the latter stages of sperm cell development, affecting the electron transport chain and GSH metabolism, thereby underscoring the essential role of TAL in maintaining mitochondrial function and critical sperm signalling pathways for fertility [104].

Building on prior research, Sotolongo and Ward [106] provided evidence that spermatozoa reach the epididymis and contain a less compact DNA strand, making them inert and unable to perform their functions effectively. However, under the influence of hydrogen peroxide, nuclear proteins are oxidised, leading to DNA condensation, making the sperm more motile; this aligns with the conclusions reached by other authors [107, 108]. It is widely acknowledged that this process is essential for sperm to swim towards the egg [109] and penetrate the egg [110]. Notably, hydrogen peroxide at low concentrations also induces tyrosine phosphorylation, which increases the area on the sperm membrane that interacts with the egg, ultimately increasing the chances of fertilisation; this is essential for sperm to capacitate and successfully fertilise an egg [111, 112].

There is also evidence that hydrogen peroxide is necessary for the acrosomal reaction, although the exact mechanism by which this ROS influences this process remains unclear [109]. The acrosomal reaction is a crucial event in fertilisation, as it allows the sperm to penetrate the egg's outer layers. While ROS, including hydrogen peroxide, can play critical physiological roles, their accumulation must be tightly regulated, as excessive levels of ROS can damage sperm and other cells.

It is imperative to comprehend the intricate interplay between redox imbalance and antioxidant defence systems in a physiological milieu that fosters normal spermatogenesis. The importance of such research stems from its ability to show that seminal plasma and sperm exhibit a notable abundance of protective antioxidants [113, 114]. These antioxidants are instrumental in ensuring that ROS, indispensable for crucial functions, such as antimicrobial defence and intracellular signalling, do not inflict harm upon the body's cells. Antioxidants, e.g., SOD, catalase, and glutathione peroxidase, neutralise excess ROS, thereby preventing oxidative damage to sperm DNA and membrane integrity [115]. This balance is essential for preserving sperm quality and function and maintaining male fertility [116].

It has been established in the scientific literature that both spermatozoa and seminal plasma contain natural antioxidants that protect against OS, particularly after testicular production, which is crucial for maintaining sperm quality [15]. Several high-molecular-weight enzymatic antioxidants in seminal plasma are fascinating, as they are essential in neutralising ROS [116]. This field of research provides valuable insights and shows that, during spermatogenesis and epididymal maturation, spermatozoa acquire key antioxidant enzymes, including SOD, catalase, GPx, peroxiredoxins, glutathione-S-transferases, thioredoxins, and thioredoxin reductase, working collectively to prevent oxidative damage [79]. The absence of any of these enzymes, as evidenced by studies utilising knockout models, has been demonstrated to have a detrimental effect on sperm quality, resulting in compromised motility, fertilisation ability, and DNA integrity due to increased redox

imbalance [79]. Another vital aspect is connected with the activity of SOD, which catalyzes the conversion of superoxide anion into less harmful compounds, while catalase functions by breaking down hydrogen peroxide into water and oxygen. Notably, the seminal plasma and the sperm itself contain SOD, with the former protecting the sperm from oxidative damage [99], thus illustrating its crucial role in safeguarding sperm from the potentially deleterious effects of OS [79].

As a vital component of a protective antioxidant triad, GPx plays a pivotal role in preventing oxidative damage to sperm DNA and membranes [118, 115] through interaction with hydroperoxides using glutathione as an electron donor and reduction of peroxides using glutathione as a substrate. A seminal study conducted by this scientific team underscored the pivotal function of selenium-containing GPxs, particularly GPx4, in preserving male fertility. This is achieved by forming the mitochondrial sheath of spermatozoa, which is imperative for their structure and function. Furthermore, GPx4 has been identified as a pivotal regulator of oxidative imbalance, functioning by reducing lipid hydroperoxides and silencing lipoxygenases. This process is vital in preventing oxidative damage, which has the potential to compromise sperm integrity and viability, as shown in the experiments carried out by [118].

Seminal plasma contains non-enzymatic antioxidants as well as enzymatic antioxidants [119]. Examples include ascorbic acid (vitamin C), α -tocopherol (vitamin E), pyruvate, glutathione, L-carnitine, taurine, and hypotaurine. These non-enzymatic antioxidants directly affect free radicals, scavenging them and preventing cell damage. For instance, ascorbic acid is a highly effective free radical scavenger, capable of directly neutralising ROS. At the same time, α -tocopherol is a potent antioxidant that protects the sperm membrane from lipid peroxidation. L-carnitine and taurine maintain sperm motility and function by stabilising the mitochondrial membrane and protecting against oxidative damage [120]. In addition, seminal plasma contains other antioxidant components, including urate, pyruvate, albumin, β -carotene, and ubiquinol [121]. Urate, for example, acts as a powerful antioxidant, scavenging peroxynitrite and other reactive species [122]. Pyruvate helps maintain mitochondrial function by buffering excess ROS production. Albumin has also been demonstrated to bind and neutralise potentially damaging free radicals, while the role of β -carotene and ubiquinol in the overall antioxidant defence system is to protect against lipid peroxidation and antioxidant deficiency [123, 124].

The antioxidant capacity of seminal plasma has been shown to be significantly greater than that of blood plasma [125], being approximately ten times higher. In a study employing the Total Radical Trapping Antioxidant Potential (TRAP) method, Rhemrev and colleagues demonstrated that the antioxidant properties of seminal plasma are primarily attributed to the presence of vitamin C, uric acid, and tyrosine, which together protect sperm cells from oxidative damage. While other known antioxidants, such as glutathione and taurine, demonstrate some antioxidant activity, their contribution is limited due to their relatively low concentrations [125]. These findings suggest that the fast and slow TRAPs are valuable infertility biomarkers and potential targets for antioxidant-based therapeutic interventions. However, it is essential to note that, during spermatogenesis and in the epididymis, spermatozoa are not directly exposed to the antioxidants present in the seminal plasma [79]. Instead, protection against oxidative damage is provided by the antioxidants produced in the testes and epididymis and by the intrinsic antioxidant capacity of the sperm. Consequently, spermatozoa exhibit heightened vulnerability to OS as they traverse the epididymis, particularly in inflammatory conditions.

Another study [126] demonstrated that tyrosine, with its strong antioxidant capacity and high plasma concentration, plays a significant role in the total antioxidant capacity of seminal plasma. The post-addition method using ABTS radical scavenging revealed a distinctive antioxidant profile in seminal plasma, which quenches radicals continuously and slowly. Some compounds, such as ascorbic acid, α -tocopherol, and uric acid, have been shown to exhibit rapid radical scavenging properties. In contrast, others, including hypotaurine and tyrosine, have contributed to the same slow radical scavenging pattern as in the case of seminal plasma, as demonstrated by Pérez-Pé et al. [127].

Thus, spermatozoa rely on a delicate balance of enzymatic and non-enzymatic antioxidants to protect them from oxidative damage. Depletion or dysfunction of these antioxidant systems can lead to irreversible damage to sperm, including chromosome fragmentation and loss of fertility. The complex interplay of these antioxidants highlights the importance of managing redox imbalance in maintaining male fertility and reproductive health.

Implications of ROS production and male fertility

It is well-documented in the literature that, in men, controlled production of ROS plays a critical role in essential reproductive processes, such as sperm hyperactivation, capacitation, acrosome reaction, and natural fertilisation, as revealed earlier by Aitken [99] and Aitken et al. [100]. Spermatozoa exhibit heightened vulnerability to redox imbalance, as evidenced by the capacity of ROS to inflict damage to the sperm membrane and DNA, culminating in lipid peroxidation and sperm DNA fragmentation (SDF), as elucidated in their studies by Cannarella et al. [128] and Jeng et al. [92]. These functions rely on moderate levels of ROS to facilitate key signalling pathways required for successful reproduction. However, excessive ROS production under OS, particularly by immature germ cells and leukocytes, has detrimental effects. These include increased lipid peroxidation, reduced sperm motility, and cellular damage, all of which contribute to male infertility. The imbalance between ROS production and antioxidant defences is a central mechanism in the pathogenesis of OS-induced reproductive dysfunction [96, 101].

ROS induce oxidative DNA damage in sperm through single- and double-strand breaks and base modifications, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), as demonstrated by Jeng et al. [92]. These alterations compromise genetic integrity, potentially affecting fertilisation outcomes and increasing the risk of inherited defects. A study carried out by authors [92] found that coke oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) showed a significant increase in bulky DNA adducts and 8-oxo-dGuo, compared to the control group, indicating DNA damage. However, no significant differences were observed in sperm DNA fragmentation or denaturation between the exposed and control groups, despite a positive correlation between 8-oxo-dGuo levels and DNA fragmentation. The findings of this study suggest that ROS overload may contribute to sperm DNA fragmentation. In contrast, bulky DNA adducts appear independent of oxidative DNA damage and fragmentation in sperm integrity [93].

Lipid peroxidation, another critical process, targets the polyunsaturated fatty acids (PUFAs) of the sperm membrane, resulting in MDA production [25]. This process leads to membrane remodelling, dissipation of mitochondrial membrane potential, electron leakage via increased ROS production and reduced energy production, accumulation of cytotoxic byproducts like 4-hydroxynonenal, dysregulation of bioenergetic pathways, and disruption of structural and signalling components of the motility apparatus, all of which contribute to sperm dysfunction and genomic lesions. This toxic byproduct also weakens membrane integrity, reduces fluidity, and impairs sperm motility, which is critical for successful fertilisation [116]. In addition, ROS-induced mitochondrial dysfunction exacerbates oxidative damage and disrupts ATP production, which is essential for sperm energy and motility [129].

ROS are integral to sperm function via redox signalling, which modulates protein phosphorylation critical for capacitation and the acrosome reaction. The mechanisms described above are consistent with the findings reported by de Lamirande and O'Flaherty [130], who demonstrated that ROS, such as superoxide anion, hydrogen peroxide, and nitric oxide, act as second messengers regulating sperm capacitation, and this process is essential for sperm-oocyte interaction, acrosome reaction, and fertilisation. Their research highlighted that ROS modulate key signalling events during capacitation, including the increase in cAMP, PKA activation, phosphorylation of specific substrates, MEK-like protein phosphorylation, and late tyrosine phosphorylation of fibrous sheath proteins, with these

processes being regulated by various kinases, including protein kinase C, PKA, and protein tyrosine kinases. Furthermore, the role of ROS in regulating sperm motility and the acrosome reaction was elucidated, with these effects likely being achieved through sulfhydryl/disulfide interactions on sperm proteins. The proper timing and function of spermatozoa is ensured by redundancies and cross-talk [130].

Phosphorylation of tyrosine residues is a redox-sensitive process for sperm to acquire fertilising capacity. One of the critical molecular mechanisms is the effect of OS on Leydig cells, which are responsible for testosterone production. OS can disrupt the steroidogenic pathway in these cells by inhibiting enzymes responsible for testosterone synthesis, such as 17β -hydroxysteroid dehydrogenase and cytochrome P450 side chain cleavage enzyme (P450scc), as demonstrated by Miller and Auchus [131]. This results in reduced testosterone levels, directly affecting sperm production and motility. In addition, a decrease in testosterone levels further exacerbates infertility by impairing spermatogenesis [4]. A comprehensive elucidation of the mechanisms of steroidogenesis is imperative for comprehending a broad spectrum of physiological functions, as it governs the synthesis of biologically active steroid hormones indispensable for sexual differentiation, reproduction, fertility, and overall homeostasis. A more profound comprehension of the enzymes, cofactors, and genetic factors implicated in steroidogenesis provides valuable insights into various disorders, including reproduction, metabolic conditions such as obesity, and hypertension [131].

In obese men, elevated ROS levels also cause damage to sperm DNA, which can significantly reduce sperm fertility. ROS-induced DNA damage can lead to mutations, chromosome fragmentation, and overall loss of sperm quality, making it difficult to achieve a successful pregnancy [26, 29, 30]. Furthermore, the adverse effects of obesity on sperm DNA integrity have been shown to reduce the success rates of assisted reproductive technologies, such as *in vitro* fertilisation, where sperm quality is critical for achieving fertilisation and subsequent embryo development [132].

Infertile men often have reduced activity of enzymatic antioxidants, such as GPx, SOD, and catalase (CAT). The depletion of non-enzymatic antioxidants, including reduced GSH, ascorbic acid (vitamin C), and tocopherol (vitamin E), further exacerbates oxidative damage. As demonstrated by Kopalli et al. [96], exposure to heat stress elicits substantial alterations in diverse parameters, encompassing body and organ weight, sperm motility, and lipid metabolism markers in both serum and testicular tissue. Concurrently, this exposure affects the expression of testicular antioxidant enzymes, inflammatory cytokines, sex hormone receptors, and genes associated with spermatogenesis [91, 92, 93]. Together, these deficiencies weaken the ability of sperm to maintain redox balance, resulting in increased susceptibility to oxidative damage. Therapeutic approaches with antioxidants are an essential way to combat male infertility. Therefore, given the role of ROS in sperm dysfunction, antioxidant therapy offers a promising approach to the treatment of male infertility. Antioxidants work by neutralising ROS, increasing the activity of enzymatic defence systems, stabilising sperm membranes, and protecting DNA from oxidative damage. Supplementation with key antioxidants, such as vitamins C and E, selenium, and coenzyme Q10, can help restore redox balance, reduce lipid peroxidation, and improve sperm quality. By lowering oxidative imbalance, these interventions improve fertility potential and the likelihood of successful conception [121, 123, 124].

Endogenous and exogenous sources of ROS in the male reproductive system

The primary ROS in human spermatozoa is superoxide anion ($\cdot\text{O}_2^-$), which can undergo enzymatic or spontaneous dismutation to form hydrogen peroxide (H_2O_2) [133]. In the presence of transition metals, such as iron or copper, H_2O_2 can participate in Fenton or Haber-Weiss reactions, forming the highly reactive hydroxyl radical ($\text{OH}\cdot$). These hydroxyl radicals are among the most destructive ROS, initiating lipid peroxidation (LPO) cascades [134]. LPO significantly compromises the integrity and fluidity of the sperm plasma membrane, affecting critical functions, such as motility, capacitation, and the acrosome reaction [135, 136].

In a study conducted by Gil-Guzmán et al. [137], significant variations in ROS production among subsets of spermatozoa at different stages of maturation were observed, and it was hypothesised that oxidative damage to mature spermatozoa by ROS-producing immature spermatozoa during their migration from the seminiferous tubules to the epididymis may be a significant cause of male infertility. In addition to membrane damage, ROS also causes oxidative DNA damage, contributing to fragmentation in sperm nuclei [138]. A study conducted by authors [138] demonstrated that the chromosomal architecture within the mouse sperm nucleus exhibits a distinct and organised arrangement, where certain chromosomes preferentially occupy specific positions. Employing a combination of the fluorescence in situ hybridisation method, confocal microscopy, and three-dimensional reconstruction techniques, the authors conclusively demonstrated that chromosome positioning is non-random. Their investigation of a transgenic mouse model (Gpx5^{-/-}) revealed that oxidative DNA damage does not disrupt the organization of chromosomes but significantly alters specific 3D nuclear parameters. The findings of this study suggest that oxidative DNA damage, which was previously underappreciated, may have a profound impact on chromatin quality and potentially influence post-fertilisation processes [138].

As Cassina et al. [8] suggested, mitochondria are likely the primary sources of ROS in sperm cells. Their study employed high-resolution respirometry, ROS production analysis, and the evaluation of oxidative and nitritative stress markers in intact human sperm cells. The findings demonstrated a correlation between mitochondrial dysfunction, as indicated by a reduced respiratory control ratio, and decreased sperm motility. ROS also cause oxidative DNA damage that can compromise genetic integrity and reduce fertilisation potential. Furthermore, mitochondrial dysfunction exacerbates ROS production, creating a vicious cycle that amplifies oxidative burden. This cascade disrupts the electron transport chain, further increasing ROS generation and impairing ATP production, which is essential for sperm motility, as demonstrated earlier by Koppers et al. [101].

The primary endogenous sources of ROS in the male reproductive system are leukocytes and immature spermatozoa. This is confirmed by the direct correlation between the production of ROS and cytokines in seminal fluid, such as interleukin IL-6, IL-8, and tumour necrosis factor (TNF), as shown by Jiang et al. [139] and Collodel et al. [140]. Among leukocytes, polymorphonuclear neutrophils and macrophages are the major contributors to ROS production [141], particularly during infections or inflammatory processes in the prostate and seminal vesicles. A study by Rosales [142] highlights neutrophils' diverse and critical roles, i.e., the most abundant leukocytes in circulation. These cells are rapidly mobilised to sites of inflammation or infection, where they perform essential antimicrobial functions, including degranulation, ROS production, phagocytosis, and the formation of neutrophil extracellular traps. While the traditional view regards these cells as short-lived cells primarily tasked with pathogen elimination, recent findings have revealed their capacity to modulate adaptive immune responses through cytokine production and interactions with lymphocytes and dendritic cells, as shown earlier by the author [142].

Furthermore, neutrophil heterogeneity, with distinct functional phenotypes, has been observed in pathological conditions, such as cancer and chronic inflammation. These findings align with studies on sperm cells, where ROS also play a dual role, being crucial for physiological processes yet harmful when produced excessively, contributing to cellular dysfunction and pathologies [142]. In these conditions, leukocytes can produce up to 100 times more ROS than normally as part of the immune defence response [143]. This increased ROS production is exacerbated by activation of the NADPH oxidase system and increased NADPH levels through the hexose monophosphate shunt, which supports ROS generation [144].

In addition to leukocytes, immature spermatozoa contribute to ROS production [145], which is essential for sperm maturation. However, excessive ROS in spermatozoa can lead to oxidative damage, particularly to the sperm plasma membrane, DNA, and mitochondria, impairing sperm motility and fertilisation potential [137]. Furthermore, inflammation in the reproductive tract increases pro-inflammatory mediators, such as cytokines and

prostaglandins, while simultaneously depleting antioxidant defences, as shown by the authors [140] in a rabbit model.

In summary, the impaired function of antioxidant systems in the context of chronic inflammation is a crucial factor in the maintenance of a persistent OS environment. This imbalance between the production of ROS and antioxidant defence mechanisms can severely impact male fertility, leading to disruptions in sperm motility, DNA integrity, and overall reproductive health. A deeper understanding of the endogenous sources of ROS emphasises the necessity of managing inflammation effectively and maintaining optimal antioxidant status in the male reproductive system. Therefore, interventions such as antioxidant supplementation and regulation of inflammatory processes may prove vital in mitigating the adverse effects of ROS on male fertility and reproductive health.

Role of sperm DNA fragmentation in male infertility

As demonstrated in a seminal study by Krausz et al. [146], a significant proportion of the male population, approximately 7%, is afflicted by male infertility. The condition, marked by its intricate genetic underpinnings, encompasses considerable heterogeneity in semen parameters and testicular histological phenotypes, with at least 2,000 genes implicated in the complicated process of spermatogenesis. As highlighted by authors [147], the most prevalent genetic factors contributing to male infertility, amounting to 25% of cases, are observed in azoospermia. Baszyński et al. [69] emphasise that male infertility is a complex multifactorial condition influenced by both environmental and genetic factors, accounting for 20-50% of infertility cases worldwide. Nevertheless, the identification of genetic abnormalities in other semen parameters and aetiological categories continues to increase. The findings of these studies emphasise the increasing complexity of male infertility, with the continued discovery of genetic variations playing a crucial role in its diagnosis and treatment.

The role of the process of sperm DNA fragmentation (SDF) in the development of male infertility is well documented in the research literature [148, 149]. DNA fragmentation represents the final stage of intracellular changes leading to apoptosis, a programmed cell death process primarily responsible for eliminating damaged cells [150]. A seminal study conducted by Collodel et al. [140] and Jiang et al. [139] demonstrated that OS is a major trigger of this process. SDF is increasingly being recognised as a critical factor in male infertility [151]. SDF represents the culmination of intracellular damage that precedes apoptosis, a biological process essential for the elimination of defective cells [148], and OS is a primary driver of this programmed cell death. Elevated ROS levels in sperm cells initiate oxidative damage that disrupts DNA integrity and triggers molecular pathways that lead to fragmentation. Muratori et al. [152] provided valuable insights into the mechanisms underlying SDF, highlighting apoptosis as the primary pathway responsible for DNA breaks in sperm cells. The findings indicate that defects in chromatin maturation, combined with redox dysfunction, contribute significantly to the fragmentation process, particularly during sperm transit through the male genital tract. The results of this study are of significant clinical importance, as they contribute to a more comprehensive understanding of the effects of drugs and OS on SDF in infertile men, and they inform the development of new therapeutic strategies that target these mechanisms [152].

SDF leads to intracellular damage and ultimately triggers apoptosis in sperm cells. ROS, particularly hydroxyl radicals, induce DNA breaks by oxidising nucleotide bases, resulting in single-strand and double-strand breaks, as Cannan and Pederson show [153]. Their study demonstrated that exposure to ionising radiation can induce double-strand breaks (DSBs) in DNA, and such DSBs may also arise when replication forks encounter DNA lesions or repair intermediates. Namely, the processing and repair of these breaks can lead to mutations. Such alterations can potentially result in cell death or cancer, with the most common repair pathway in metazoans, non-homologous DNA end joining, being more mutagenic than homologous recombination-mediated repair [153]. As emphasised by Dianov and Hübscher

[154], base excision repair (BER) is paramount in maintaining genome integrity, a concept directly relevant to sperm DNA repair processes. BER plays a crucial role in mitigating DNA damage caused by endogenous and exogenous mutagens, ensuring the maintenance of sperm quality and preventing premature aging, cancer, and infertility. A study by Dianov and Hübscher [154] highlights the necessity of precise coordination between BER and cell cycle progression to avoid the replication of damaged DNA, a process particularly significant in sperm cells, where reactive oxygen species overload and DNA fragmentation are prevalent.

As demonstrated by Stinson and Loparo [155], non-homologous DNA end joining (NHEJ) has been identified as the predominant repair pathway for DSBs in mammalian cells during the cell cycle. This repair pathway is particularly crucial in the S and G2 phases when DNA replication is active and homologous recombination is less feasible due to the absence of an undamaged template. It is therefore vital to understand the role of NHEJ in maintaining genomic stability by facilitating the rapid repair of DSBs, a function that 8-oxo-7, 8-dihydroguanine, especially in such cells as sperm, is especially important in such cells as sperm, which are highly susceptible to redox imbalance and DNA damage [155].

Redox imbalance also forms a specific DNA lesion that leads to mutagenesis, as Fleming and Burrows show [156]. Their study highlights the mutagenic potential of oxidative DNA damage, particularly the formation of 8-oxo-7, 8-dihydroguanine, which can lead to G→T transversion mutations, a process relevant to sperm cells exposed to oxidative imbalance. In sperm, oxidative modifications of DNA, including OG formation, can result in polymerase misreading, leading to mutations that affect sperm DNA integrity and contribute to infertility. Additionally, the involvement of base excision repair in removing 8-oxo-7, 8-dihydroguanine and its potential regulatory role in gene activation underscores the complex balance between DNA repair and the epigenetic functions of oxidative DNA damage in maintaining reproductive health. As a result of this damage, chromatin remodelling is impaired, and incomplete protamination of sperm DNA increases susceptibility to oxidative damage, making DNA more prone to fragmentation [148, 154].

Redox dysfunction has been demonstrated to activate intrinsic apoptotic pathways in sperm cells, as evidenced by experimental studies [150]. This process is characterised by the release of cytochrome c from the mitochondria and the subsequent activation of caspases (particularly caspase-3 and caspase-9). Another study [157] has shown that the process of apoptosis induced by the administration of staurosporine leads to the fragmentation of nuclear DNA mediated by endonucleases, such as caspase-activated DNase. The analysed molecular processes lead to sperm DNA fragmentation, which is a key factor in male infertility.

Agarwal et al. [149] emphasise the significance of SDF tests as clinical markers in the assessment of male fertility, as SDF serves as a critical indicator of sperm quality and potential infertility. It is known that SDF is associated with early embryonic arrest and miscarriage and reduces the success of assisted reproductive technologies by impairing sperm fertility and embryo development [158]. These tests are categorised into two main types: direct and indirect. Direct assays operate probes and dyes to measure SDF directly. In contrast, indirect assays evaluate existing DNA breaks and the susceptibility of DNA to denaturation, a characteristic commonly observed in fragmented DNA. The most widely used methods for assessing SDF include TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labelling), which detects DNA strand breaks by labelling exposed DNA ends with nucleotides. The SCSA test (Sperm chromatin structure assay), which analyses sperm DNA integrity by examining the structural configuration of sperm chromatin, is also commonly used. The SCSA test measures the dispersion of sperm chromatin, with fragmented DNA displaying a broader dispersion pattern. These tests are of top importance in the identification of sperm with compromised DNA integrity, thus providing valuable information to guide infertility treatment strategies and clinical interventions [149].

Researchers have been actively investigating the relationship between sperm DNA integrity and male fertility. In approximately 20% of cases of idiopathic male infertility, the inability of a sexually active couple to achieve pregnancy can be attributed to single- and/or double-stranded DNA breaks in the sperm head [159]. These DNA breaks often

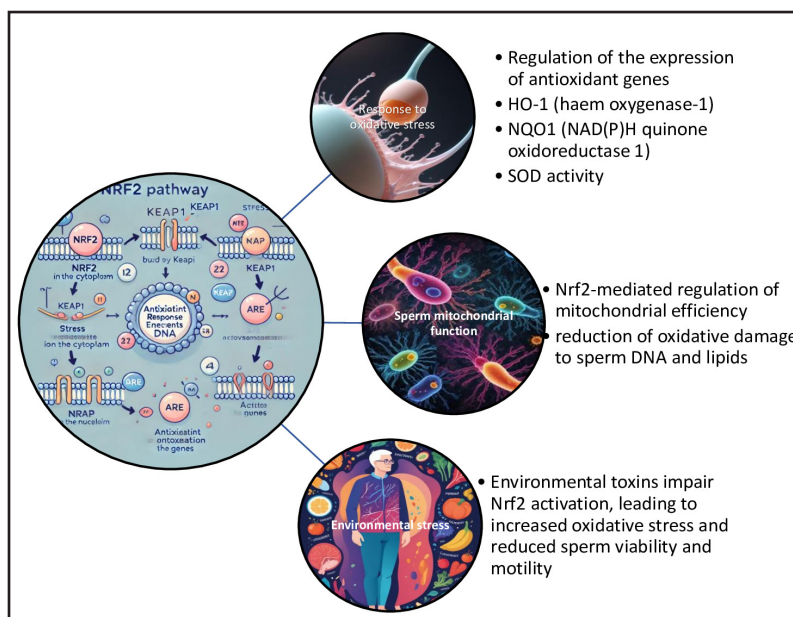
correlate with the severity of pathozoospermia [160, 161]. In addition, in many cases, SDF is responsible for the arrest of embryonic development and subsequent elimination of the embryo during the early stages of embryogenesis, resulting in missed pregnancies, as shown by Sahin et al. [161]. Importantly, this condition significantly reduces the effectiveness of assisted reproductive technologies in treating infertile couples [163]. The persistence of DNA fragmentation in sperm cells highlights the importance of targeted interventions to improve DNA integrity, thereby improving reproductive outcomes and supporting successful pregnancies [161].

Thus, SDF has been identified as a pivotal factor in male infertility, given its direct impact on sperm quality and fertilisation capacity. Assessment of sperm DNA fragmentation using such methods as terminal deoxynucleotidyl transferase dUTP nick end labelling, sperm chromatin structure assay, and sperm chromatin dispersion provides valuable insights into the extent of DNA damage. It is therefore a reliable means of assessing sperm integrity. The identification of elevated levels of DNA fragmentation is paramount for the diagnosis of infertility, the guidance of clinical interventions, and the development of targeted therapeutic strategies aimed at the enhancement of male reproductive health.

Role of Nrf2 in male infertility via ROS

The significance of this research lies in its potential to elucidate phenomena that, in normal physiological conditions, demonstrate the vital role of ROS as secondary messengers in diverse signalling pathways within cells [164]. However, it is essential to note that their excessive accumulation can lead to oxidative damage to cellular components, including lipids, proteins, and DNA [165]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a pivotal regulator of antioxidant and cytoprotective genes, playing a crucial role in mitigating redox imbalances by orchestrating cellular defence mechanisms [62] and particularly impacting reproductive success [166]. Studies highlight factors contributing to sperm membrane and DNA damage, dysregulated RNA processing, and telomere destruction via Nrf2 [163, 166].

Fig. 4. Nrf2 (nuclear factor erythroid 2-related factor 2) pathway and male infertility. The Nrf2 pathway plays a significant role in male infertility by protecting sperm cells from oxidative stress, which is a major contributor to male reproductive dysfunction. Oxidative damage can impair sperm motility, DNA integrity, and overall sperm function, leading to infertility. Activation of the Nrf2 pathway can enhance antioxidant defence mechanisms in the testes and improve sperm quality, suggesting that targeting Nrf2 signalling may offer therapeutic potential in treating male infertility related to oxidative stress.



The studies emphasise the regulatory function of Nrf2 in maintaining the balance between oxidants and antioxidants, thereby protecting male fertility. The authors established a link between Nrf2 signalling pathways and the regulation of spermatogenesis and sperm quality. This provides valuable insights into potential therapeutic strategies for modulating oxidative imbalance and inflammation to improve male reproductive health [163].

A study highlighting the central role of Nrf2 in male fertility, particularly in the context of oligospermia, was conducted by Han et al. [167]. It elucidated the protection offered by Nrf2 against ferroptosis, emphasising its importance in maintaining sperm quality and overall male reproductive health. The research findings indicate that Nrf2 and GPX4 protein levels are significantly reduced in sperm from patients diagnosed with oligospermia. Furthermore, Nrf2 knockout mice exhibit reduced sperm concentrations, motility, and fertility. The study further demonstrated the presence of ferroptosis-related biomarkers, such as elevated malondialdehyde (MDA) levels, and altered expression of ferroptosis-related genes, including GPX4 and ferroportin 1 (FPN1), in Nrf2 knockout mice. Treatment with a ferroptosis inhibitor was found to reverse these effects, proving that Nrf2 deletion induces ferroptosis in spermatogenic cells, thereby contributing to oligospermia pathogenesis. This finding underscores the critical role of Nrf2 in maintaining sperm quality, as demonstrated by many authors [167] and shown in Fig. 4.

A study conducted by Akino et al. [168] demonstrated the potential of dimethylfumarate (DMF) in mitigating age-related infertility by activating the Nrf2/Keap1-antioxidant response element (ARE) signalling pathway. The authors showed that DMF plays a key role in reducing OS and is a significant factor contributing to ovarian aging and infertility by upregulating antioxidant defences and reducing DNA damage. The study's findings indicate that DMF administration enhances Nrf2 expression, increases antioxidant activity, and preserves the ovarian reserve. This process is characterised by improved oocyte quality and maintenance of primordial follicles. The study results suggest that DMF could be a therapeutic approach with great potential in combating age-related infertility. The mechanism of action appears to involve protection of ovarian function from oxidative damage and enhancement of cellular resilience, both of which are crucial for successful fertilisation [168].

Valipour et al. [169] explained the mechanisms underlying OS and its impact on infertility, underscoring the pivotal role of Nrf2 in cellular defence mechanisms. Specifically, their findings demonstrate that, upon release from its inhibitor, Kelch-like ECH-associated protein 1 (Keap1), Nrf2 translocates to the nucleus, activating the expression of genes involved in antioxidant production and detoxification processes. The experimental models applied in this study have demonstrated that Nrf2-mediated pathways can mitigate oxidative damage in reproductive tissues, offering protection against such conditions as sperm DNA fragmentation, ovarian follicle development disruption, and testicular tissue damage. This emphasises the therapeutic potential of targeting the Nrf2 signalling pathway in treating OS-related infertility.

The research conducted by Falvo et al. [170] offers a compelling perspective on the deleterious impact of short-term high-fat diets on male reproductive function. Their findings underscore an association between the consumption of high-fat diets and a range of adverse outcomes, including the impairment of steroidogenesis, the induction of spermatogenic cell apoptosis, and the disruption of spermatogenesis. The mechanisms by which these effects occur are elucidated in the study, which finds that there is compromised blood-testis barrier integrity due to the downregulation of structural proteins (N-cadherin, ZO-1, occludin, connexin 43, and VANG2) and altered phosphorylation of regulatory kinases, such as Src and FAK. Furthermore, the mitochondrial dynamics, including fission, fusion, and biogenesis of spermatogenesis, is disrupted, as are the SIRT1/NRF2/MAPKs signaling pathways. This finding derived from experiments conducted on 10-week-old male Wistar rats is particularly concerning in the context of the WHO estimates that 50% of adults and 30% of children and adolescents are overweight or obese [170]. Concurrently, a global decline in sperm quality and male fertility has been observed [171], underscoring the imperative to investigate the molecular underpinnings connecting metabolic disorders and reproductive health, to identify potential therapeutic interventions.

Therefore, the results highlight the critical role of oxidative balance in male fertility, where excessive reactive oxygen species can lead to cellular damage, particularly in sperm cells via Nrf2, which plays a key role in maintaining redox homeostasis by regulating antioxidant defense mechanisms, thereby protecting sperm quality. Disruption of Nrf2 signalling, e.g., by ferroptosis, has been associated with impaired spermatogenesis and reduced sperm quality, particularly in such conditions as oligospermia. These findings highlight the potential for therapeutic strategies targeting Nrf2 pathways to alleviate OS and improve male reproductive health.

Antioxidant-rich diets, male fertility, and Nrf2, AMPK/PGC-1 α , and NF- κ B pathways

Numerous studies have shown that antioxidants are crucial in protecting cells from OS, which has been identified as a significant contributing factor to male infertility [12]. The Nrf2 pathway (nuclear factor erythroid 2-related factor 2) is the central regulator of the body's antioxidant response [172]. In normal conditions, Nrf2 is bound to a molecule known as Keap1 (Kelch-like ECH-associated protein 1), and this binding results in the degradation of Nrf2 [173]. However, during periods of redox dysfunction or following the

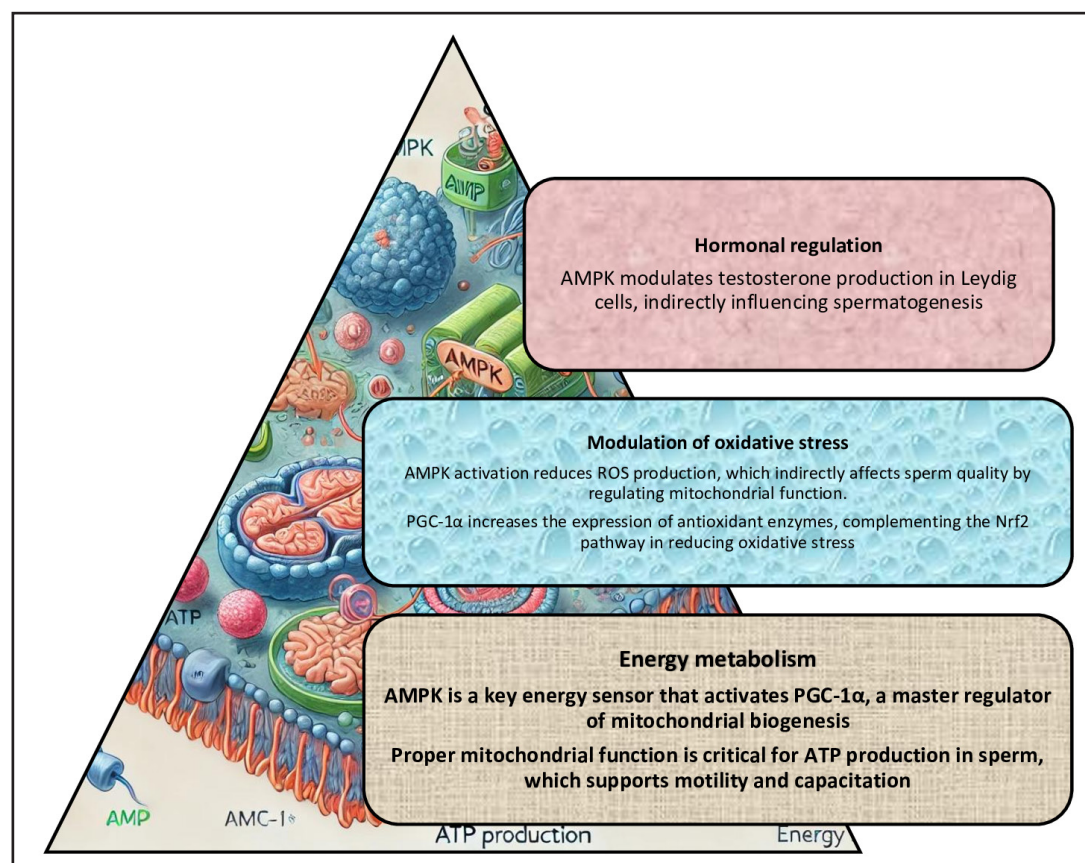


Fig. 5. AMPK/PGC-1 α pathway (AMP-activated protein kinase and peroxisome proliferator-activated receptor gamma coactivator-1 α) and male infertility. The AMPK/PGC-1 α pathway has been demonstrated to play a crucial role in regulating cellular energy balance and metabolic processes; these processes are essential for the proper function of reproductive cells; dysregulation of this pathway impairs spermatogenesis and mitochondrial function, which can contribute to male infertility.

intake of antioxidants, Nrf2 is released and transported to the nucleus, where it activates the expression of antioxidant-related genes, including SOD, CAT, and GPx [173]. The function of these genes is to neutralize ROS. This, as demonstrated by Valipour et al. [169], results in a reduction of OS, improvement in sperm motility, and, consequently, enhancement of DNA integrity and overall fertilising capacity.

Recent studies have indicated that the enhancement of mitochondrial function via the AMPK/PGC-1 α pathway is another significant factor in this process [174]. It has been established that antioxidants also influence other metabolic pathways via AMPK (AMP-activated protein kinase) and PGC-1 α (peroxisome proliferator-activated receptor-gamma coactivator 1-alpha), both of which are crucial for mitochondrial function, as demonstrated by [174]. This is related to the fact that mitochondria present in spermatozoa are critical for generating the energy required for motility [175]. AMPK has been reported to be activated by resveratrol [176] and quercetin [177], which enhanced mitochondrial biogenesis and improved mitochondrial function via PGC-1 α . The improved mitochondrial performance resulting from this process has been shown to reduce the production of ROS in sperm cells, which aligns with the conclusions reached by other authors [165]. In turn, this, in synergy with the NRF2 pathway, has been demonstrated to mitigate OS, as shown in Fig. 5.

Studies investigate the potential of inflammation reduction, focusing on NF- κ B pathway regulation, as previously demonstrated by authors [178]. It was previously understood that the NF- κ B (nuclear factor kappa B) pathway plays an instrumental role in regulating inflammatory responses in the body. Excessive activation of NF- κ B has been linked to

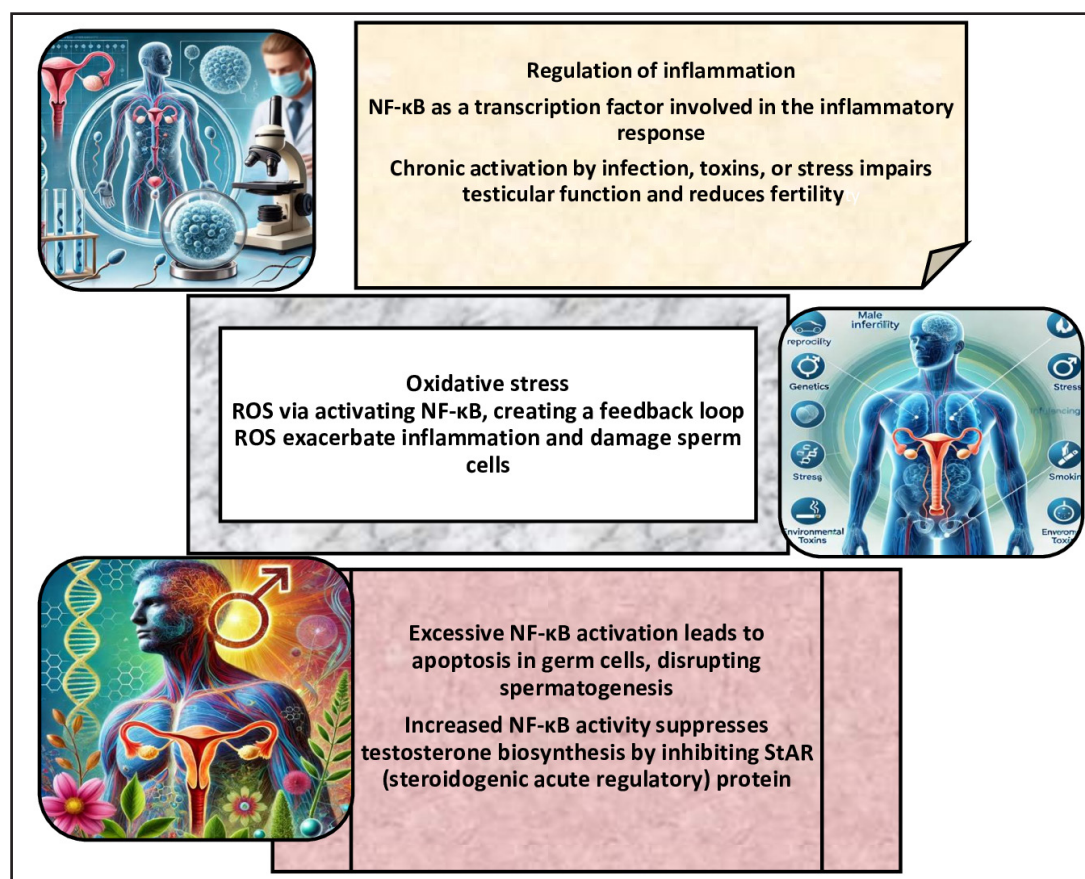


Fig. 6. Nuclear factor kappa B (NF- κ B) signalling pathway and male infertility. The NF- κ B signalling pathway plays a pivotal role in the regulation of immune responses, inflammation, and cell survival, which are critical processes for maintaining normal spermatogenesis. Dysregulated NF- κ B activity can lead to chronic inflammation, oxidative stress, and impaired testicular function, contributing to male infertility.

chronic inflammation, exerting detrimental effects on testicular function, spermatogenesis, and semen quality [179], as shown in Fig. 6. Some investigations have provided valuable insights into such antioxidants as curcumin, vitamin E, and polyphenols from green tea, which have been shown to inhibit NF- κ B activation, thereby reducing inflammation in reproductive tissues [180]. Consequently, the combined modulation of the NRF2, AMPK, and NF- κ B pathways by an antioxidant-rich diet triggers an integrated protective mechanism that improves semen quality, reduces sperm DNA fragmentation, and enhances sperm fertilising ability, thus supporting the treatment of male infertility.

Thus, by improving the function of key regulatory pathways, such as Nrf2, AMPK/PGC-1 α , and NF- κ B, an antioxidant-rich diet plays a crucial role in supporting male fertility. Nrf2, in particular, is essential for maintaining redox balance and protecting sperm quality by activating antioxidant defences. Disruption of these pathways, including OS-induced ferroptosis, can impair spermatogenesis and fertility. Therefore, dietary interventions rich in antioxidants may offer a promising strategy to mitigate oxidative damage and support male reproductive health by modulating these critical pathways.

Prevention and treatment of oxidative stress in the reproductive system

The human body has highly effective mechanisms to combat OS, with particular emphasis on protecting the reproductive system [181]. In addition to sperm, the ejaculate of a healthy adult male contains many essential components, including natural antioxidants, such as vitamin C, sodium, calcium, zinc, citric acid, fructose, and various proteins [182]. These components help neutralise free radicals and prevent cell damage. A deficiency of these compounds can be replenished through the diet. Research suggests that fruit and vegetables, especially those with yellow, orange, and red pigments, e.g., carrots and tomatoes, can improve male fertility significantly [183].

The findings presented by Britton and Khachik [184] categorise carotenoid patterns based on the colour of plant tissues into five distinct groups characterised by 1) high levels of the acyclic carotene lycopene, resulting in a red colour, as seen in tomatoes; 2) high levels of β -carotene and/or its hydroxyl derivatives, such as β -cryptoxanthin and zeaxanthin, contributing to an orange colour; 3) a combination of β -carotene with α -carotene and/or lutein, resulting in a yellow-orange colour; 4) a predominance of carotenoid epoxides, leading to a yellow colour; and 5) carotenoids unique to specific species, such as capsanthin and capsorubin in red peppers or crocetin in saffron, contributing to yellow, orange, or red hues. Notably, lycopene, a key component of the first pattern, is typically accompanied by colourless carotenoids like phytoene and phytofluene, as discussed by Dias et al. [185]. These foods are rich in antioxidants, such as β -carotene (provitamin A) and lycopene, essential in reducing oxidative damage, especially in idiopathic oligoasthenoteratozoospermia [186].

As reported by Ramgir et al. [187], the mechanisms of action of phytomedicinal therapeutics exert an impact on the testosterone pathway that is involved in the stimulation of spermatogenesis, the reduction of redox imbalance, the inhibition of inflammation, and the activation of signalling pathways in the testes via the extracellular-regulated kinase (ERK)/protein kinase B (PKB)/transformation of growth factor-beta 1 (TGF- β 1)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signalling pathways and mediation of sexual behaviour. It has been demonstrated that ashwagandha can inhibit lipid peroxidation, improve sperm count and motility, and regulate reproductive hormone levels [188]. Selected studies of these effects and proposed mechanisms of action are presented in Table 1.

Micronutrients and energy boosters further support male fertility by enhancing key biological processes. Zinc and folic acid are essential for spermatogenesis, DNA synthesis, and testosterone production, while selenium contributes to sperm structural integrity, as shown by Li et al. [211]. Supplements, such as L-carnitine and omega-3 fatty acids, promote energy metabolism in sperm cells, improving their motility and vitality [212]. A study conducted by

Table 1. Effects, mechanisms, and active ingredients of natural herbs and other ingredients used to support male fertility

N	Name of herb/ingredient	Primary effect on male fertility	Mechanism of action	Key bioactive compounds responsible for the effects	Source
1	Korean ginseng (Panax ginseng)	Improves sperm quality, enhances sperm motility, and increases testosterone levels; stimulation of sperm production, restoration of sperm quality and motility, and improvement of testicular function	Adaptogen reducing oxidative stress and improving hormonal balance, thereby improving reproductive function; supports overall energy levels, reduces stress, and improves immune function, making it a well-rounded option for general health	Ginsenosides (RG1, RB1), polysaccharides, and antioxidants; Bisphenol A is recognised as an endocrine disruptor that has adverse effects on both male and female reproductive systems	96, 189-191
2	Ginkgo biloba	improves blood circulation to the reproductive organs, enhances sperm motility and overall fertility	improves circulation and reduces oxidative stress; protective effect against oxidative stress and apoptosis via pretreatment, alleviated doxorubicin-induced damage in rat testes by reducing lipid peroxidation, restoring antioxidant enzyme activities, and decreasing apoptotic such markers as pro-apoptotic Bcl-2 family proteins, p53, Apaf-1, cytochrome c release, and caspase-3 activation	ginkgolides and flavonoids	192-194
3	Ashwagandha (Withania somnifera)	increases sperm production and motility while reducing stress and cortisol levels; inhibits lipid peroxidation, improves sperm count and motility, and regulates reproductive hormone levels	Adaptogen supporting the hypothalamic-pituitary-adrenal (HPA) axis, reducing stress-induced hormonal imbalances, and improving reproductive health; calming effects and ability to support general physical and mental well-being; its direct oxidative mechanism involves mitigation of oxidative stress and the indirect action involves a gamma-aminobutyric acid-like-mimetic pathway	withanolides, alkaloids, and steroidal lactones	195-197
4	Maca root (Lepidium meyenii)	enhances sperm count and motility, while supporting sexual desire; quality and quantity of semen, sexual behaviour and disorders of the male genital tract	rich in nutrients that support hormonal balance and improve testicular function; natural energy booster and stamina enhancer in physical activities; possible mechanisms of action by specific oxindole alkaloids through inhibition of nuclear factor (NF)-kappaB activation	glucosinolates, macamides, and flavonoids, oxindole and indole alkaloids, glucosinolates, sterols, polyunsaturated fatty acids, and carbolines	198-200
5	Puncture Vine (Tribulus terrestris)	improves sperm quality and libido by enhancing testosterone production, sperm count, improved sperm viability and motility, significant changes in the structure of seminiferous tubules, increase in spermatogonia, Leydig and Sertoli cell numbers, epithelial thickness, and higher numbers of spermatogonia and spermatid cells	stimulates the release of luteinizing hormone (LH), which in turn increases natural testosterone production; protodioscin converts testosterone into dihydrotestosterone; used by athletes to boost performance and improve physical recovery; semen quality and physiological parameters	protodioscin and saponins	201-203
6	Shilajit	increases sperm count and motility while reducing oxidative stress; improves general energy levels and cognitive function, making it a versatile natural supplement; spermatogenic nature, as attributed in Ayurvedic medicine	provides essential trace minerals and fulvic acid, which enhance mitochondrial function and cellular energy production; antioxidant properties and an effective immuno-modulator	fulvic acid, humic acid, and trace minerals, e.g. selenium	204-205
6	Fenugreek (Trigonella foenum-graecum)	enhances testosterone levels and improves sperm count by balancing the endocrine system; improves sperm quality, motility, and testosterone levels	anti-inflammatory properties that support overall reproductive health	furostanolic saponins, flavonoids, and alkaloids, which are believed to enhance male fertility	206
7	Saw palmetto (Serenoa repens)	supports prostate health, enhances testosterone metabolism, and reduces inflammation in the reproductive system, improving fertility	prostate health, improving testosterone metabolism, anti-estrogenic properties in prostatitis	plant sterols, flavonoids, free fatty acids (FFA), methyl and ethyl esters, glycerides, and flavonoids	18, 207
8	Epimedium herba	increases libido and sperm quality; aphrodisiac properties; improves sperm count and motility by increasing blood flow and testosterone levels	increases blood flow and testosterone levels, improves nitric oxide production, increases blood flow and sperm function; leads to a reduction in testicular damage, improvement in testosterone production and spermatogenesis, and alleviation of both apoptosis and impairment of the blood-testis barrier in mice with streptozotocin-induced diabetes; increases cell proliferation and decreases cell apoptosis in testis; regulation of the glycolysis pathway	via the flavonoid icariin	220, 208,209
9	Red clover (Trifolium repens)	helps balance hormone levels and supports fertility; improves sperm quality by detoxifying reproductive organs helps enhance fertility; improves sperm characteristics and antioxidant status	balances hormones and improves sperm health	phyto-oestrogens, contains isoflavones (genistein and daidzein), which mimic oestrogen	210

authors [212] through a comprehensive literature search has underscored the advantageous effects of various nutritional supplements on sperm parameters. Selenium, zinc, omega-3 fatty acids, coenzyme Q10, and carnitines have been observed to positively influence such parameters as total sperm concentration, sperm count, motility, and morphology. Specifically, omega-3 fatty acids and coenzyme Q10 significantly enhanced sperm count and

motility, while carnitines were particularly effective in improving sperm progressive motility. Additionally, selenium, omega-3 fatty acids, coenzyme Q10, and carnitines contributed to improvements in sperm morphology, demonstrating the potential of these supplements in supporting male reproductive health [212].

The microbiome and its effect on male fertility

The human microbiome is a diverse ecosystem comprising bacteria, fungi, viruses, archaea, and protozoa [19, 36]. Bacteria are taxonomically classified into phyla, classes, orders, families, genera, and species, with the gut microbiota dominated by a limited number of phyla, as shown by Laterza et al. [213], comprising over 160 species. *Firmicutes* and *Bacteroidetes* account for approximately 90% of the gut microbiota [214], with *Firmicutes* including over 200 genera, such as *Lactobacillus*, *Bacillus*, and *Clostridium*. In contrast,



Fig. 7. Mechanisms and biomarkers in multifaceted links between the microbiome and male fertility. The relationship between the microbiome and male fertility involves a dynamic interplay of hormonal regulation, oxidative stress modulation, immune response control, nutrient absorption, and the gut-brain axis, with dysbiosis and microbial imbalances emerging as critical factors influencing sperm quality, DNA integrity, and overall reproductive health. Abbreviations: SCFAs - short-chain fatty acids; TMAO - trimethylamine-N-oxide; ROS - reactive oxygen species; MDA - malondialdehyde; SOD - superoxide dismutase; IL-6 - interleukin-6; TNF-alpha - tumour necrosis factor-alpha; IgG - Immunoglobulin G.

Bacteroidetes and *Actinobacteria* are comprised of genera such as *Bacteroides*, *Prevotella*, and *Bifidobacterium*. These findings, supported by Rinninella et al. [215], highlight the diversity and dominance of these phyla within the gut microbial community. These dependencies are shown in Fig. 7.

These bacteria contribute to vital functions, such as nutrient metabolism, immune modulation, and the production of metabolites, e.g., short-chain fatty acids [33, 34, 37, 216]. The fungal mycobiome, comprising such genera as *Candida*, *Saccharomyces*, and *Malassezia*, influences digestive processes and immune function [217]. The virome, primarily consisting of bacteriophages, regulates bacterial populations and maintains microbial diversity. Archaea, including *Methanobrevibacter*, play a role in fermentation and methane production, while protozoa, such as *Blastocystis hominis*, interact with other microbes and influence immune responses [218, 219].

This intricate community functions collectively to maintain homeostasis [33, 34, 37]. The contribution of bacteria and fungi to nutrient absorption and inflammation regulation, the role of viruses in shaping microbial dynamics, the assistance of archaea in metabolic processes, and the interaction of protozoa with the immune system are all pivotal in this regard. Collectively, these elements constitute a balanced ecosystem, which is imperative for



Fig. 8. Complex relationships between the microbiome and male fertility.

overall health. However, disruptions in this composition can lead to dysbiosis and associated systemic conditions (Fig. 8).

The microbiome plays a significant role in male fertility through its direct influence on the gut-brain axis, immune system, hormonal regulation, and inflammation. The vagus nerve is a key component in the gut-brain axis [35, 220]. An imbalance in the microbiome can contribute to hormonal disruptions, increased inflammation, and altered brain function, negatively affecting reproductive health. Bonaz et al. [220] reported that the vagus nerve serves as a crucial mediator in the bidirectional communication between the gut and brain. It plays a critical role in maintaining homeostasis by monitoring the “milieu intérieur” and coordinating nervous and endocrine responses to support gastrointestinal health. Its anti-inflammatory functions are achieved through activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to cortisol release, and through the vagovagal reflex, which exerts an anti-tumour necrosis factor (TNF) effect, known as the cholinergic anti-inflammatory pathway. As a result, vagus nerve stimulation is emerging as a promising non-drug therapeutic strategy for treating gastrointestinal disorders characterised by impaired brain-gut communication, such as inflammatory bowel disease and related conditions [220].

Recent research highlights the importance of a healthy gut microbiome in male fertility. In their study, Lv et al. [216] emphasised the critical role of the gut microbiota in improving sperm quality, testicular structure, and sexual behaviour, primarily through the production of metabolites and the regulation of host metabolism. Endotoxemia, linked to gut microbiota imbalance, is identified as a key factor in testicular damage and disruption of the blood-testis barrier, which can lead to orchitis. The gut microbiota has also been demonstrated to influence sex hormone levels by modulating sex hormone-related enzymes and enterohepatic circulation, impacting the hypothalamic-pituitary-testicular axis and promoting sexual arousal and behaviour [216].

Diets high in fibre and probiotics improve reproductive health via the gut-testis axis, as shown by authors [221]. Beneficial gut flora can reduce systemic inflammation, while microbial metabolites influence hormone levels and sperm quality [222]. Molecular pathways include modulation of inflammatory markers and hormone synthesis by short-chain fatty acids (SCFAs) and other microbial products, as demonstrated earlier by Tan et al. [223]. Moreover, dietary and lifestyle choices that promote a healthy microbiome can support male fertility by optimising the hypothalamic-pituitary-gonadal axis function, reducing systemic inflammation, and enhancing sperm production [224]. A comprehensive understanding of the intricate relationship between the microbiome, brain, behaviour, and fertility can yield novel therapeutic interventions for male infertility.

The microbiome's influence on male fertility is attributable to its effects on systemic inflammation, OS, and hormonal balance [225]. Corral-Vazquez et al. [226] demonstrated that a healthy gut microbiome can promote anti-inflammatory states and support testosterone production by regulating the hypothalamic-pituitary-gonadal (HPG) axis. Bacteria, such as *Lactobacillus* and *Bifidobacterium*, can produce SCFAs and other metabolites that enhance gut barrier integrity and modulate immune responses, thereby creating an environment conducive to healthy spermatogenesis [227]. Conversely, dysbiosis, defined as an overgrowth of pathogens like *Escherichia coli* and *Enterococcus*, has increased systemic inflammation and OS, resulting in damaged sperm DNA and impaired motility [228]. In addition to inflammation, disruptions in the microbiome have been demonstrated to impair hormone regulation (Fig. 9). The overproduction of inflammatory cytokines and endotoxins, such as lipopolysaccharides, has been shown to disrupt the HPG axis, reducing testosterone levels and hindering sperm production [97]. The presence of pathogens and a concomitant reduction in the abundance of beneficial bacteria in the seminal microbiome directly impact sperm quality and viability. Therapeutic interventions addressing microbial imbalances through probiotics, prebiotics, and dietary modifications can mitigate infertility [222, 225].

Synergistic approaches to gut microbiota and dietary interventions

Developing supplements targeting the gut microbiota and male fertility involves combining ingredients with proven benefits for both gut health and reproductive function. Probiotics play a central role, with *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* strains being particularly effective in improving gut health, reducing systemic inflammation, and positively influencing testosterone levels and sperm quality, as reported by Raheem et al. [229]. Prebiotics, including inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS), also promote the growth of beneficial bacteria, which indirectly supports fertility by reducing OS and balancing hormones [230].

In addition to gut-focused ingredients, fertility-specific ingredients are essential [26, 29, 30]. Antioxidants, e.g., selenium, vitamin E, CoQ10, and N-acetylcysteine, protect sperm from oxidative damage, while amino acids, such as L-carnitine and L-arginine, also improve

Fig. 9. Pathways demonstrating the synergistic effect of the gut microbiota and dietary interventions in maintaining and enhancing male fertility through the modulation of both physiological and hormonal mechanisms.



motility and overall sperm function [9]. Omega-3 fatty acids reduce inflammation and improve the structural integrity of sperm membranes, providing a further layer of support. Adaptogens, such as ashwagandha, maca root, and ginseng, reduce stress while boosting fertility, making them a valuable addition to a comprehensive formula as shown earlier in this review.

In the context of male fertility, the influence of the gut microbiota and dietary interventions on various molecular pathways has been demonstrated. These pathways illustrate the synergistic effect of the gut microbiota and nutritional interventions in maintaining and enhancing male fertility by modulating physiological and hormonal mechanisms (see Fig. 8). Such mechanisms include the regulation of the hypothalamic-pituitary-testicular axis, which controls testosterone and sperm production, and the modulation of inflammatory pathways, where gut imbalances can cause endotoxemia and disrupt the blood-testis barrier, as shown by authors [231]. Furthermore, microbiota-derived metabolites have been shown to impact metabolic processes vital for sperm quality. At the same time, gut bacteria have also been demonstrated to regulate sex hormone synthesis and influence brain areas that are responsible for sexual behaviour and arousal [232]. Together, these findings underscore the pivotal role of gut health in male reproductive function (Fig. 9).

When designing supplements to improve male fertility, it is crucial to consider synergistic effects and precise delivery mechanisms, as highlighted by Sánchez et al. [233]. For example, combining probiotics with antioxidants has been shown to reduce inflammation in both the gut and reproductive organs, while combining prebiotics with adaptogens addresses stress regulation and gut health [190]. A comprehensive formulation, i.e., a capsule or powder integrating probiotics, prebiotics, antioxidants, amino acids, omega-3 fatty acids, and adaptogens, offers a dual-action strategy to improve the gut microbiota and male fertility [189, 191]. Ensuring proper dosing and clinical validation enhances the efficacy of such supplements, providing a science-based approach to male infertility [96].

When developing male fertility supplements, it is essential to consider the synergistic effects of combining different ingredients that target gut health and reproductive function [234]. Probiotics and prebiotics work together to improve the gut microbiota, which can improve overall systemic health. Probiotics, such as *Lactobacillus* and *Bifidobacterium* strains, help to balance the gut microbiota, reduce inflammation, and support hormonal regulation, especially in mood health, as demonstrated previously [235]. Prebiotics, such as inulin and FOS, feed beneficial gut bacteria, promoting a healthy gut environment that can improve metabolic and reproductive health. Combined, these ingredients can provide a dual benefit by addressing gut and fertility issues [230].

Oxidative stress is a key factor in male infertility, leading to impaired sperm function and DNA damage [236, 237], and is closely linked to gut microbiota dysbiosis. Excessive ROS not only damages sperm function but also disrupts the gut microbiome, exacerbating systemic inflammation and oxidative imbalance [238]. Environmental exposures, such as heavy metals, further impair the composition of the gut microbiota, which amplifies oxidative damage [238, 239]. On the other hand, intense physical activity has similarly altered microbial diversity, affecting systemic oxidative stress and sperm health [240, 241]. It is known that, in normal conditions, leukocytes produce 1,000 times more free radicals than spermatozoa, making leukocytes the dominant producers of free radicals in semen [241]. The level of activity of leukocytes plays an essential role in forming free radicals [241]. Therapies that target oxidative stress, including antioxidants and adaptogens such as *Withania somnifera*, have shown promise in improving sperm quality and fertility outcomes [242, 243]. Therefore, antioxidant supplementation and adaptogens may improve gut microbiota and reproductive outcomes by mitigating oxidative stress.

The use of antioxidants and amino acids is another synergistic combination. Antioxidants, such as CoQ10, selenium, and N-acetylcysteine, protect sperm from oxidative damage, a significant cause of male infertility [9, 205, 219]. By helping to reduce oxidative stress in the body, these antioxidants also help to support the functioning of the reproductive organs. Combined with amino acids such as L-carnitine and L-arginine, they further improve sperm

motility and function. L-carnitine helps enhance mitochondrial energy production in sperm cells, while L-arginine supports nitric oxide production, which promotes better blood flow to reproductive organs [72, 120, 125]. Together, these ingredients provide a comprehensive approach to combating oxidative damage and supporting sperm function. Complementary effects are also seen when omega-3 fatty acids are combined with such adaptogens as ashwagandha, maca root, and *Tribulus terrestris*. Omega-3 fatty acids reduce inflammation and support sperm membrane integrity, improving sperm quality and motility, as shown by Montano et al. [74]. Adaptogens help the body manage stress and regulate hormones, critical factors in maintaining optimal fertility. These herbs and supplements can help restore hormonal balance, reduce cortisol levels, and support ovulation [244].

Emerging systemic and lifestyle factors in male infertility: future research directions

In addition to direct testicular or oxidative mechanisms, there is an increasing recognition of several systemic and lifestyle-related factors as significant contributors to male infertility. These include metabolic dysfunctions such as lipid imbalance and obesity, socioemotional stress and unhealthy lifestyle patterns, as well as mitochondrial genetic polymorphisms that may compromise sperm function. This section provides a comprehensive overview of the multifactorial impact of these emerging determinants on male reproductive health, to inform future studies in this area. It is evident from the extant literature that lipid metabolism disorders and obesity represent well-documented risk factors for male infertility.

Firstly, excess adipose tissue, particularly visceral fat, has been demonstrated to contribute to a pro-inflammatory and hormonally imbalanced state that has a detrimental effect on testicular function. Obesity has been demonstrated to be associated with hypogonadism, reduced testosterone levels, and elevated aromatisation of androgens into oestrogens, thereby disrupting the HPG axis. Excess body fat has been demonstrated to exert a substantial influence on the reproductive health of males, through its capacity to disrupt hormonal regulation, to engender increased oxidative stress, and to impair testicular function. Obesity-related changes, including elevated estrogenic levels resulting from aromatase activity in adipose tissue, have been demonstrated to induce hypogonadotropic hypogonadism and diminished fertility potential in males [245]. Furthermore, dyslipidaemia has been demonstrated to compromise the structural integrity of sperm membranes by modifying their lipid composition, thereby impacting motility and fertilisation capacity. In men diagnosed with non-obstructive azoospermia (NOA), both basal and stimulated levels of inhibin B and anti-Müllerian hormone (AMH) are significantly lower than in fertile control subjects. However, dynamic testing using exogenous FSH (EFSERT) does not provide additional diagnostic value beyond baseline measurements of these hormones [246]. Emerging evidence also links obesity-related oxidative stress and insulin resistance with reduced sperm quality and DNA fragmentation. Given the intricacy of these mechanisms, further research is indicated to elucidate precise pathways and therapeutic interventions targeting lipid metabolism in male infertility.

It was shown that mitochondrial DNA (mtDNA) polymorphisms and mutations have garnered increasing attention as potential contributors to male infertility. Mitochondria have been demonstrated to play a pivotal role in sperm bioenergetics, particularly in the generation of ATP, which is essential for flagellar movement. A single nucleotide polymorphism (rs527236194) in the mitochondrial cytochrome B gene (MT-CYB) has been associated with impaired sperm parameters, particularly in men diagnosed with asthenoteratozoospermia. This finding highlights the potential role of mitochondrial genetic variation in the pathophysiology of male infertility and underscores the need for further investigation in broader populations [247]. Variations in mtDNA have been demonstrated to impair mitochondrial respiratory chain function, resulting in diminished ATP availability, augmented generation of ROS, and, in turn, oxidative damage to both

nuclear and mitochondrial genomes. Specific mtDNA haplogroups or point mutations have been associated with impaired asthenozoospermia, abnormal morphology, and reduced fertilisation potential. Although research in this area is still evolving, future studies focusing on mitochondrial genetics show great promise in advancing diagnostics and targeted therapies in the field of male reproductive health.

The role of chronic socioemotional stress and adverse lifestyle behaviours in the development of male reproductive dysfunction is a significant yet often underestimated factor. Psychological stress activates the HPA axis, leading to elevated cortisol levels, which have been demonstrated to suppress gonadotropin release and impair spermatogenesis. Furthermore, several studies have shown a link between certain lifestyle factors and sub-optimal male reproductive health. These factors, which include a lack of physical activity, inadequate sleep, smoking, alcohol consumption, and unhealthy dietary choices, have been shown to contribute to oxidative stress, endocrine function imbalances, and reduced sperm quality. While the associations are increasingly recognised, further detailed studies are required to define causal relationships and develop effective interventions to mitigate lifestyle-related risks in infertile men.

Throughout the manuscript, we have outlined several mechanisms by which the gut microbiota may influence male infertility, including the gut-brain axis, modulation of the immune system, and hormonal regulation. While these mechanisms highlight potential interaction pathways, we have been careful to present them as possible contributors rather than definitive causal factors, emphasising the need for further research to validate these relationships. Thus, future directions in lifestyle interventions for male infertility connected with innovative approaches include wearable technology to monitor physical activity, stress, health parameters, and personalised dietary plans. Epigenetic therapies, which use lifestyle modifications to alter gene expression associated with fertility, represent an innovative avenue for intervention. Integrating dietary strategies, fitness programmes, and psychological support offers a holistic and effective solution to improve male fertility outcomes.

Conclusion

The identification of reliable biomarkers of male infertility is crucial for both early diagnosis and personalised treatment. The assessment of sperm quality, including such parameters as motility, morphology, and DNA integrity, remains essential for evaluating male reproductive health. Advances in molecular biology have provided insights into how epigenetics and lifestyle interact to influence sperm quality.

Common pathological conditions, such as varicocele, infections, and testicular hyperthermia, significantly contribute to male infertility. Additionally, stress responses, particularly due to testicular hyperthermia, further exacerbate oxidative stress, leading to sperm damage and impaired fertility. The role of ROS in spermatogenesis is discussed in this study, and it is demonstrated that ROS play a dual role in spermatogenesis, being necessary for normal sperm function but resulting in sperm DNA fragmentation and diminished fertility potential through excessive ROS production from both endogenous and exogenous sources. Understanding the balance between ROS production and antioxidant defences is crucial for male infertility management.

Antioxidants and pathways involved in male infertility are discussed. An analysis of antioxidant-rich diets and key molecular pathways, including Nrf2, AMPK/PGC-1 α , and NF- κ B, reveals their vital role in mitigating oxidative stress and improving sperm quality. Targeting these pathways could offer novel therapeutic options for improving male fertility. Male infertility is often associated with endothelial dysfunction and cardiovascular diseases, suggesting a systemic impact on reproductive health. Addressing these comorbidities through lifestyle changes and interventions may enhance male fertility outcomes, as the gut microbiota and synergistic dietary approaches require further investigation to understand this complex relationship better.

The findings of this review highlight the complexity of male infertility and underscore the need for further research to develop effective therapeutic interventions and improve reproductive outcomes. Consequently, a multifaceted therapeutic approach holds considerable promise for enhancing fertility outcomes.

Disclosure Statement

The authors have no competing interests to declare.

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