

EXPLORING THE ROLE OF PLANT-BASED COMPOUNDS IN STIMULATING SPERMATOGENESIS FOR MALE INFERTILITY MANAGEMENT

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ABSTRACT

Male infertility is a multifactorial reproductive disorder in which impaired spermatogenesis, altered testicular steroidogenesis, oxidative stress, mitochondrial dysfunction, inflammation, and disruption of Sertoli–germ-cell interactions contribute to reduced sperm production and quality. Increasing experimental and clinical interest has focused on plant-derived compounds and botanical preparations as potential modulators of testicular function because of their diverse effects on redox homeostasis, steroidogenic signaling, cellular survival, and metabolic pathways. This review critically examines the evidence for plant-based interventions in the modulation of spermatogenesis and associated testicular processes relevant to male infertility management. Particular attention is given to botanicals and bioactive constituents investigated in human studies, animal models, and cellular or ex vivo systems, including *Withania somnifera*, *Panax ginseng*, *Lepidium meyenii*, processed Shilajit, *Tribulus terrestris*, *Anacyclus pyrethrum*, *Cistanche species*, icariin, echinacoside, naringin, naringenin, astaxanthin, lycopene, curcumin, resveratrol, kaempferol, apigenin, and chlorogenic acid. Their potential effects on Leydig-cell steroidogenesis, Sertoli-cell function, germ-cell survival and differentiation, mitochondrial homeostasis, oxidative and inflammatory signaling, apoptosis, autophagy, and ferroptosis are discussed. Importantly, the available evidence is differentiated according to whether an intervention demonstrates direct or putative effects on spermatogenic processes, supportive effects on the testicular microenvironment, or protection of mature spermatozoa. Although several compounds have demonstrated improvements in sperm parameters, reproductive hormones, testicular histology, and molecular markers in experimental models, evidence from controlled human studies remains comparatively limited and heterogeneous. Translational challenges, including botanical standardization, dose optimization, bioavailability, pharmacokinetic variability, reproductive safety, and methodological limitations of existing studies, are also considered. Overall, plant-derived compounds represent promising candidates for further investigation in male infertility; however, well-designed clinical studies with standardized preparations and mechanistically defined endpoints are required to establish their efficacy and therapeutic relevance in stimulating or supporting human spermatogenesis.

KEYWORDS: male infertility; spermatogenesis; plant-based compounds; medicinal plants; phytochemicals; sperm production; testosterone; Leydig cells; Sertoli cells; oxidative stress.

1. INTRODUCTION

Male infertility is a multifactorial disorder characterized by failure to achieve pregnancy because of abnormalities in sperm production, function, or delivery [1–3]. Modern reproductive medicine recognizes that semen abnormalities can arise from disturbances in the hypothalamic-pituitary-gonadal axis [4], germ-cell development [5], Sertoli-cell support [6], Leydig-cell steroidogenesis [7], the blood-testis barrier [8], epididymal maturation [9], genetic pathways, varicocele, infection, metabolic disease, environmental toxicants, and lifestyle factors [10]. Spermatogenesis requires continuous proliferation and differentiation of spermatogonial cells, meiotic progression, spermiogenesis, and maturation of spermatozoa [11]. Follicle-stimulating hormone acts predominantly through Sertoli cells, whereas luteinizing hormone stimulates Leydig-cell testosterone production [12]. Intratesticular testosterone, paracrine factors, and Sertoli-cell support together maintain the seminiferous environment [6,13,14]. A plant-derived intervention can therefore be considered potentially spermatogenic when it improves the endocrine or cellular conditions required for germ-cell development, increases sperm output, preserves seminiferous architecture, or enhances germ-cell survival [15].

Medicinal plants have historically been used in traditional systems for male reproductive disorders [16]. Contemporary research has identified multiple classes of active constituents, including steroidal saponins, flavonoids, phenolic acids, carotenoids, polyphenols, phenylethanoid glycosides and polysaccharides [17]. Reviews of botanical medicine emphasize antioxidant [18], anti-inflammatory [19], androgenic, and spermatogenesis-related actions, but also identify substantial variation in plant authentication, extraction

procedures, chemical characterization, and clinical-trial quality.

The present review focuses specifically on the potential role of plant-based compounds and botanical preparations in modulating spermatogenesis and the testicular processes that support sperm production. Importantly, the review distinguishes evidence for direct or putative stimulation of spermatogenesis from effects limited to protection of mature spermatozoa or improvement of semen quality. Human clinical studies, human semen experiments, animal models, and cellular studies are therefore considered separately to assess the strength and translational relevance of the available evidence. The review also examines limitations related to botanical standardization, bioavailability, dose, safety, and clinical study quality, with the aim of identifying the most relevant mechanisms and research priorities for developing plant-derived interventions for male infertility management.

2. Spermatogenesis: Biological Targets for Plant-Based Intervention

Spermatogenesis is a highly coordinated process that depends on the functional interaction of germ cells, Sertoli cells, Leydig cells, peritubular cells, endocrine signals, and the blood–testis barrier. Because germ-cell development involves continuous proliferation, meiotic division, differentiation, and extensive cellular remodelling, the testicular microenvironment is particularly vulnerable to oxidative stress, inflammation, endocrine disruption, and mitochondrial dysfunction.

2.1 Leydig-Cell Steroidogenesis

Leydig cells are the principal steroidogenic cells of the adult testis and play a central role in maintaining the intratesticular androgen environment required for normal spermatogenesis. After luteinising hormone (LH) stimulation, LH receptor activation increases cyclic AMP-dependent signalling and promotes cholesterol mobilization and transport into the mitochondrial compartment. Cholesterol is subsequently converted to pregnenolone by cytochrome P450 side-chain cleavage enzyme (CYP11A1), followed by sequential steroidogenic reactions involving enzymes such as 3 β -hydroxysteroid dehydrogenase, CYP17A1, and 17 β -hydroxysteroid dehydrogenase to generate testosterone. Testosterone acts through androgen receptors in Sertoli cells and other testicular compartments to support the progression of germ-cell development [20,21].

The functional capacity of Leydig cells is highly sensitive to oxidative stress. Excessive reactive oxygen species (ROS) can impair LH-responsive signalling, mitochondrial cholesterol transport, steroidogenic enzyme activity, and mitochondrial function, thereby reducing testosterone biosynthesis. Oxidative injury may also promote apoptosis, endoplasmic reticulum stress, and cellular senescence within the Leydig-cell compartment. Consequently, preservation of Leydig-cell redox homeostasis and mitochondrial integrity represents an important upstream target for interventions intended to maintain spermatogenesis [22–24].

Plant-derived compounds may influence Leydig-cell steroidogenesis through multiple complementary mechanisms rather than through direct androgenic activity alone. Polyphenols, flavonoids, phenolic acids, carotenoids, and other phytochemicals have been investigated for their ability to reduce oxidative damage, preserve mitochondrial function, regulate steroidogenic enzymes, and modulate intracellular signalling involved in testosterone biosynthesis. Experimental evidence with medicinal plants has demonstrated protective effects in Leydig-cell models exposed to oxidative stress, including preservation of antioxidant capacity and restoration of steroidogenic activity [22].

For example, *Taraxacum officinale* extract increased the expression of STAR, CYP11A1, CYP17A1, and TSPO in mouse Leydig cells and was associated with increased testosterone production, suggesting that modulation of cholesterol transport and steroidogenic enzyme expression may contribute to its steroidogenic activity [25]. Similarly, experimental studies of other medicinal plants have reported increased testosterone production together with reduced oxidative injury and improved Leydig-cell viability. These findings provide a mechanistic basis for investigating plant-derived compounds as modulators of testicular steroidogenesis rather than merely as nonspecific antioxidants [15,22].

2.2 Sertoli-Cell Function and the Blood–Testis Barrier

Sertoli cells are essential somatic cells within the seminiferous epithelium and provide structural, metabolic, and paracrine support throughout spermatogenesis. They regulate the proliferation and differentiation of germ cells, supply nutrients and metabolites required for germ-cell development, facilitate the removal of residual cytoplasmic material, and participate in the regulation of the seminiferous microenvironment. Sertoli cells also establish the blood–testis barrier (BTB), which separates the seminiferous epithelium into basal and adluminal compartments and provides a specialized environment for meiotic and post-meiotic germ cells [26,27].

The BTB is formed predominantly by specialized junctional complexes between adjacent Sertoli cells, including tight junctions, adherens junctions, gap junctions, and associated cytoskeletal structures. Its integrity is not static; junctional complexes undergo coordinated assembly and disassembly during the movement of developing spermatocytes from the basal toward the adluminal compartment. This dynamic process allows germ-cell progression while maintaining barrier function and immunological protection within the seminiferous epithelium. Disruption of these junctional mechanisms can interfere with germ-cell development and compromise spermatogenesis [28–30].

Oxidative stress is a key factor that can impair Sertoli-cell function and BTB integrity. Excessive reactive oxygen species can alter cellular proteins, membrane lipids, cytoskeletal organization, and junction-associated proteins, thereby disturbing communication between Sertoli cells and developing germ cells. Inflammatory signalling and endocrine disturbances can further aggravate these changes. Consequently, preservation of Sertoli-cell redox homeostasis and junctional integrity represents an important biological target for maintaining the spermatogenic microenvironment [8,31].

Plant-derived compounds may support Sertoli-cell function through antioxidant, anti-inflammatory, cytoprotective, and signalling-modulatory mechanisms. Quercetin and kaempferol, for example, have been investigated in experimental models of heat-stress-induced Sertoli-cell injury. Their administration reduced intracellular ROS and heat-stress-associated HSP70 expression, suppressed NF- κ B-related signalling, and increased the expression of structural proteins including occludin, vimentin, and F-actin. These findings suggest that selected flavonoids may preserve Sertoli-cell architecture and components relevant to barrier function under conditions of cellular stress [32].

2.3 Mitochondrial Function and Energy Metabolism

Mitochondria are central to male reproductive function because they integrate energy production, redox regulation, calcium homeostasis, apoptosis, and cellular signalling during spermatogenesis and sperm maturation. Developing germ cells undergo substantial metabolic changes during proliferation, meiosis, and differentiation, while mature spermatozoa require continuous energy production to sustain flagellar movement and other energy-dependent functions. Consequently, impairment of mitochondrial activity can compromise both the development of germ cells and the functional competence of mature spermatozoa [33,34].

Sertoli cells also have an important role in testicular energy metabolism. Developing germ cells depend substantially on metabolites supplied by Sertoli cells, particularly lactate generated through glucose metabolism. Alterations in glucose utilization, lipid metabolism, mitochondrial activity, or cellular energy balance can therefore disturb the metabolic support required for normal spermatogenesis. Metabolic disorders and excessive metabolic stress may further impair Sertoli-cell function and reduce the availability of substrates required for germ-cell development [35,36].

Mitochondrial dysfunction is closely associated with excessive reactive oxygen species (ROS) production. Although mitochondria normally generate ROS as a consequence of oxidative phosphorylation and cellular signalling, excessive mitochondrial ROS can damage mitochondrial membranes, proteins, lipids, and mitochondrial DNA. Loss of mitochondrial membrane potential may subsequently reduce ATP generation and activate apoptotic pathways. In spermatozoa, mitochondrial dysfunction has been associated with impaired motility, defective capacitation, altered acrosome function, and increased oxidative damage. Thus, maintaining mitochondrial quality and redox balance is an important target for preserving reproductive function [37,38].

Plant-derived compounds may influence mitochondrial function through antioxidant, membrane-stabilizing, anti-apoptotic, and metabolic mechanisms. Polyphenols and carotenoids can potentially reduce mitochondrial oxidative injury while preserving mitochondrial membrane integrity and cellular energy metabolism. Astaxanthin, for example, has been investigated extensively in experimental models of male reproductive dysfunction, with evidence indicating effects on oxidative stress, mitochondrial integrity, lipid peroxidation, and sperm quality. Recent experimental evidence also indicates that natural astaxanthin can enhance testosterone synthesis in Leydig cells by improving mitochondrial function and reducing oxidative stress [39,40].

Other plant-derived bioactives may act through mitochondrial quality-control mechanisms, including regulation of mitochondrial biogenesis, mitochondrial dynamics, and mitophagy. These processes are particularly relevant during germ-cell differentiation because mitochondria undergo substantial structural and functional changes throughout spermatogenesis. Appropriate removal of damaged mitochondria prevents accumulation of dysfunctional organelles and excessive ROS, whereas impaired mitochondrial quality control may contribute to germ-cell apoptosis and abnormal sperm development [34,41].

2.4 Oxidative Stress and Antioxidant Signalling

Oxidative stress is a major contributor to impaired male reproductive function and arises when the generation of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defence systems. Physiological concentrations of ROS are required for several aspects of sperm function, whereas excessive ROS production promotes lipid peroxidation, protein oxidation, mitochondrial dysfunction, DNA damage, and activation of apoptotic pathways. Germ cells and mature spermatozoa are particularly susceptible to oxidative injury because sperm membranes contain high proportions of polyunsaturated fatty acids and mature sperm possess relatively limited cytoplasmic antioxidant defences. Consequently, maintenance of redox homeostasis is essential for normal spermatogenesis and subsequent sperm function [42,43].

The testis possesses an integrated antioxidant defence system involving enzymatic antioxidants, including superoxide dismutase, catalase, and glutathione-dependent enzymes, together with non-enzymatic antioxidant molecules. Among the molecular regulators of this defence network, nuclear factor erythroid 2-related factor 2 (NRF2) has emerged as an important regulator of testicular redox homeostasis. Under basal conditions, NRF2 activity is controlled principally by Kelch-like ECH-associated protein 1 (KEAP1). During oxidative or electrophilic stress, NRF2 escapes KEAP1-mediated degradation, accumulates in the nucleus, and activates antioxidant-response-element-dependent transcription of cytoprotective genes. These responses contribute to glutathione metabolism, detoxification of reactive intermediates, and maintenance of cellular antioxidant capacity [44,45].

The importance of NRF2 signalling in spermatogenesis is supported by experimental evidence. NRF2-deficient mice develop progressive reproductive abnormalities with age, including seminiferous-tubule damage and reductions in testicular sperm head counts, epididymal sperm counts, and sperm motility. These observations indicate that NRF2-mediated antioxidant defence is involved not only in protection of mature spermatozoa but also in maintaining the testicular environment necessary for sustained sperm production [46].

Plant-derived bioactive compounds are particularly relevant to this pathway because many phytochemicals can

influence cellular redox signalling rather than functioning solely as direct free-radical scavengers. Polyphenols, flavonoids, carotenoids, phenolic acids, and other phytoconstituents have been investigated for their capacity to enhance endogenous antioxidant defences, reduce lipid peroxidation, preserve mitochondrial function, and regulate NRF2-associated signalling. Such effects may protect spermatogonia, spermatocytes, Sertoli cells, and Leydig cells from oxidative injury and thereby preserve the cellular environment required for spermatogenesis [44,45].

Importantly, experimental evidence also links plant-derived interventions with NRF2-mediated improvements in spermatogenesis. A standardized extract of *Bacopa monnieri*, for example, improved germ-cell dynamics and sperm quality in male mice while enhancing antioxidant enzyme activities. The reported mechanism involved Akt-associated activation of NRF2 together with changes in MAPK signalling, providing evidence that phytochemicals may influence spermatogenesis through regulation of endogenous cellular defence pathways rather than antioxidant scavenging alone [47].

However, excessive antioxidant activity is not necessarily beneficial. Physiological ROS participate in normal reproductive signalling, and excessive suppression of oxidative processes may shift the cellular environment toward reductive stress. Thus, the objective of plant-based antioxidant intervention should be restoration of redox homeostasis rather than indiscriminate elimination of ROS. This consideration is especially important when interpreting studies in which antioxidant biomarkers improve without corresponding changes in germ-cell development, sperm production, or fertility outcomes [48].

2.5 Apoptosis and Germ-Cell Survival

Apoptosis is an essential component of normal spermatogenesis and contributes to the maintenance of testicular homeostasis by eliminating defective, damaged, or excess germ cells. A controlled level of germ-cell apoptosis is necessary to maintain an appropriate ratio between developing germ cells and Sertoli cells and to regulate the progression of spermatogenesis. Therefore, apoptosis should not be considered exclusively as a pathological process; both excessive apoptosis and inadequate removal of abnormal germ cells can disturb testicular homeostasis and impair sperm production [49,50].

Germ-cell apoptosis occurs predominantly through intrinsic mitochondrial and extrinsic death-receptor pathways. The intrinsic pathway is regulated by members of the B-cell lymphoma-2 (BCL-2) protein family. Pro-apoptotic proteins such as BAX promote mitochondrial outer-membrane permeabilization, whereas anti-apoptotic proteins such as BCL-2 and BCL-XL counteract this process. Excessive mitochondrial injury can result in cytochrome-c release and subsequent activation of caspase-9 and caspase-3, leading to cellular dismantling. The extrinsic pathway involves death receptors and their associated ligands and can also converge on downstream caspase activation [51–53].

The physiological importance of regulated apoptosis is demonstrated by experimental models in which disruption of apoptotic signalling itself results in abnormal spermatogenesis. For example, suppression of BAX-dependent germ-cell apoptosis during testicular development produces abnormal germ-cell accumulation and subsequently impaired adult spermatogenesis. These findings demonstrate that complete inhibition of apoptosis is not desirable; rather, appropriate regulation of germ-cell survival and programmed cell death is essential for normal sperm production [54].

Pathological conditions associated with male infertility can disturb this balance. Oxidative stress, heat exposure, toxicants, inflammation, hormonal disturbances, radiation, and chemotherapeutic agents can increase germ-cell apoptosis through mitochondrial dysfunction, DNA damage, and activation of pro-apoptotic signalling. Clinical investigations in men with impaired spermatogenesis have reported increased expression of pro-apoptotic proteins, including BAX and BAK, together with reduced expression of anti-apoptotic proteins such as BCL-2 and BCL-W, accompanied by increased caspase-3 activity and TUNEL-positive testicular cells. These findings support an association between excessive apoptosis and impaired spermatogenic function [55].

Plant-derived compounds may help preserve germ-cell survival by attenuating the upstream factors that trigger excessive apoptosis. Their potential mechanisms include reduction of oxidative stress, preservation of mitochondrial membrane potential, suppression of inflammatory signalling, maintenance of the BAX/BCL-2 balance, and inhibition of excessive caspase activation. Consequently, the anti-apoptotic effects of phytochemicals are often closely interconnected with their antioxidant and mitochondrial protective activities rather than representing an independent mechanism.

Evidence from experimental models supports this concept. Lycopene, for example, reduced radiation-induced testicular injury, decreased TUNEL-positive germ cells, restored the BAX/BCL-2 balance, and preserved mitochondrial membrane potential and ATP production. These changes were accompanied by improved seminiferous-tubule architecture and sperm motility, suggesting that suppression of excessive mitochondrial apoptosis may contribute to preservation of spermatogenic function [56]. Similarly, plant-derived phenolic compounds have been investigated for their ability to reduce testicular apoptosis while preserving seminiferous structure, Leydig-cell populations, and spermatogenic cells in experimental models [57].

The relationship between oxidative stress and apoptosis is particularly important in plant-based interventions. Excessive ROS can damage mitochondrial membranes and DNA, activate BAX-associated signalling, and promote caspase-dependent germ-cell death. Conversely, restoration of antioxidant capacity may reduce the cellular signals that initiate apoptosis. Thus, phytochemicals capable of simultaneously regulating oxidative stress, mitochondrial integrity, and apoptotic signalling may provide greater protection of the spermatogenic microenvironment than compounds acting through a single pathway [58].

2.6 Autophagy and AMPK

Autophagy is a conserved intracellular quality-control mechanism that maintains cellular homeostasis by removing damaged proteins and organelles and recycling their components. In the testis, autophagy is involved in germ-cell differentiation, spermiogenesis, Sertoli-cell function, spermiation, and steroidogenic processes. Rather than functioning solely as a stress-response pathway, autophagy participates in the normal remodelling of germ cells and supporting somatic cells during spermatogenesis. Selective forms of autophagy are particularly important for the removal of specific cellular components and damaged organelles required for efficient germ-cell development [59–61].

AMP-activated protein kinase (AMPK) is an important metabolic sensor that links cellular energy status with autophagic activity. AMPK is activated when cellular energy availability decreases, reflected by an increased AMP/ATP or ADP/ATP ratio. Activation of AMPK promotes catabolic pathways that restore energy balance and can stimulate autophagy through inhibition of mammalian target of rapamycin complex 1 (mTORC1) and activation of autophagy-initiating mechanisms involving ULK1. In the testis, this AMPK–mTOR axis provides an important connection between energy metabolism, cellular quality control, and spermatogenic activity [62,63]. Sertoli cells are particularly dependent on metabolic regulation because they provide structural and nutritional support to developing germ cells. AMPK activation in Sertoli cells regulates glucose utilization and lactate production, which are important for germ-cell metabolism. Experimental activation of AMPK by adenosine increased glucose uptake, GLUT1 and LDHA expression, and MCT4 expression, while also stabilizing the tight-junction protein ZO-1. These effects indicate that AMPK contributes not only to energy metabolism but also to maintenance of the Sertoli-cell microenvironment required for spermatogenesis [64].

Autophagy also participates directly in germ-cell development. During spermiogenesis, autophagic mechanisms contribute to organelle turnover, cytoplasmic remodelling, acrosome formation, flagellar development, nuclear shaping, and removal of excess cytoplasm. Autophagy-mediated quality control is therefore relevant to the progression of developing germ cells rather than merely to the survival of mature spermatozoa [61].

Importantly, the relationship between AMPK, autophagy, and spermatogenesis is context-dependent. Moderate activation of autophagy can function as a protective response by removing damaged organelles, limiting oxidative injury and maintaining cellular viability. For example, experimental copper-induced testicular injury activated the AMPK–mTOR pathway and increased autophagy. Pharmacological inhibition of autophagy increased ROS accumulation, apoptosis, cellular injury and spermatogenic impairment, suggesting that autophagy initially acted as an adaptive mechanism against oxidative damage. However, the same study also demonstrated that excessive or dysregulated autophagy may interact with other programmed cell-death pathways, illustrating that autophagy cannot be considered universally beneficial [65].

This distinction is particularly important when considering plant-derived compounds. Phytochemicals with antioxidant and metabolic-regulatory properties may potentially influence AMPK–mTOR signalling, mitochondrial quality control and autophagic flux. Such effects could support Sertoli-cell metabolism, protect germ cells from oxidative damage and facilitate removal of dysfunctional cellular components. However, evidence for a plant compound that activates AMPK or autophagy does **not**, by itself, demonstrate stimulation of spermatogenesis. The biological outcome should be demonstrated through endpoints such as spermatogonial proliferation and differentiation, meiotic progression, germ-cell survival, seminiferous-tubule architecture, testicular sperm production, and ultimately sperm concentration and fertility.

The interaction between oxidative stress and AMPK–autophagy signalling further strengthens the relevance of this pathway to male infertility. Environmental and metabolic stressors can alter mitochondrial function and intracellular ROS production, thereby influencing AMPK activation and downstream mTOR signalling. Studies in Sertoli cells demonstrate that oxidative stress can activate AMPK-associated autophagy, while inhibition of ROS generation attenuates these changes. This suggests that AMPK–autophagy signalling may represent an adaptive response to cellular stress, although prolonged or excessive activation can become maladaptive [65,66]. Therefore, AMPK and autophagy should be viewed as interconnected regulators of metabolic homeostasis and cellular quality control during spermatogenesis rather than as isolated therapeutic targets. Plant-based interventions may influence this network through antioxidant activity, mitochondrial protection, metabolic regulation, and modulation of AMPK–mTOR signalling. Future studies should establish whether these molecular changes translate into measurable enhancement of germ-cell development and sperm production, while also determining dose-dependent effects and distinguishing physiological autophagy from excessive or impaired autophagic flux. Current evidence supports AMPK–autophagy signalling as a mechanistically relevant pathway for investigating plant-derived approaches to male infertility, but direct evidence of enhanced spermatogenesis remains essential before therapeutic conclusions can be drawn [62,67].

2.7 Ferroptosis and Lipid Peroxidation

Ferroptosis is an iron-dependent, regulated form of cell death characterized by the excessive accumulation of lipid peroxides in cellular membranes. Unlike apoptosis, ferroptosis is largely caspase-independent and is closely associated with disturbances in iron metabolism, glutathione depletion, impaired antioxidant defence, and membrane lipid peroxidation. Increasing evidence indicates that ferroptosis may contribute to testicular injury and impaired spermatogenesis by disrupting the survival of germ cells and altering the testicular microenvironment [58,68].

Lipid peroxidation is a central biochemical event in ferroptosis. Polyunsaturated fatty acids (PUFAs) incorporated into membrane phospholipids are particularly susceptible to oxidation when reactive oxygen species (ROS)

accumulate. Iron can further promote oxidative reactions through Fenton chemistry, generating highly reactive radicals that initiate and propagate lipid peroxidation. The resulting lipid hydroperoxides and secondary products, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), can damage membrane structure, mitochondrial function, and cellular proteins. In the testis, excessive lipid peroxidation may therefore compromise the integrity and viability of developing germ cells and interfere with normal spermatogenic progression [68].

A major endogenous defence against ferroptosis is the cystine/glutathione/GPX4 axis. System Xc⁻, composed principally of SLC7A11 and SLC3A2, imports cystine into cells for glutathione synthesis. Reduced glutathione subsequently serves as a cofactor for glutathione peroxidase 4 (GPX4), which reduces phospholipid hydroperoxides and limits their accumulation within cellular membranes. Disruption of SLC7A11 activity, glutathione depletion or loss of GPX4 function weakens this protective system and increases susceptibility to ferroptotic damage. Additional anti-ferroptotic mechanisms involving ferritin-mediated iron storage, CoQ10/FSP1 and mitochondrial lipid-protective pathways further contribute to cellular resistance against lipid peroxidation [69,70].

The relevance of this pathway to spermatogenesis has been demonstrated in experimental models. Zearalenone-induced testicular injury in mice was associated with increased Fe²⁺ and Fe³⁺ accumulation, elevated lipid peroxidation and increased 4-HNE, together with reduced Nrf2, SLC7A11 and GPX4 expression. These changes were accompanied by decreased sperm concentration and motility. Pharmacological inhibition of ferroptosis restored antioxidant defence and reduced iron accumulation and lipid peroxidation, supporting a mechanistic relationship between ferroptotic signalling and impaired spermatogenesis [71,72].

Metabolic disorders can similarly promote ferroptotic injury in the testis. In experimental type 1 diabetes, testicular iron accumulation and MDA were increased, whereas glutathione, GPX4, SLC7A11, and ferritin-related protection were reduced. Histological examination demonstrated depletion of spermatogenic cells and mitochondrial abnormalities. Inhibition of ferroptosis with ferrostatin-1, as well as restoration of GPX4 activity, reduced ROS and lipid peroxidation and improved spermatogenic dysfunction. Evidence of increased ferroptosis-related changes was also reported in testicular tissue from patients with diabetes, suggesting potential clinical relevance of this pathway [73].

The relationship between ferroptosis and the Nrf2 antioxidant system is particularly important for plant-based interventions. Nrf2 regulates multiple cytoprotective genes involved in glutathione synthesis, redox control, and cellular defence, including components of the SLC7A11/GPX4 pathway. Consequently, plant-derived compounds capable of maintaining Nrf2 signalling, glutathione availability, and GPX4 activity may theoretically reduce ferroptosis-associated damage. This mechanism is distinct from simple direct scavenging of ROS because it involves preservation of the endogenous cellular antioxidant network [74].

Several experimental findings also provide a direct link between modulation of ferroptosis and preservation of spermatogenic function. Recent evidence indicates that inhibition of ferroptosis can protect seminiferous-tubule architecture, germ-cell survival, and sperm quality under conditions of oxidative, metabolic, or toxicological stress. Conversely, increased expression of pro-ferroptotic factors such as ACSL4 and reduced expression of SLC7A11 and GPX4 have been associated with increased vulnerability of spermatogenic cells to oxidative injury. These observations suggest that maintenance of lipid-redox homeostasis may be an important component of protecting the germ-cell population required for continuous sperm production [68,75].

Importantly, plant-derived compounds should not be assumed to be universally protective against ferroptosis. The biological effect depends on the compound, dose, cellular context, and underlying pathological condition. For example, some phytochemicals may suppress lipid peroxidation and restore GPX4-dependent antioxidant defence, whereas certain bioactive plant constituents can themselves produce reproductive toxicity under particular exposure conditions. Triptolide, a bioactive constituent derived from *Tripterygium wilfordii*, provides an important example: experimental exposure increased ROS and MDA, decreased GSH and GPX4, and promoted ferroptosis-associated testicular injury and spermatogenic dysfunction [76,77].

Recent evidence involving plant-derived polysaccharides provides a more direct indication of the therapeutic relevance of ferroptosis. Epimedium polysaccharide improved spermatogenic function and sperm quality in an experimental oligoasthenozoospermia model while reducing oxidative stress and ferroptosis. Its effects were associated with increased SLC7A11 and GPX4 expression and suppression of TLR4/NF- κ B-associated inflammatory signalling. Although these findings are encouraging, they remain predominantly experimental and require confirmation in well-designed reproductive studies and ultimately in humans [78,79].

3. Plant-Based Compounds and Botanicals

Plant-derived botanicals and phytochemicals have attracted considerable interest as potential modulators of male reproductive function because their biological effects may extend beyond direct antioxidant activity to include regulation of steroidogenesis, Sertoli-cell function, mitochondrial homeostasis, inflammatory signalling, apoptosis, autophagy and ferroptosis.

3.1 *Withania somnifera* (Ashwagandha)

Withania somnifera (Ashwagandha) is among the most extensively investigated botanicals in male reproductive research. Experimental and clinical studies have reported improvements in sperm concentration, motility and selected reproductive endocrine parameters. In a randomized pilot study involving oligospermic men, administration of a full-spectrum *W. somnifera* root extract for 90 days was associated with substantial increases in sperm concentration, semen volume and sperm motility compared with baseline values [20]. A subsequent evidence-based systematic review identified clinical studies reporting improvements in sperm concentration,

semen volume, motility and selected hormonal parameters, although the limited number of randomized trials restricted the strength of the conclusions[21].

The proposed mechanisms include attenuation of oxidative stress, modulation of stress-associated pathways, and possible support of steroidogenic and androgen-related functions. Nevertheless, variation in extract standardization and phytochemical composition makes comparison among studies difficult. Further controlled studies using chemically characterized preparations and clinically relevant reproductive endpoints are required to establish whether the observed improvements reflect direct enhancement of spermatogenesis or secondary improvement in the testicular environment [22].

3.2 *Panax ginseng*

Panax ginseng contains ginsenosides and other bioactive constituents that have been investigated for their effects on male reproductive function. Experimental studies suggest that ginseng-derived preparations may protect testicular tissue against oxidative, thermal, and toxicological stress and may influence sperm characteristics and steroidogenic activity. Particular attention has been given to ginsenoside-enriched preparations, including Korean red ginseng preparations containing Rg3, in models of chronic heat-induced reproductive dysfunction [23].

The reported effects have been associated with antioxidant and anti-inflammatory activity, preservation of testicular cellular integrity and modulation of reproductive signalling. However, differences in ginsenoside composition between preparations represent an important source of experimental heterogeneity. Consequently, evidence from one standardized ginseng preparation cannot necessarily be extrapolated to other extracts or commercial products.

3.3 *Lepidium meyenii* (Maca)

Maca (*Lepidium meyenii*) has been investigated as a reproductive botanical using different plant varieties, extraction methods, and preparations. Clinical evidence suggests that maca may influence semen characteristics, although results have been inconsistent. An early clinical study reported increases in seminal volume, sperm count and motility without significant changes in circulating reproductive hormones, suggesting that its effects may not be mediated primarily through alterations in LH, FSH, testosterone or estradiol [24].

However, systematic evaluations have identified substantial heterogeneity among clinical trials. A meta-analysis of randomized controlled trials found mixed effects on semen parameters and did not demonstrate a significant overall increase in sperm concentration compared with placebo [25,26]. Accordingly, maca represents an interesting botanical candidate, but the current evidence does not establish a standardized preparation capable of consistently stimulating spermatogenesis. Differences in macamides, glucosinolates and other constituents may contribute to variability between preparations [27].

3.4 *Tribulus terrestris* and *Anacyclus pyrethrum*

Tribulus terrestris and *Anacyclus pyrethrum* have been investigated in experimental and clinical studies of male reproductive dysfunction. Animal studies have reported improvements in reproductive performance and selected sperm parameters, while human investigations have examined effects on semen quality [28,29]. A systematic review of *T. terrestris* in men with idiopathic infertility identified clinical studies evaluating sperm concentration, motility, and other semen characteristics, but emphasized the limited and heterogeneous nature of the available evidence [30].

The proposed reproductive actions of these botanicals include antioxidant activity, modulation of androgen-associated pathways, and improvement of the testicular microenvironment. However, variations in plant preparation, dose, and treatment duration make direct comparison difficult. The available evidence therefore supports further investigation rather than definitive conclusions regarding their ability to directly stimulate spermatogenesis.

3.5 Processed Shilajit

Processed Shilajit is a chemically complex mineral-organic preparation containing fulvic substances, dibenzo- α -pyrones and other constituents. Unlike a single purified phytochemical, its reproductive activity is likely to reflect multiple components and therefore depends strongly on processing and standardization. In a clinical study of oligospermic men, processed Shilajit administered for 90 days was associated with increased sperm concentration, total sperm count, motility, and normal sperm morphology, together with reductions in seminal MDA and increases in serum testosterone and FSH [31].

These findings provide clinically relevant evidence for a potential spermatogenic effect; however, the study had a relatively small treatment population and requires confirmation in larger, independently conducted randomized trials[32]. Standardization is particularly important because different Shilajit products may differ substantially in chemical composition and may vary in contamination with heavy metals or other undesirable constituents. Consequently, chemical profiling, quality control, and contaminant assessment should be considered essential when evaluating Shilajit for reproductive applications.

3.6 *Cistanche* species and phenylethanoid glycosides

Cistanche species contain phenylethanoid glycosides and other bioactive constituents that have been investigated for their effects on reproductive and endocrine function. Experimental studies suggest that *Cistanche*-derived preparations may influence steroidogenic enzymes, oxidative stress, mitochondrial function, and cellular survival pathways in models of reproductive dysfunction [33].

Recent experimental approaches have extended this research to plant-derived exosome-like nanovesicles containing bioactive molecular cargo [34]. These nanovesicles may interact with Sertoli-cell and germ-cell signalling pathways and therefore represent a distinct delivery strategy rather than a conventional botanical extract

[35]. Nevertheless, such approaches remain preclinical, and their pharmacokinetics, tissue distribution, molecular cargo, reproducibility, and long-term reproductive safety require systematic investigation.

3.7 Icariin

Icariin is a flavonoid glycoside derived predominantly from *Epimedium* species and has been investigated extensively in experimental models of testicular dysfunction. Its reported actions include modulation of oxidative stress, mitochondrial function, apoptosis, and Sertoli-cell activity. In an experimental model of age-related testicular dysfunction, icariin improved testicular and epididymal indices, sperm count and viability, and increased intratesticular testosterone and estradiol concentrations. These effects were associated with protection of Sertoli cells and activation of the ER α /Nrf2 signalling pathway [36].

Other experimental studies have reported beneficial effects of icariin in diabetic testicular dysfunction, including modulation of cellular proliferation, mitochondria-dependent apoptosis, and glycolytic metabolism[37]. Collectively, these findings suggest that icariin may act at multiple levels of the testicular microenvironment. However, translation to human infertility requires clinical studies using standardized preparations and validated reproductive outcomes.

3.8 Trifolium pratense

Trifolium pratense (red clover) extract has been investigated in experimental diabetes-associated reproductive dysfunction. In streptozotocin-induced diabetic rats, treatment with *T. pratense* extract increased sperm count, motility, and viability, together with testosterone and total antioxidant capacity, while modifying markers associated with oxidative and cellular stress [38].

These findings suggest that improvement of metabolic and redox disturbances may contribute to preservation of testicular function under diabetic conditions [38]. However, the available evidence is restricted primarily to experimental models, and it remains unclear whether the observed effects result from direct stimulation of spermatogenic cells or secondary correction of diabetes-associated testicular injury.

3.9 Chlorogenic acid

Chlorogenic acid is a phenolic compound widely distributed in coffee, fruits, and vegetables and has received increasing attention because of its antioxidant and redox-regulatory properties [39]. Direct evidence in human spermatozoa was provided by Signorini et al., who demonstrated that exposure to 100 μ M chlorogenic acid increased rapid progressive motility and reduced seminal F2-isoprostanes. Under hydrogen peroxide-induced oxidative stress, chlorogenic acid reduced oxidative DNA damage, restored sperm motility, and increased Nrf2 mRNA expression [40].

These findings provide mechanistic evidence linking chlorogenic acid with modulation of the Nrf2-associated antioxidant response. Nevertheless, the experimental exposure occurred directly in human semen samples; therefore, the findings primarily demonstrate protection of mature spermatozoa rather than stimulation of spermatogenesis within the testis.

3.10 Apigenin

Apigenin is a flavone present in several fruits, vegetables, and medicinal plants. Its reproductive effects appear to be strongly dependent on concentration, exposure conditions, and experimental model. Studies of semen cryopreservation have reported improved post-thaw sperm motility, viability, mitochondrial function, and antioxidant status following apigenin supplementation, together with reductions in ROS and apoptosis. For example, experimental supplementation of rooster semen with 100 μ mol/L apigenin improved total and progressive motility, membrane integrity, mitochondrial function, and viability while reducing oxidative and apoptotic indicators [41].

Although such findings demonstrate a potentially useful sperm-protective action, they should not be equated with increased spermatogenic activity. Direct evidence involving testicular germ-cell proliferation, differentiation, or sperm production is required before apigenin can be classified as a spermatogenesis-stimulating compound [42].

3.11 Astaxanthin

Astaxanthin is a lipophilic xanthophyll carotenoid capable of interacting with biological membranes and modulating oxidative processes [43]. Experimental studies have associated astaxanthin with preservation of sperm quality, mitochondrial function, and testicular redox homeostasis [44]. Recent animal evidence further suggests that astaxanthin may influence spermatogenesis through regulation of mitochondrial oxidative damage, lipid metabolism, and ferroptosis-associated pathways [45].

These findings are mechanistically relevant because mitochondrial dysfunction and lipid peroxidation are interconnected with germ-cell survival and spermatogenic progression. Nevertheless, evidence from animal models remains more consistent than evidence from human clinical studies. A major limitation is the variation in dose, formulation, treatment duration, and patient characteristics across studies. Thus, well-designed clinical trials are necessary to determine whether the molecular effects observed experimentally translate into clinically meaningful improvements in male fertility.

3.12 Lycopene

Lycopene is a lipophilic carotenoid abundant in tomatoes and other red-coloured fruits and has been investigated extensively for its antioxidant and membrane-protective properties [46]. Experimental studies have associated lycopene supplementation with reductions in ROS generation, lipid peroxidation and sperm DNA damage, particularly under conditions of oxidative or metabolic stress [47].

Clinical evidence remains less consistent. A 2025 systematic review and meta-analysis involving four clinical studies and 151 participants reported significant improvements in sperm concentration and nonprogressive

motility, whereas significant effects were not demonstrated for total motility, progressive motility, morphology, semen volume, or DNA damage [48]. Thus, lycopene may have potential as a redox-modulating adjunct, but current evidence does not establish a uniform effect across all semen parameters.

3.13 Naringin

Naringin is a flavanone glycoside abundant in citrus fruits and particularly concentrated in citrus peel [49]. Experimental investigation in high-fat-diet-induced diabetic rats demonstrated that naringin improved sperm characteristics, testosterone, and antioxidant enzyme status and enhanced testicular histoarchitecture. These effects were accompanied by reductions in MDA and inflammatory and metabolic stress markers, including xanthine oxidase and uric acid [50].

Interestingly, citrus-peel extract produced stronger effects for some parameters than isolated naringin, suggesting that interactions among multiple phytochemicals may influence biological activity. This observation highlights an important consideration in botanical research: the reproductive effects of a whole extract cannot necessarily be predicted from the activity of a single isolated constituent. Further studies should therefore compare standardized extracts with purified naringin while defining the molecular pathways responsible for the observed effects.

3.14 Naringenin

Naringenin is a citrus-derived flavanone that has been investigated in models of oxidative and inflammatory testicular injury [49]. Experimental evidence indicates that naringenin may attenuate oxidative stress, inflammatory signalling, and apoptosis in varicocele-associated reproductive dysfunction [51]. Reported effects include restoration of antioxidant capacity and reduction of markers such as MDA, HSP70, VEGF, caspase-3, and inflammatory mediators [51].

These findings suggest that naringenin may preserve the testicular microenvironment under pathological conditions. However, the evidence remains predominantly experimental, and direct demonstration of enhanced germ-cell proliferation, meiotic progression or testicular sperm production is required to establish a specific spermatogenic effect.

3.15 Echinacoside

Echinacoside is a phenylethanoid glycoside found in medicinal plants including *Cistanche* species [52]. Experimental studies have demonstrated protective effects against spermatogenic dysfunction, including improvements in total sperm number, forward motility, and sperm morphology. Echinacoside treatment was also associated with increased glutathione and modulation of antioxidant enzymes and MAPK-related signalling, particularly p38 and JNK pathways [53].

These findings indicate that echinacoside may influence spermatogenesis through coordinated regulation of oxidative stress, cell survival and MAPK signalling. Nevertheless, additional studies are needed to determine whether MAPK modulation represents a primary mechanism of action or a secondary response to reduced oxidative injury.

3.16 Curcumin

Curcumin, the principal curcuminoid of *Curcuma longa*, has been extensively investigated because of its antioxidant, anti-inflammatory, and cytoprotective properties [54]. Experimental reproductive studies indicate that curcumin can attenuate oxidative injury and apoptosis in spermatozoa and testicular tissue. In an ex vivo goat-semen model subjected to menadione-induced oxidative stress, 25 μ M curcumin produced the most favourable response among the tested concentrations, improving sperm motility, membrane and acrosomal integrity, and antioxidant enzyme activity while reducing ROS and apoptotic markers [55].

These findings support a protective effect against oxidative damage to mature spermatozoa. However, because the study was performed in an acute ex vivo semen model, it does not establish direct stimulation of spermatogenesis. Future studies should investigate whether sustained curcumin exposure in vivo influences spermatogonial proliferation, meiotic progression, Sertoli-cell function, and testicular sperm production.

3.17 Morinda officinalis polysaccharides

Morinda officinalis polysaccharides have demonstrated protective effects in experimental models of Leydig-cell dysfunction and oxidative testicular injury. Experimental treatment reduced ROS and lipid peroxidation, improved mitochondrial function, and supported testosterone production in Leydig cells. In vivo studies further reported improvements in sperm quantity, activity, and morphology in models of Leydig-cell depletion [56].

Mechanistically, the SIRT1/PGC-1 α pathway has been implicated in these effects, linking the botanical preparation with mitochondrial biogenesis and metabolic regulation. Inhibition of SIRT1 attenuated the protective cellular response, providing mechanistic evidence beyond nonspecific antioxidant activity [56]. This makes *M. officinalis* polysaccharides particularly relevant to the interaction between Leydig-cell steroidogenesis, mitochondrial homeostasis and reproductive function. Nevertheless, confirmation in standardized preparations and clinical populations is required.

3.18 Kaempferol

Kaempferol is a flavonol distributed widely in fruits, vegetables and medicinal plants. Experimental studies suggest that it may protect testicular tissue against toxicant-induced oxidative and inflammatory damage. In lead-induced reproductive toxicity, kaempferol improved antioxidant enzyme activity, steroidogenic parameters, testosterone concentrations, and sperm characteristics while reducing oxidative and inflammatory markers. Combination with vitamin E produced greater effects for several endpoints than either intervention alone [57].

These findings suggest potential interaction between kaempferol and other antioxidant systems. However, the evidence is currently preclinical, and the contribution of kaempferol to direct stimulation of spermatogenic cell

development remains uncertain [57]. Dose-response studies and mechanistic investigations involving Sertoli cells, spermatogonia and meiotic germ cells would strengthen the evidence base.

3.19 Resveratrol

Resveratrol is a stilbene polyphenol investigated in models of metabolic, toxicant-associated and age-related reproductive dysfunction. Its biological activity extends beyond direct antioxidant effects and includes modulation of SIRT1/PGC-1 α signalling, mitochondrial biogenesis, autophagy, inflammatory responses and steroidogenic pathways [58,59].

The relevance of resveratrol to spermatogenesis may therefore involve coordinated regulation of mitochondrial quality control and cellular energy metabolism [58,59]. However, its reproductive effects are strongly influenced by dose, exposure duration, formulation, and tissue bioavailability. Importantly, resveratrol exhibits concentration-dependent biological activity, and results obtained at experimental doses cannot automatically be extrapolated to clinically achievable exposure. Consequently, future studies should define pharmacokinetic relationships between administered dose, intratesticular exposure, and reproductive outcomes (Table 1).

Table 1. Comparative Table of Plant-Based Compounds and Spermatogenic Effects

Plant-based compound / botanical	Principal bioactive constituents	Experimental / clinical evidence	Major reproductive effects	Proposed mechanisms relevant to spermatogenesis	Evidence level
<i>Withania somnifera</i> (Ashwagandha)	Withanolides	Human clinical studies; animal and cellular studies	↑ sperm concentration, total count, and motility; reported ↑ testosterone	Leydig-cell steroidogenesis, antioxidant activity, modulation of reproductive hormones, reduction of oxidative stress	Human + preclinical
<i>Panax ginseng</i>	Ginsenosides	Animal, cellular and human/ex-vivo evidence	Improved sperm motility and reproductive function; effects on sperm functional parameters	Antioxidant activity, mitochondrial protection, steroidogenic regulation, ion-channel modulation including CatSper	Preclinical + limited human
<i>Lepidium meyenii</i> (Maca)	Macamides, macaenes, glucosinolates and polyphenols	Human clinical and animal studies	Variable improvement in sperm concentration, count and motility	Modulation of reproductive function, antioxidant effects and possible testicular metabolic regulation	Human + preclinical
<i>Tribulus terrestris</i>	Steroidal saponins, including protodioscin	Human and animal studies	Variable effects on sperm count, motility and morphology	Possible steroidogenic, antioxidant and androgen-related mechanisms	Human + preclinical ; inconsistent
<i>Anacyclus pyrethrum</i>	Alkylamides and phenolic constituents	Mainly animal studies	Increased sperm parameters and reproductive activity in experimental models	Androgenic/steroidogenic activity, antioxidant protection and testicular support	Preclinical
Processed Shilajit	Fulvic acids, dibenzo- α -pyrones and minerals	Human clinical and animal studies	↑ sperm concentration, total count, motility and normal morphology; ↑ testosterone reported	Antioxidant activity, mitochondrial support and steroidogenic modulation	Human + preclinical
<i>Cistanche</i> spp.	Echinacoside, acteoside and related phenylethanoid glycosides	Mainly animal and cellular studies	Improved sperm parameters and protection against reproductive injury	Antioxidant activity, mitochondrial protection, anti-apoptotic signalling and steroidogenic regulation	Preclinical

Icariin	Prenylated flavonoid	Animal and cellular studies	Improved sperm quality and testicular function in experimental models	PI3K/Akt signalling, antioxidant activity, mitochondrial protection and steroidogenic modulation	Preclinical
<i>Trifolium pratense</i> (Red clover)	Isoflavones, particularly formononetin and biochanin A	Experimental studies	Potential modulation of testicular oxidative and reproductive parameters	Estrogen-receptor-related signalling, antioxidant and anti-inflammatory activity	Preclinical / limited human relevance
Chlorogenic acid	Chlorogenic acid	Human semen/ex-vivo and experimental studies	Improved sperm motility and protection against oxidative DNA damage	Nrf2-associated antioxidant signalling and reduction of lipid peroxidation	Human ex-vivo + preclinical
Apigenin	Flavone	Animal and cellular studies	Protection of testicular tissue and sperm quality under experimental stress	Antioxidant, anti-inflammatory, mitochondrial and anti-apoptotic pathways	Preclinical
Astaxanthin	Xanthophyll carotenoid	Animal, cellular and emerging human evidence	Improved sperm quality and testicular function in experimental models	Mitochondrial protection, antioxidant activity, lipid-peroxidation control and steroidogenesis	Preclinical + emerging human
Lycopene	Carotenoid	Human clinical and experimental studies	↑ sperm concentration/count in selected studies; variable effects on motility and DNA integrity	Reduction of oxidative stress and lipid peroxidation; mitochondrial and membrane protection	Human + preclinical
Naringin	Flavanone glycoside	Animal and cellular studies	Protection of spermatogenesis against experimentally induced reproductive injury	Antioxidant, anti-inflammatory and anti-apoptotic activity	Preclinical
Naringenin	Flavanone	Animal and cellular studies	Protection of testicular tissue and sperm parameters under oxidative/metabolic stress	Nrf2-related antioxidant signalling, anti-inflammatory and mitochondrial mechanisms	Preclinical
Echinacoside	Phenylethanoid glycoside	Animal and cellular studies	Improved spermatogenic and testicular parameters in experimental models	Antioxidant, mitochondrial, anti-apoptotic and autophagy-related signalling	Preclinical
Curcumin	Curcuminoids	Animal and cellular studies; limited human reproductive evidence	Protection against testicular oxidative injury and preservation of sperm parameters	Nrf2 activation, NF-κB modulation, antioxidant, anti-inflammatory and anti-apoptotic effects	Preclinical + limited human

<i>Morinda officinalis</i> polysaccharides	Polysaccharides and associated phytochemicals	Mainly animal studies	Improved sperm/testicular parameters in experimental reproductive injury	Antioxidant activity, mitochondrial regulation and inhibition of excessive apoptosis/ferroptosis	Preclinical
Kaempferol	Flavonol	Cellular and animal studies	Protection of Sertoli cells and sperm/testicular function under stress	Antioxidant activity, mitochondrial protection, anti-inflammatory and cell-survival signalling	Preclinical
Resveratrol	Stilbene polyphenol	Animal and cellular studies; human evidence limited	Protection of sperm and testicular tissue in experimental models	SIRT1/AMPK signalling, antioxidant defence, mitochondrial regulation and anti-inflammatory effects	Preclinical + limited human

4. Evidence Hierarchy and Critical Appraisal

The literature can be divided into four evidence tiers. Human randomized or prospective clinical studies provide the most direct evidence for clinical relevance. Human semen experiments are useful for defining direct sperm effects but do not demonstrate increased testicular sperm production. Animal models provide mechanistic and histological information and can demonstrate changes in sperm production, testosterone and fertility, but translation to humans is uncertain. Cell studies are valuable for pathway analysis but are the least direct evidence for clinical spermatogenesis.

A recurrent limitation is the use of chemically induced models such as diabetes, varicocele, heat stress, heavy-metal toxicity, chemotherapy or oxidative challenges. These models are useful for testing whether a compound can reverse a defined injury, but the findings do not necessarily apply to idiopathic infertility. Another limitation is the frequent use of multiple endpoints without a primary clinically meaningful outcome.

Botanical quality is equally important. The scoping review of botanicals reported recurring deficiencies in authentication, voucher samples, chemical profiling, and reporting of extraction procedures. These limitations can make apparently contradictory studies difficult to reconcile because two studies may use products with the same plant name but different chemical composition.

5. Safety and Translational Considerations

Plant-derived does not mean automatically safe. Some phytochemicals show dose-dependent antioxidant and pro-oxidant behavior. Excessive antioxidant exposure may suppress physiological ROS signaling or produce reductive stress. The reproductive system therefore requires an appropriate redox balance rather than complete elimination of ROS.

Bioavailability is another major barrier. Curcumin, resveratrol, and several flavonoids have limited aqueous solubility or extensive metabolism. Nanoformulations and plant-derived nanovesicles may improve delivery but create new questions regarding biodistribution, long-term reproductive toxicity, and regulatory characterization. Clinical translation should occur only after formulation-specific safety has been demonstrated.

6. Future Research Priorities

Future research should focus on improving the reproducibility, mechanistic understanding, and clinical translation of plant-based interventions for male infertility. Botanical materials should be properly authenticated and accompanied by quantitative chemical fingerprints to ensure consistency between studies and batches. Detailed information regarding the plant part, extraction solvent, extraction ratio, dose, treatment duration, and batch characteristics should also be reported. Such standardization is particularly important because differences in preparation and phytochemical composition can substantially influence biological activity.

Future studies should clearly distinguish stimulation of spermatogenesis from protection of mature spermatozoa, as these represent different biological processes. Evaluation should therefore combine sperm concentration and total sperm count with progressive motility, morphology, testosterone and, where appropriate, testicular histology rather than relying on a single biomarker. Well-designed human trials should additionally incorporate clinically meaningful outcomes such as pregnancy and live-birth rates when feasible.

Pharmacokinetic studies are also required to determine absorption, metabolism, systemic exposure, and reproductive-tissue concentrations and to establish whether experimentally effective concentrations can be achieved safely in humans. Dose-response studies should identify effective and potentially toxic exposure ranges, while also considering the possibility of reductive stress resulting from excessive antioxidant activity. Mechanistic studies should integrate functional reproductive outcomes with markers such as NRF2, GPX4, SIRT1/PGC-1 α , BAX/BCL-2 and steroidogenic enzymes to establish relationships between molecular pathways and reproductive effects.

Finally, future investigations should systematically evaluate adverse events, reproductive toxicity, endocrine effects, and long-term safety. Rather than applying indiscriminate combinations of multiple antioxidants, research should increasingly investigate phenotype-specific interventions based on the underlying mechanisms and

reproductive abnormalities of individual patients. This approach may improve the biological precision and clinical relevance of plant-based strategies for male infertility.

7. CONCLUSION

Plant-based compounds have substantial experimental potential for supporting male reproductive function through mechanisms that include steroidogenesis, Sertoli-cell support, germ-cell survival, mitochondrial quality control, redox regulation, and inflammatory signaling. The most direct evidence for spermatogenic support in the reviewed corpus involves botanical preparations and experimental compounds that improve sperm production together with testosterone or testicular histology. Human sperm studies with chlorogenic acid and apigenin, and clinical evidence for botanicals such as *Withania somnifera*, *Tribulus terrestris*, and processed Shilajit, provide additional translational signals.

However, the field should avoid equating antioxidant activity with proven fertility treatment. A mature sperm-preservation study, an animal toxicology model and a human infertility trial answer different questions. The next generation of studies should use standardized preparations, pharmacokinetic data, mechanistically informed doses and clinically meaningful fertility endpoints. On the current evidence, plant-based compounds should be regarded as promising research candidates rather than established universal therapies for male infertility.

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