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Physiological and pathological functions of Reactive Nitrogen Species (RNS) and Reactive Sulphur Species (RSS) on Male Reproductive functions

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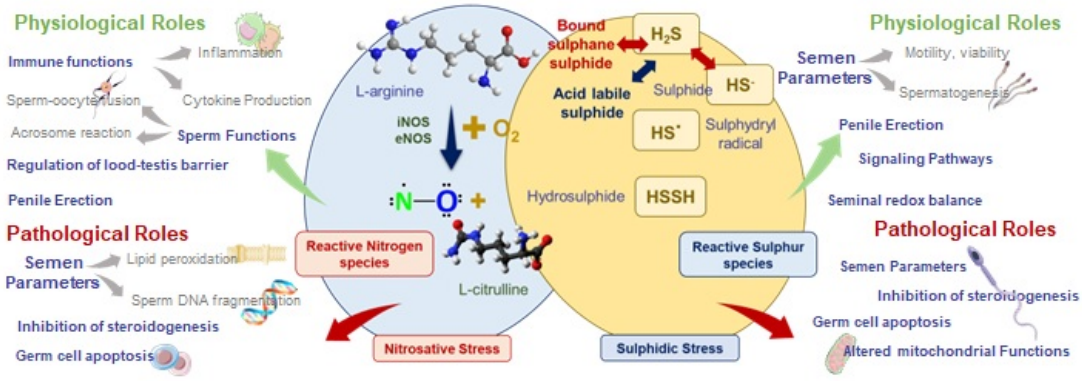
Review

ABSTRACT



Reactive Nitrogen Species (RNS) and Reactive Sulphur Species (RSS) play crucial roles in numerous physiological pathways, including cellular signaling, metabolic cascades, and redox balance maintenance. In male reproduction, these molecules serve dual purposes: they support essential physiological functions but can cause harm if unregulated. This review explores the roles of RNS and RSS in male reproduction, emphasizing their beneficial and detrimental impacts. Physiologically, RNS and RSS aid in sperm maturation, capacitation, and initiating the acrosome reaction. Regulated synthesis of these species is vital for redox-regulated events essential for optimal male reproductive outcomes. Specifically, nitric oxide, a primary RNS, regulates sperm functions and penile erection mechanisms. However, imbalances, leading to excessive RNS and RSS levels, can cause oxidative and nitrosative stresses, which can trigger lipid peroxidation, protein alterations, and sperm DNA fragmentation, affecting sperm vitality, motility, and genomic integrity.

Keywords: male infertility; nitrosative stress; reactive nitrogen species; reactive oxygen species; reactive sulphur species

INTRODUCTION

Reactive species, especially those derived from oxygen, have been notably studied in biomedicine due to their ambivalent influence in both physiological and pathological contexts.[1-3] Conversely, the roles of Reactive Nitrogen Species (RNS) and Reactive Sulphur Species (RSS) are relatively understudied, especially concerning male reproductive functions. RNS and RSS are groups of molecules that originate from their primary nitrogen and sulphur precursors, respectively. These species are integral to various biological processes, functioning as signaling agents that orchestrate cellular activities.[4, 5]

Within the male reproductive framework, physiological concentrations of RNS and RSS are pivotal for a spectrum of processes from spermatogenesis to influencing sperm motility and efficacy.[5, 6] For example, nitric oxide (NO), an essential RNS constituent, plays a central role in penile erection and sperm functionality, underscoring its importance in male fertility.[7] In parallel, RSS, while a more nascent research area, holds potential to reveal novel physiological roles pertaining to male reproduction.

Conversely, an excessive accumulation of these species, stemming from external influences like environmental contaminants or inherent causes such as oxidative stress, can be injurious.[8] Heightened RNS and RSS levels may culminate in nitrosative or sulphidic stress, respectively.[4, 9] These stressors are associated with multiple maladies in the male reproductive system,

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encompassing diminished sperm quality, compromised sperm movement, and even instances of male infertility.[9]

The objective of this literature review is to provide an exhaustive examination of the physiological and pathological connotations of RNS and RSS within male reproductive mechanisms. By elucidating both their salutary and deleterious impacts, this review seeks to address present knowledge deficits and foster forthcoming research directions. Such endeavors could conceivably enhance diagnostic and therapeutic methodologies pertaining to male reproductive wellbeing.

Reactive Nitrogen Species

Reactive nitrogen species (RNS) constitute a diverse group of molecules, each exhibiting unique chemical characteristics and reactivity, all originating from a shared precursor: ˙NO. This ˙NO is recognized as a free radical and a mild oxidizing agent, playing a pivotal role in regulating critical cellular processes across a spectrum of organ systems, encompassing the cardiovascular, neural, immune, reproductive, gastrointestinal, and secretory systems.[10,11] ˙NO synthesis predominantly arises from an enzymatic process mediated by nitric oxide synthase (NOS). There exist three distinct isoforms of NOS: endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS).[10] These isoforms vary in their affinities for calmodulin and the magnitude of ˙NO production. Intracellularly, ˙NO can manifest dual roles, ranging from physiological to pathological outcomes. The specific effects imparted by ˙NO are contingent upon its concentration, which is intrinsically linked to its rate of synthesis and the prevailing cellular redox environment.[10, 12] At nanomolar concentrations, ˙NO operates through the traditional cGMP-dependent signaling pathway, which entails the activation of soluble guanylyl cyclase (sGC), cGMP generation, followed by the stimulation of particular cGMP-dependent enzymatic targets.[13] In contrast, at elevated, micromolar concentrations, ˙NO employs non-traditional, indirect signaling mechanisms independent of cGMP.[14, 15] This mode of action involves covalent post-translational modifications of proteins, notably S-nitrosylation, S-glutathionylation, and tyrosine nitration. Such non-traditional signaling arises from ˙NO interactions with prevalent intracellular reactants like O2 or O2•−, leading to the generation of various RNS (like nitrogen dioxide, ˙NO2; peroxynitrite, ONOO−; nitroxyl, HNO; dinitrogen trioxide, N2O3; and dinitrogen tetroxide, N2O4). These RNS exhibit higher reactivity compared to ˙NO and have the capability to modify cysteine and tyrosine residues within proteins.[16,17] Notably, S-nitrosylation and S-glutathionylation are dynamic processes, and their equilibrium can be modulated by specific reductases, namely thioredoxin and S-nitrosoglutathione reductase.[18,19] Conversely, tyrosine nitration, predominantly facilitated by ONOO−, is largely an irreversible process and can often lead to cytotoxic implications of ˙NO by potentially altering key protein functions.[19, 20]

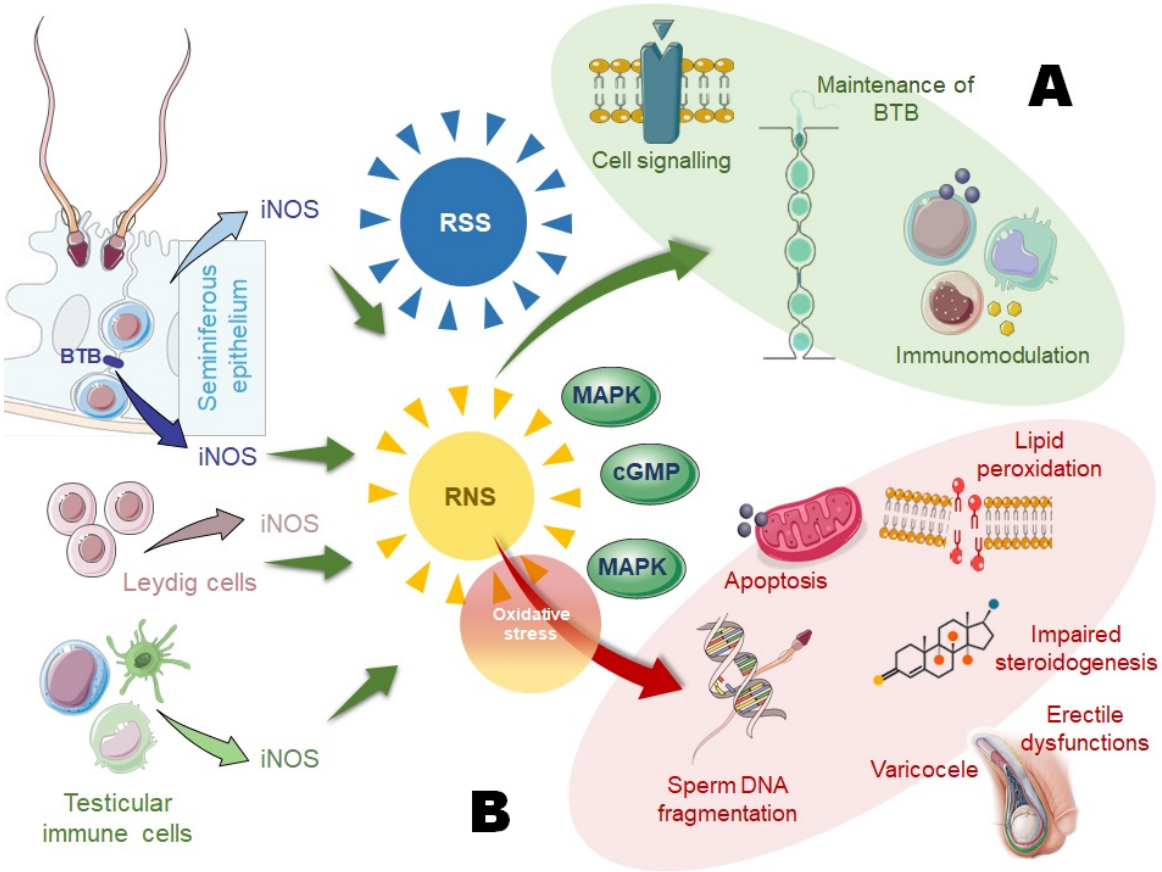

Figure 1. Physiological (A) and pathological roles of reactive nitrogen species (RNS) and reactive sulphur species (RSS) on male reproduction.

Sources of RNS in male reproductive system

In the human reproductive system, ˙NO synthesis pathway plays a pervasive role, with ˙NO modulating numerous reproductive processes.[21, 22] Within the testicular tissue, all three NOS isoforms are present across various cell categories—namely Leydig, endothelial, myoid, Sertoli, and germ cells situated in the seminiferous epithelium.[23, 24] In penile tissue, the neuronal form of NOS (nNOS) is identified in the pelvic plexus, dorsal penile and cavernous nerve, and within the smooth muscle cells of the corpus cavernosum.[25, 26] In contrast, endothelial NOS (eNOS) is detected in both cavernous endothelial cells and corpus cavernosum smooth muscle cells.[26] Human sperm cells synthesize ˙NO through the function of all known NOS isoforms, which are localized in either the sperm head or flagellum.[21, 22] Notably, as sperm undergo maturation, there is a variability in the enzymatic activity of sperm-associated NOS, highlighting the intrinsic physiological significance of ˙NO in sperm functions (**Fig. 1)**.[27]

Cellular signaling of RNS in male reproductive events

RNS are instrumental in modulating diverse cellular activities. Within this category, ˙NO stands out as a crucial mediator in male reproductive biology. This composition explores the complex signaling pathways of ˙NO related to male reproduction, with a particular focus on its integral involvement in sperm activity and male reproductive tissue dynamics. ˙NO, produced within the reproductive system, is a multifunctional entity that performs numerous roles. At physiological levels, ˙NO is generated by Leydig cells, facilitating unimpeded diffusion across cell boundaries, thus enhancing testicular functionalities. Its regulatory scope over the male reproductive apparatus spans processes like spermatogenesis, sperm maturation, motility, and germ cell programmed cell death.[23, 25] The predominant mechanism through which ˙NO enforces these controls is primarily by triggering the soluble guanylate cyclase (sGC) and subsequently signaling through the cyclic guanosine monophosphate (cGMP) protein kinase G (PKG) route.[24] Moreover, penile tissues significantly benefit from ˙NO's actions, where it plays a foundational role in the erectile mechanism. This functional attribute is rooted in the generation of ˙NO by constitutive NOS, especially eNOS and nNOS.[28, 29] Interference with these NOS variants, through genetic or drug-based means, highlights the indispensable nature of ˙NO in mediating erectile capabilities.[30, 31] Within this scope, ˙NO's regulatory authority primarily hinges on the sGC/cGMP signaling framework.[32]

Concerning spermatozoa, ˙NO is a proactive modulator of numerous molecular pathways pivotal for assorted sperm operations. Such operations encompass sperm motility, survival, hyperactivation, capacitation, acrosome reaction (AR), oocyte fusion, and the subsequent programmed cell death, all pivotal phases for effective fertilization and procreation.[21, 22, 33] While the sGC/cGMP pathway remains recurrent for many of these roles, an essential mechanism merits attention: the tyrosine nitration of distinct sperm proteins. This nitration has dual implications in sperm biology, with research indicating both detrimental.[34, 35] and advantageous[21, 36] outcomes, implying a sophisticated and harmonized regulation. Beyond these roles, ˙NO has been proven to serve a defensive role against oxidative stress in spermatozoa. It offers protection to sperm cells against lipid peroxidation, predominantly by safeguarding protein sulfhydryl clusters, thereby averting potential oxidative membrane damage.[37, 38] Thus, as an integral component of the RNS group, ˙NO wields profound effects on diverse facets of male reproductive phenomena. From its deep-rooted cellular signaling in sperm cells to its cardinal role in erectile dynamics, the ubiquitous nature of ˙NO within male reproductive biology is evident, underscoring the need for deeper research for potential therapeutic prospects.

Sperm motility and viability

RNS, notably ˙NO, are integral to the complex biochemical mechanisms that govern sperm movement and survival. Throughout the intricate physiological journey within the reproductive system, ˙NO's involvement becomes progressively vital, safeguarding the prime functioning of sperm imperative for effective fertilization. Numerous studies have highlighted the crucial modulatory function of ˙NO in dictating sperm motility. Research that pharmacologically adjusted ˙NO concentration reinforced this interrelation.[39, 40] For instance, the application of sodium nitroprusside (SNP), a recognized NO donor, at concentrations between 25-100 nM, beneficially supported the maintenance of post-thaw human sperm activity and survival.[40] Conversely, introducing NG-nitro-L-arginine methyl ester (L-NAME) to the sperm culture medium resulted in a marked decrease in human sperm motility.[41, 42] Such outcomes accentuate the fundamental role of ˙NO in steering sperm kinetics. A noteworthy revelation was the reduced nitrite production in asthenozoospermic individuals compared to normozoospermic ones, emphasizing the crucial nature of intrinsic ˙NO not only in modulating sperm movement but also its fertilization capability.[43, 44] On a molecular level, ˙NO modulates sperm motility by triggering soluble guanylate cyclase (sGC), which in turn activates cGMP-dependent protein kinases. Recent insights reveal the function of ˙NO in amplifying sperm movement through the bolstering of energy generation within sperm mitochondria.[45] Specifically, a 3-hour exposure of spermatozoa in Tyrode's medium led to reductions in sperm movement, mitochondrial membrane potential, ˙NO content, and eNOS expression. Yet, the incorporation of M40403, a superoxide dismutase (SOD) analogue, prominently rejuvenated eNOS expression, raising ˙NO concentrations. The operational state of sperm mitochondria, indicated by the proportion of positive MitoTracker®Green FM sperm cells, increased significantly in sperm samples with enhanced ˙NO concentration. Additionally, a rise in ˙NO concentration in semen samples favorably affected another dimension of sperm mitochondrial functionality. This related to heightened mRNA expression of the nuclear-encoded subunits of both complex I and IV of the Electron Transport Chain (ETC), with subunits dictated by the mitochondrial DNA returning to baseline values. Such findings correspond positively with sperm movement, a chief indicator of semen quality, clarifying that ˙NO improves the sperm's capability to fertilize.[46, 47] These results also unveiled, unprecedentedly, the effectiveness of redox-active compounds in enhancing sperm fertilization potential during sperm extraction and preparation for in vitro fertilization. Beyond movement and energy production, ˙NO is pivotal for upholding standard sperm morphology, an essential metric for the precise

prediction of fertility potential and in vitro fertilization pregnancy outcomes. Thus, the diverse functionalities of ˙NO and RNS in sperm physiology underline their significance in reproductive medicine, emphasizing the imperative for ongoing research in this domain.[48]

Sperm capacitation

Sperm capacitation represents an essential physiological transformation that equips spermatozoa with the capability to fertilize an ovum. This metamorphosis is delineated by a multitude of biophysical and biochemical modifications.[49] Contemporary research has illuminated the pivotal function of RNS, particularly ˙NO, in orchestrating this complex procedure (**Fig. 1)**. When human spermatozoa were exposed to minor concentrations of ˙NO-releasing agents, it elucidated the importance of ˙NO in capacitation.[49] Such experimentation underscored that ˙NO donors augment capacitation, while in contrast, NOS antagonists considerably diminish this process.[50] These outcomes authenticate the essential influence of intrinsic ˙NO in empowering spermatozoa to reach optimal fertilizing capability. Concurrently, this research unveiled a consistent ˙NO-driven modulation of capacitation, regardless of the specific stimulus, whether albumin, progesterone, or fetal cord serum ultrafiltrate.[51, 52]

Examining the molecular details, it was ascertained that ˙NO synthesis in spermatozoa, which commences at the beginning of capacitation, presides over several pivotal biochemical phenomena. Such phenomena encompass the amplification in cyclic AMP (cAMP) concentrations and the comprehensive serine/threonine phosphorylation of protein kinase A (PKA) targets. In addition, ˙NO adjusts the tyrosine phosphorylation of fibrous sheath constituents and concurrently induces tyrosine nitration in assorted proteins.[22] Notably, these ˙NO-driven changes persist throughout the capacitation procedure, emphasizing its essential role in preparing the sperm for efficacious fertilization. Another dimension to the ˙NO-mediated mechanism in capacitation pertains to the genesis of its synthesis. Evidence suggests the involvement of multiple NOS isoforms in capacitation, hinting at a coordinated effort of several NOS variants to regulate ˙NO concentrations and consequently influence the capacitation trajectory.[53] Thus, the revelations regarding the influence of ˙NO, an integral component of the RNS category, have revolutionized our perspective on sperm capacitation. Its role as a chief regulatory entity, directing spermatozoa through requisite biochemical transformations, and its continuous involvement during capacitation, accentuates its vital character.[5] The diverse effects of ˙NO, from influencing cAMP concentrations to managing phosphorylation episodes, firmly establish it as a key molecule in ensuring the reproductive efficacy of spermatozoa. As the molecular dynamics of sperm capacitation continue to be elucidated, the prominence of ˙NO and the larger RNS family is poised to persistently dominate discussions in reproductive biology investigations.[54, 55]

Hyperactivation

RNS encompass a collection of highly active free radicals that originate from nitrogen. In the field of reproductive biology, these molecular entities have attracted substantial consideration, especially in decoding the complex mechanisms that underpin sperm activity.[5] An essential function of sperm is hyperactivation, a distinct and intense motility pattern marked by irregular flagellar oscillations, which is crucial for the ability of the sperm to fertilize.[5] The influence of RNS, particularly ˙NO, in modulating sperm hyperactivation has become a prime focus of contemporary scientific iinvestigation.[7] Traditionally, the association between ˙NO and sperm motility was underscored, with a significant differentiation made depending on the ˙NO concentration. NO exerts bifunctional effects on sperm hyperactivation contingent on its concentration.[41, 56] At concentrations below 1 μM, ˙NO appears to enhance sperm hyperactivation. This might be a result of various molecular processes such as increased intracellular calcium, alteration in adenylate cyclase activity, or possible activation of specific protein kinases. These processes potentially impact axonemal proteins and dynein arms, which dictate the propulsive force and specialized oscillatory patterns of hyperactivated sperm. In contrast, when ˙NO concentrations exceed 1 μM, a decline in hyperactivated sperm motility is documented.[42] Elevated ˙NO levels might induce oxidative stress, potentially causing lipid peroxidation in the sperm membrane and subsequent reduction in membrane fluidity.[57] Such disturbances might negatively impact cellular signaling mechanisms that sustain hyperactivation. Moreover, excessive ˙NO could hinder mitochondrial operations, jeopardizing the ATP generation essential for the energy-demanding hyperactivated movement.[41] It is noteworthy that the influence of ˙NO on sperm hyperactivation is analogous to its effect on standard sperm motility, as highlighted in various research works.[42, 58] This emphasizes the critical equilibrium sperm cells must preserve in their intracellular milieu to function optimally. Clearly, an intricate balance of ˙NO is vital, serving almost as a molecular regulator, either enhancing or suppressing hyperactivated movement based on its concentration. Nonetheless, it's imperative to recognize that while ˙NO is a decisive factor, sperm motility, including hyperactivation, arises from a complex interplay of numerous molecular interactions and signaling pathways.[42, 59] RNS represent just one component in this sophisticated orchestration. Future studies should center on elucidating the wider interactions of RNS with other reactive entities and their collective impacts on the diverse facets of sperm activity. Thus, RNS, especially ˙NO, are crucial in shaping sperm hyperactivation.[42] This revelation not only offers a more profound understanding of the intricate mechanisms of sperm activity but also paves the way for potential therapeutic strategies in addressing male infertility issues.[43, 45, 46]

Acrosome reaction

RNS are integral to a myriad of physiological functions, with a pronounced importance in reproductive biology, especially concerning the acrosome reaction (AR). The AR represents a critical phase during fertilization, wherein the acrosomal membrane of sperm merges with the adjacent plasma membrane, leading to the discharge of enzymes crucial for penetrating the zona pellucida (ZP) and facilitating sperm-egg fusion. Among the RNS group, ˙NO stands out, having been associated with modulating ZP adhesion and the AR. Multiple pieces of evidence reinforce the relevance of ˙NO in this arena.[21] A prominent research finding indicates that enriching the capacitation medium of human sperm with modest amounts of SNP augments the spermatozoa adhering

to hemizona.[60] This hints at direct impact of ˙NO on fortifying sperm-ZP adhesion. Additionally, this enrichment seems to bolster sperm's affinity toward the ZP, underscoring ˙NO's potential role as an enhancer in this mechanism. On the contrary, the introduction of L-NAME, a NO synthesis inhibitor, to capacitated sperm led to a significant decline in the fusion capability.[61] Noteworthily, this impact did not encompass ZP adhesion, implying that while ˙NO is crucial for the fusion mechanism, its involvement in ZP adhesion is more complex.[61] Additionally, tests employing ˙NO-releasing agents have revealed their capacity to trigger the AR in human spermatozoa in a dose-responsive manner.[60] These inductions, when compared with their respective controls, exhibit pronounced distinctions, emphasizing ˙NO's indispensable role in the AR.[48, 50] In contrast, hemoglobin, serving as a ˙NO neutralizer, has demonstrated the capability to hinder the AR in spermatozoa pre-treated with follicular fluid. This observation bolsters the foundational role ˙NO assumes in modulating the acrosome reaction.[62] Thus, the nuanced interactions between RNS, especially ˙NO, and the mechanisms of ZP adhesion and acrosome reaction illuminate the intricate chemical communication inherent in human reproductive processes. Grasping these molecular pathways presents potential therapeutic breakthroughs in reproductive medicine.[48, 63]

Changes in sperm redox status change gene transcription?

Sperm cells, specifically spermatozoa, have been traditionally understood as transcriptionally inactive due to their highly compacted chromatin structure. This has been attributed to the replacement of histones with protamines in mature sperm, which was thought to suppress transcriptional activity. However, recent studies have indicated that approximately 15% of the sperm nuclear material still maintains a connection with histones, which adopt a characteristic nucleosomal configuration.[64] This preservation implies a potential for transcriptional processes in these nucleosomal areas, particularly since they show specific associations with transcription factors and RNA polymerase. Recent studies emphasize the significance of this frequently disregarded nucleosomal section in male reproductive cells. These discoveries signify a reevaluation of sperm cell biology, suggesting these regions might be more critical than previously believed. A pivotal observation was the association between RNS, especially ˙NO, and gene transcription in sperm cells. Studies on this relationship have elucidated the connection between the redox state and gene expression modulation in spermatozoa. Specifically, sperm samples with increased ˙NO levels displayed enhanced mRNA expression of antioxidative enzymes, such as catalase and MnSOD.[46] This implies that ˙NO could enhance the antioxidative response in sperm by promoting the transcription of catalase and MnSOD.[22] Additionally, alterations in the redox equilibrium of spermatozoa seem to have broader consequences for gene transcription. This is supported by the documented upregulation of nucleus-encoded components of both complex I and IV of the Electron Transport Chain (ETC) in sperm samples with heightened ˙NO levels.[62] The relationship between ˙NO and gene transcription modulation emphasizes the interrelation between RNS and the sophisticated redox system in sperm cells, pointing to new areas of exploration in male fertility research.[65]

Pathophysiological roles of RNS on male reproduction

RNS are integral to numerous physiological and pathological mechanisms within the organism. ˙NO, a key constituent of the RNS group, possesses a dual functionality of significant interest, especially concerning male reproductive functions. The interaction of ˙NO with fertility and sperm activity is multifaceted and depends on its concentration, making it a multifunctional entity in reproductive biology. The dualistic action of ˙NO is not limited to reproductive systems but is observed in multiple physiological systems. At physiological, lower concentrations, ˙NO facilitates essential events related to fertilization. Conversely, at augmented levels, its impacts are harmful. This bidirectional effect has led to inconsistencies in scientific findings about ˙NO's exact relationship with fertility and sperm performance.[22, 29]

The theory suggesting ˙NO toxicity proposes that excessive quantities of this compound can inhibit mitochondrial respiration, reduce ATP reserves, and initiate DNA oxidative, nitration, and deamination processes.[37, 66] Research has documented reduced sperm motility in the context of elevated pure ˙NO concentrations and other ˙NO sources like SNP or S-nitroso-N-acetylpenicillamine. Such results are consistent with clinical data showing a relationship between high ˙NO levels in semen and infertility or diminished sperm motility.[37, 47, 66, 67] Additionally, an increase in ˙NO concentration in sperm cells from infertile individuals corresponds with a decline in sperm metabolism.[5, 68] Both sperm viability and structural integrity are negatively influenced by high ˙NO concentrations.[68, 69] For instance, specific SNP concentrations have been shown to significantly decrease sperm viability, while elevated ˙NO levels result in structural anomalies in sperm.[70] Furthermore, the effects of excess ˙NO aren't restricted to sperm. In testicular tissues, increased ˙NO, followed by peroxynitrite formation, curtails testosterone release. Excessive production can also lead to blood vessel vasodilation and extended myofibroblast relaxation, which might obstruct essential sperm transport movements.[59, 71] Within Sertoli cells, surplus ˙NO can modify the patterns of actin-associated proteins crucial for cellular processes like adhesion, proliferation, migration, and differentiation.[72, 73] In the context of penile tissue, elevated ˙NO concentrations, particularly from inducible NOS (iNOS), can instigate pathophysiological alterations.[55]

While the negative implications of augmented ˙NO levels are clear, reproductive mechanisms also display vulnerability to decreased ˙NO concentrations.[74] Vasculogenic erectile disorders, for instance, have been linked to lowered NOS activity, indicating potential erectile issues due to decreased ˙NO availability. Seminal plasma concentrations of ˙NO and 3-nitrotyrosine (3-NT) are critical for sperm performance. Discrepancies in these concentrations in infertile individuals' seminal plasma have been documented, although the exact nature of these variations remains debated.[75,76] Increased 3-NT concentrations in seminal plasma from men with idiopathic infertility hint at the role of intensified oxidative stress in reduced reproductive capacity.[35,77] Some research, such as that conducted by Huang and colleagues[76], suggests that even marginally reduced ˙NO levels in seminal plasma could increase infertility risk, irrespective of statistical significance. The complex role of ˙NO in male reproductive

biology emphasizes its significance in ensuring ideal sperm functionality. Both excessively low and high ˙NO concentrations can negatively impact sperm count, movement, and structure, potentially leading to infertility. This nuanced balance accentuates the urgency for a more comprehensive understanding the role of RNS in reproduction, offering potential avenues for advanced therapeutic strategies aimed at addressing male infertility.[5, 78]

REACTIVE SULFUR SPECIES

RSS is characterized as redox-reactive sulfur-bearing entities capable of either oxidizing or reducing biological macromolecules under standard physiological conditions.[79] Analogous to ROS and RNS, RSS originate intracellularly and have gained recognition as pivotal biomolecular regulators in the biological domain, playing roles in redox-mediated signal transduction by modulating cysteine-based redox alterations in proteins, genomic modulation, ion flux, mitochondrial operations, metabolic activities, among other functions.[65] Notable members of these entities encompass thiyl radicals (RSS), disulfide-S-oxides [RS(O)2SR], and sulfenic acids (RSOH). Additionally, H2S, representing a fully reduced sulfur variant, qualifies as an RSS due to its ability to undergo redox interactions with proteins, influencing their functional activities.[4] The chemical biology inherent to these diverse sulfur redox states governs their characteristics and modus operandi. This discourse places an emphasis on H2S, given its intriguing implications across a spectrum of biological platforms realized through a variety of operational mechanisms.[80, 81] Naturally, H2S formation within mammalian cellular structures results from L-cysteine processing, facilitated by two pyridoxal-50-phosphate reliant enzymes: cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). Beyond L-cysteine, CBS has the capability to utilize homocysteine for cystathionine synthesis, which subsequently, under CSE's influence, gives rise to H2S. Another H2S biosynthesis pathway involves the pyridoxal-50-phosphate independent enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST), in conjunction with cysteine aminotransferase.[81] Intrinsic H2S formation also occurs through non-catalytic routes and via elemental sulfur derivatives, such as thiosulfate and thiocysteine, which can be reduced to H2S within the glycolytic cascade.[79] Post-biosynthesis, H2S partakes in reactions with an assortment of biological entities like ROS/RNS, cysteine-related compounds, and metalloproteins.[80, 81] Such interactions elucidate the biological roles attributed to H_2S.

Synthesis of RSS in male reproductive system

Hydrogen sulfide (H2S) within the reproductive system has historically been recognized for its cytotoxic effects and negative correlations with fecundity.[82] Yet, recent investigations indicate that H2S is intrinsically synthesized and plays a pivotal role in orchestrating a multitude of reproductive functions across both sexes.[83, 84] The enzymes responsible for H2S biosynthesis have been identified in diverse sections of the male reproductive anatomy. In human testicular tissues, immunohistochemical analyses have demarcated a specific locational distribution of two key H2S biosynthetic enzymes: CBS is localized in Leydig, Sertoli, and germinal cells, whereas CSE is predominantly observed in Sertoli cells and progenitor germinal cells like spermatogonia.[83] This particular spatial segregation of CSE and CBS is similarly discerned in human penile tissues. To elucidate further, both CBS and CSE are present within the trabecular muscular structures. Additionally, CSE is distinctly present in the vascular smooth muscle cells and peripheral neural structures, with their enzymatic activity being manifested in homogenates derived from penile tissue.[85]

Physiological Signaling of RSS in Male Reproductive Events

Reactive sulfur species (RSS) are integral to many physiological processes, including those related to the male reproductive system. Hydrogen sulfide (H_2S) stands out as a crucial RSS in regulating male reproductive activities. The physiological significance of H_2S in male reproductive events includes its roles in erectile function, spermatogenesis, and sperm motility, underlining its importance in male fertility. The association between H_2S and the male reproductive system was initially evidenced through studies using sodium hydrosulfide (NaHS), an H_2S donor. In primates, NaHS demonstrated the beneficial impact of H_2S on erectile functionality.[86] Subsequent research further corroborated this, showing that exogenous application of H_2S to human corpus cavernosal tissues improved erectile response.[87] Conversely, when the internal generation of H_2S was obstructed using CSE and CBS inhibitors, a significant reduction in erectile functionality was observed. This highlights the essential role of internally generated H_2S in erectile function and suggests potential therapeutic applications of H_2S modulation for addressing erectile dysfunction in males.[87] However, the influence of H_2S is not limited to erectile functionality. Research led by Wang et al. highlighted its pivotal role in testicular function, especially spermatogenesis.[88] Conditions inducing stress, which resulted in decreased endogenous H_2S production and CBS expression in testes, led to hindered spermatogenesis and a disrupted blood-testicular barrier. Remarkably, introducing H_2S exogenously displayed therapeutic properties. H_2S provided protection against lipopolysaccharide-induced testicular abnormalities and rectified spermatogenic deficiencies. The primary mechanisms for these protective actions were attributed to anti-inflammatory and antioxidative properties of H_2S.[88-93]

At the molecular level, the transcription factors nuclear factor-kappa B (NF-κB) and nuclear factor erythroid 2 related factor 2 (Nrf2) play a decisive role in governing function of H_2S in reproduction.[88] Along with the mitogen-activated protein kinase (MAPK) signaling cascade, these transcription factors form the regulatory framework that underlies the protective influence of H_2S on male reproductive functions. Additionally, H_2S is essential for sperm motility and overall reproductive capability. Lower levels of H_2S in seminal plasma were observed in individuals with subfertility and infertility in comparison to fertile individuals.[88] This deficiency was not merely a chemical imbalance but had physiological manifestations as diminished CBS protein expression in sperm cells of asthenospermic and oligoasthenozoospermic subjects. Given the characteristic reduced sperm motility in these subjects, this highlights the vital role of H_2S in maintaining the fertilization capacity of sperm.[88] Supporting this notion was a study by Ikeda et al., which established a positive relationship between polysulfide concentrations in seminal fluid and sperm motility[94], suggesting the potential influence of polysulfide redox activity on

sperm function. Thus, hydrogen sulfide, an RSS, has been recognized as a key molecule in various facets of male reproductive health, ranging from erectile function, spermatogenesis, to sperm motility. Its interplay with different transcription factors and signaling cascades offers a deeper insight into its intricate role in male reproductive physiology. Continued exploration in this field promises to shed more light on its potential and diverse therapeutic applications.

Pathophysiological Significance of RSS in Reproductive Functions of Males

RSS, predominantly characterized by hydrogen sulfide (H_2S), have been identified as pivotal elements in male reproductive biology, drawing parallels to well-known reactive oxygen species (ROS) and RNS. Nonetheless, the precise equilibrium in the levels of these molecules, particularly H_2S, determines whether they play a beneficial or detrimental role in male reproductive health. Once viewed solely as a hazardous gas, H_2S was initially linked to harmful effects across multiple organ systems, eclipsing its possible advantageous impacts.[95] Current scientific interpretations, however, reveal that similar to ROS and RNS, the bifurcated outcomes of RSS (specifically H_2S) are contingent on concentration. Excessive H_2S concentrations, notably those beyond physiological thresholds, can be injurious to sperm functions.[88] For instance, interventions using sodium hydrosulfide (NaHS) have been found to markedly reduce sperm motility and exert cytotoxic effects on human sperm. Likewise, sodium sulfide (Na_2S), another H_2S donor, also inhibited sperm movement in both in vitro and in vivo studies.[96] At the core of this detrimental effect is the release of the gasotransmitter by certain H_2S donors in amounts exceeding physiological levels, which has negative repercussions for sperm functionality. This toxic action primarily stems from the obstruction of mitochondrial complex IV, leading to the halt of mitochondrial electron transport and the synthesis of sperm ATP.[97] As a result, the energy required for sperm motion becomes scarce, invariably compromising sperm motility and potentially leading to infertility.[46, 98]

On a molecular level, in vitro studies using boar sperm revealed that H_2S interferes with multiple signaling cascades, subsequently compromising sperm movement.[96] Key molecular impacts include decreased ATP synthesis, activation of AMP-activated protein kinase (AMPK) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and an increase in ROS concentrations. Elevated ROS levels might activate PTEN, which in turn might inhibit protein kinase B. The combined effects of ATP shortage and protein kinase B suppression activate AMPK, resulting in reduced sperm movement and, by extension, decreased sperm quality. Confirming these results, in vivo experiments using mouse sperm mirrored the in vitro findings.[96] On the other hand, a lack of H_2S in reproductive systems has also been associated with male infertility. A striking observation found that the CBS protein concentrations in the sperm of asthenospermic patients were notably lower than in normospermic counterparts, highlighting the crucial function of H_2S in maintaining sperm motility.[88] Additionally, an H_2S shortage in human plasma and corpus cavernosum samples was linked to a significant intensification of erectile dysfunction.[99] A decline in testosterone due to aging was concurrently linked to a drop in H_2S production and concentrations in penile tissues, aligning with the symptomatic occurrences in erectile dysfunction.[99] Even with the knowledge acquired regarding the diverse roles of H_2S across both physiological and pathological domains, the holistic mechanism of action of H_2S remains elusive. As researchers continue to probe the nuances of H_2S in male reproductive physiology, it is crucial to recognize the delicate balance between its advantageous and disadvantageous effects, depending on its concentration and the specific scenario.[100,101]

CONCLUSION

The complex interaction between RNS and RSS in male reproductive biology is a fascinating field of study. RNS and RSS play dual roles in both maintaining physiological processes and instigating pathological disturbances, highlighting their importance. Under normal conditions, they regulate key aspects of male fertility, such as sperm movement, capacitation, and the acrosome response. Their involvement in redox signaling serves as a critical regulator, ensuring cellular equilibrium and functionality. Conversely, an overabundance or imbalance can induce oxidative stress, which is linked to male infertility. Excessive levels of RNS and RSS can cause DNA lesions, lipid oxidation, and protein alterations, ultimately degrading sperm health and performance. Furthermore, new insights into the interaction between RNS and RSS shed light on the intertwined nature of redox pathways in the male reproductive system. Discrepancies in this interaction could heighten or predispose the adverse effects on male reproductive capabilities, underscoring the importance of interventions aimed at these reactive species. Future research will greatly benefit from a detailed examination of the molecular pathways influenced by these reactive species and potential measures to regulate their concentrations. As our comprehension of RNS and RSS expands, recognizing their dual functions becomes vital. This knowledge allows us to leverage their physiological advantages while counteracting their pathological threats, presenting potential solutions to male reproductive issues.

Conflict of Interest

The authors declare that none of them has any conflict of interest.

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