Journal of Integrated
SCIENCE & TECHNOLOGY

Oxidants and antioxidants in male reproduction: Roles of oxidative and reductive stress

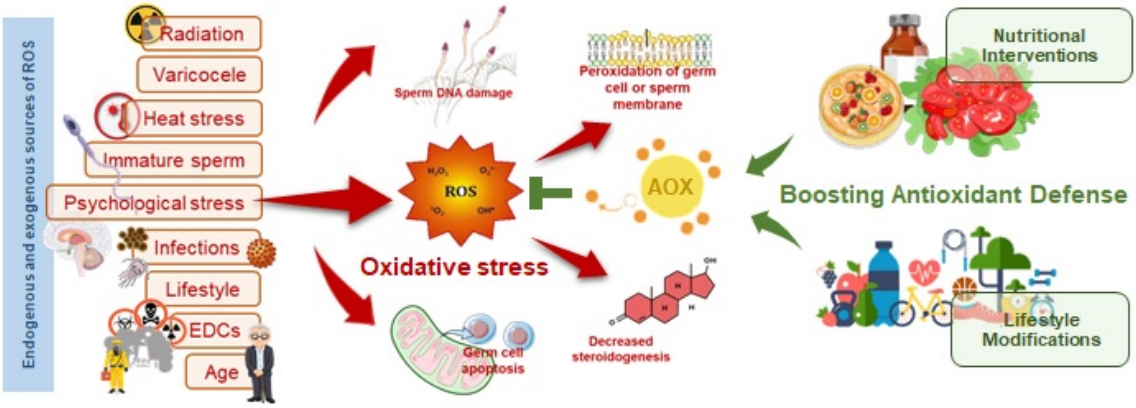
Pallav Sengupta[1*], Sulagna Dutta[2], Tulay Irez[3*]

[1]Department of Biomedical Sciences, College of Medicine, Gulf Medical University, Ajman, UAE. [2]School of Life Sciences, Manipal Academy of Higher Education (MAHE), Dubai 345050, UAE [3]Department of Clinical Embryology, Health Sciences Institute, Yeni Yuzil University, Istanbul, Turkey.

Received on: 18-Jul-2023, Accepted and Published on: 04-Nov-2023

Review

ABSTRACT



Oxidative and reductive imbalances critically impact male reproduction, necessitating a balance between pro-oxidants and antioxidants for sperm health. This paper elucidates the roles of reactive oxygen species (ROS) in male fertility. Moderate ROS levels aid physiological events like sperm capacitation and acrosomal reaction, while excessive ROS leads to oxidative stress (OS), impairing sperm quality and DNA integrity. Intrinsic antioxidant mechanisms in the male reproductive tract, including enzymes like superoxide dismutase (SOD), catalase, and glutathione, are paramount in neutralizing excess ROS, consequently shielding sperm from oxidative harm. External factors, individual lifestyle decisions, and particular medical conditions can offset the pro-oxidant-antioxidant equilibrium, leading to heightened OS and reduced male fertility potential. On the other side, reductive stress, albeit less examined, is also significant in male reproductive outcomes. Overabundance of antioxidants can create a reductive environment that adversely affects sperm functionality. This article accentuates the significance of preserving this balance for protecting male reproductive health and underlines potential modalities for addressing imbalances in oxidative-reductive stability.

Keywords: antioxidants; male infertility; reactive oxygen species; oxidative stress; varicocele

INTRODUCTION

Male reproductive processes, from spermatogenesis to fertilization, are tightly regulated, critical for species perpetuation. A crucial, yet sometimes overlooked element of these processes is the balance between oxidative and reductive stresses, governed by the interplay of oxidants and antioxidants. Maintaining this equilibrium is vital; an overabundance of reactive oxygen species (ROS) and other oxidizing agents can compromise cellular structures and molecules, jeopardizing reproductive capabilities.[1-5] Conversely, antioxidants, the inherent defense system of nature, counteract these oxidants, maintaining a suitable reproductive milieu.[6]

There is a growing understanding that oxidative stress (OS), stemming from a disproportion between oxidants and antioxidants, is central to male infertility. High ROS levels can cause sperm DNA fragmentation, lipid peroxidation, and mitochondrial anomalies, thereby reducing sperm motility, density, and general quality.[1-5] Though a foundational ROS level is indispensable for functions like sperm capacitation, hyperactivation, and the acrosome reaction, an overabundance is harmful.[7]

*Corresponding Author: Prof Dr Tulay Irez, Dr Pallav Sengupta
Email: ireztulay@yahoo.com (TI), pallav_cu@yahoo.com

Cite as: *J. Integr. Sci. Technol., 2024, 12(3), 753.*
URN:NBN:sciencein.jist.2024.v12.753

 ISSN: 2321-4635
http://pubs.thesciencein.org/jist

Both enzymatic and non-enzymatic antioxidants serve protective functions. Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, shield against the injurious effects of ROS. In contrast, non-enzymatic antioxidants like vitamins C and E, glutathione, and carotenoids offer additional protection.[8] This review aims to shed light on the intricate roles of oxidants and antioxidants in male reproductive systems. It examines the genesis and consequences of OS, the physiological significance of ROS, and antioxidants' protective actions. Through a holistic examination of oxidative and reductive stress in male reproductive physiology, this article establishes a foundation for enhanced comprehension and potential treatment strategies for male infertility.

Basic concepts: oxidants and antioxidants

Oxidizing agents, in general terms, are molecular or ionic entities with the capacity to receive electrons from other chemical species during reactions. In a biological setting, when these oxidizing agents incorporate oxygen, they are identified as ROS, which represent a collection of molecules containing oxygen exhibiting elevated reactivity due to their unpaired electrons. Prominent examples of ROS are the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$).[9]

During various cellular metabolic activities, notably within the mitochondria during the electron transport chain involved in ATP synthesis, ROS are incidentally produced. Historically, these compounds were perceived negatively due to their ability to induce oxidative damage to DNA, proteins, and lipids. However, they also serve crucial roles as regulatory signaling entities in multiple physiological functions.[7] Molecules termed antioxidants possess the ability to quench ROS by donating electrons, thereby circumventing or rectifying the potential damage inflicted by these reactive agents. These antioxidants function as protective agents against OS and help uphold the cellular redox equilibrium. They can be broadly classified into enzymatic and non-enzymatic categories based on their operational mechanisms.[6,8]

Within the realm of enzymatic antioxidants, SOD is pivotal. SOD facilitates the conversion of the superoxide anion into hydrogen peroxide and diatomic oxygen. Catalase is another instrumental antioxidant enzyme, proficient in rapidly converting hydrogen peroxide to water and diatomic oxygen, precluding the genesis of the extremely reactive hydroxyl radical.[8]

The homeostasis between oxidizing agents and antioxidants is imperative for retaining cell functionality and structural integrity, often referred to as the redox balance.[10] A deviation in this balance, whether favoring oxidizing agents or antioxidants, may result in detrimental cellular outcomes.[10]

As already mentioned, OS emerges under conditions of excessive ROS production or a weakening in antioxidant mechanisms. Such scenarios can foster oxidative alterations in cellular constituents, undermining their operational capacity and playing a role in the onset of various pathologies, including fertility disorders.[11]

On the other hand, reductive stress manifests from an over-amplification of reductive elements compared to oxidizing agents. Although reductive stress has received less investigative attention compared to OS, it can be equally adverse, interrupting cellular communication pathways and compromising protein functionality.[12]

Thus, the equilibrium between oxidizing agents and antioxidants is pivotal in cellular physiological processes. Disturbances in this balance, manifesting as either oxidative or reductive stress, can compromise cell operations, accentuating the significance of a well-regulated redox balance for cellular vitality and operations.[13]

SOURCES OF OXIDANTS IN MALE REPRODUCTIVE SYSTEM

Endogenous sources

Within the male reproductive system, the role of OS in male infertility is evident as ROS can instigate damage to spermatic DNA, lipids, and proteins. Multiple inherent sources foster the synthesis of oxidants in the male reproductive system, and recognizing these can facilitate the development of specific therapeutic strategies.[11]

Mitochondrial dysfunction

The mitochondria, commonly designated as the cell's energy hub, are central to energy generation through oxidative phosphorylation. In this mechanism, electrons traverse an array of protein assemblies (the electron transport chain) culminating in their interaction with oxygen, yielding water.[14] Occasionally, these electrons can prematurely associate with molecular oxygen, leading to the synthesis of superoxide, a variant of ROS. Although this is predominantly an effective process, occasional electron seepage transpires, culminating in ROS synthesis.[15] Within the male reproductive system, especially in sperm cells, mitochondria are profusely present in the midsection, providing the essential energy for spermatic motility. Nonetheless, any mitochondrial aberrations, resulting from genetic anomalies or exogenous determinants, can augment ROS production. This amplified ROS synthesis can inflict oxidative harm on sperm, undermining its structural and functional integrity. Sperm manifesting dysfunctional mitochondria often exhibit diminished motility, which is paramount for fertilization competence.[15]

NADPH oxidases

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases represent another salient ROS origin within the male reproductive system. These enzymatic complexes channel electrons from NADPH to molecular oxygen, engendering superoxide among other ROS types.[16] They are implicated in a plethora of cellular operations, spanning cell proliferation, differentiation, and innate immune responses. Pertaining to the male reproductive domain, NADPH oxidases, notably NOX5, find expression in both the testes and sperm cells.[17] The activation of these oxidases, elicited by diverse physiological triggers, can culminate in ROS synthesis. While a modicum of ROS is indispensable for certain spermatic functions like capacitation and the acrosome response, excessive ROS levels stemming from NADPH oxidase overactivity can inflict oxidative harm on sperm.[18, 19] This observation is underscored by the fact that spermatic membranes are replete with polyunsaturated fatty acids, which are intrinsically vulnerable to oxidative challenges.[19]

Other metabolic processes

In addition to mitochondria and NADPH oxidases, numerous metabolic pathways in the male reproductive system are

responsible for the intrinsic generation of oxidants.[20] These include: *Xanthine Oxidase:* Engaged in purine catabolism, this enzyme catalyzes reactions that produce ROS. It is detected in several sections of the male reproductive tract, notably the epididymis.[21, 22] *Cytochrome P450 Enzymes:* Located within the endoplasmic reticulum of cells, these enzymes play a role in steroidogenesis within Leydig and Sertoli cells in the testis. They have the potential to produce ROS during their metabolic actions.[23]

Lipoxygenases and Cyclooxygenases: These enzymes are implicated in arachidonic acid metabolism, which is an integral part of sperm cell membranes. Their metabolic pathways may give rise to both ROS and reactive nitrogen species (RNS).[24]

Recognizing the inherent sources of oxidants, encompassing mitochondrial anomalies, NADPH oxidases, and additional metabolic activities, offers valuable insights into the origins of male reproductive complications and paves the way for potential treatment approaches.[25]

Immature sperm

During the process of spermatogenesis, sperm cells are produced, which might carry extra cytoplasm from the germinal epithelium. In this location, sperm often remain in an immature and non-functional state. These immature sperm can be identified by the presence of cytoplasmic remnants in their mid-piece. Within this cytoplasmic region of the mid-piece, there is an enzyme called glucose-6-phosphate dehydrogenase (G6PD). This enzyme facilitates the production of intracellular β-NADPH through a pathway known as the hexose monophosphate shunt. Additionally, NADPH oxidase, located in the sperm membrane, powers the production of ROS.[26, 27] The mid-piece of the sperm also contains a significant number of mitochondria, which act as an energy reservoir for sperm motility. Within the mitochondrial respiratory chain, there is the diaphorase enzyme, which ensures a balance between the reduced and oxidized states of NADH, playing a critical role in maintaining sperm's energy equilibrium. This enzyme, belonging to the oxidoreductase family, produces superoxide anions, affecting ROS levels.[28] Elevated ROS can impair mitochondrial function and compromise the integrity of the mitochondrial membrane. Such damage amplifies ROS production. In immature sperm, the primary sites for ROS production are the mitochondrial and cell membranes.[29]

Leucocytes

Ejaculate naturally comprises a limited quantity of leucocytes. The condition where the white blood cell count exceeds one million per milliliter of semen is termed leukocytospermia.[30] Semen from individuals with leukocytospermia exhibits elevated levels of ROS, leading to sperm DNA damage.[31, 32] When there is inflammation or irritation in the reproductive system, an elevated concentration of leucocytes is observed in the seminal plasma. Peroxidase-positive leukocytes, by augmenting the production of NADPH via the hexose monophosphate shunt, can generate ROS at levels 1000 times greater than immature sperm cells.[33] There is evidence suggesting that infertile males generate more ROS compared to their fertile counterparts, establishing a connection between OS and heightened leucocyte concentrations.[34] The body's response to inflammation or infection is an increased production of leucocytes or WBCs, which are capable of producing ROS at rates up to 100 times greater than non-active cells.[32, 35-37] The activation of the myeloperoxidase (MPO) system in polymorphonuclear (PMN) leukocytes and macrophages induces a respiratory burst, leading to an amplified ROS production. Therefore, for infertile males, it is clinically imperative to evaluate potential inflammation or infection, given the potential for severe health complications arising from OS.[38]

EXOGENOUS SOURCES

Smoking

Cigarette smoking is a predominant factor in male infertility.[39] Cigarettes contain deleterious compounds like nicotine, which can interfere with hormone regulation, consequently affecting semen parameters.[40, 41] The presence of ROS in the testes due to smoking may detrimentally affect sperm DNA. Cigarette emissions are comprised of various toxins, carcinogens, and mutagenic agents, along with both stable and transient free radicals and ROS. These compounds foster the production of superoxide anions and H2O2, leading to oxidative damage on cellular lipid membranes, proteins, enzymes, and DNA, which contributes to male infertility.[42] Creatine kinase (CK) is an enzyme in sperm crucial for rapid ATP storage and regeneration and is vital for sperm mobility. Smoking decreases CK activity.[43] Moreover, ROS-induced mitochondrial DNA damage diminishes ATP synthesis and available energy, impairing sperm movement.[44] Smoking also modifies several semen attributes, including its quality, acrosin activity, and protein phosphorylation, while disturbing micro-RNA expressions and the histone-to-protamine transformation, all of which contribute to male infertility.[45] Even indirect exposure to tobacco smoke can lead to DNA aberrations and altered methylation patterns due to elevated ROS concentrations in tissues, highlighting an ancillary risk of smoking.[46]

Alcohol consumption

Exorbitant alcohol consumption adversely affects both sperm volume and viability. Studies indicate an inverse correlation between alcohol intake and sperm metrics, such as motility, concentration, and morphology.[47] Alcohol compromises sperm mobility, nuclear maturation, and DNA integrity by promoting sperm chromatin disintegration via apoptotic pathways.[29] Post ethanol ingestion, increased metabolic activity in the liver elevates ROS output, leading to mitochondrial alterations and diminished ATP synthesis.[48] Alcohol heightens cytochrome P450 enzyme (CYP2E) activity, amplifying NADPH oxidase, subsequently altering the physiological balance of metals like Cu2+ and Fe3+. This causes an upsurge in superoxide anion production.[48] In habitual alcohol consumers, nitric oxide (NO) generation also escalates due to inducible nitric oxide synthase (iNOS).[49] Agents causing mitochondrial dysfunction include NO and its derivative, peroxynitrite (ONOO-).[49, 50]

Radiation

Radiation is another exogenous contributor to ROS that impairs male fertility. The biological repercussions of radiation vary based on the radiation type, energy emitted, and exposure duration.[51] Both ionizing and non-ionizing radiation critically hinder sperm genesis. Thermal, radioactive, RF, and other detrimental radiations considerably affect male reproductive health.[52] The prevalent use

of devices like mobile phones, computers, and microwave ovens in contemporary times emit electromagnetic radiation, raising concerns over its implications for male fertility. Such radiation, by promoting OS, instigates a multitude of reproductive alterations. Specifically, mobile phone emissions have been correlated with a decrease in spermatogenic cells, sperm membrane modifications, elevated ROS, and lipid peroxidation, as well as reductions in sperm volume and structure.[53] Cellular phone radiation may harm plasma membranes and stimulate plasma membrane NADH oxidase, which plays a role in various detrimental cellular outcomes, inclusive of OS.[54] Electromagnetic radiation impairs mitochondrial DNA, disrupting the electron transport chain (ETC) and subsequently causing OS.[55] Even minor ROS fluctuations can influence sperm capacitation, acrosome response, and fertilization. Radiofrequency-induced OS can profoundly harm sperm cells.[52] Moreover, a 35-day exposure to microwave radiation for two hours daily has been linked to oxidative changes.[56] Radio frequency radiation also elevates OS, negatively impacting male fertility by decreasing glutathione concentrations and impairing sperm membrane integrity.[57]

Environmental factors

Genital heat stress is one of the most prominent sources of ROS. Long-term exposure to heat radiation causes scrotal hyperthermia, which raises ROS production substantially.[58] Heat stress has an impact on spermatogenesis, spermatozoa motility, sperm concentration, and sperm viability.[59] Subsequent increase in ROS caused by heat stress may cause over-expression of caspase 3 which leads to apoptosis in several cell types, including Sertoli and Leydig cells.[60] Pollution, which includes phthalate-like compounds, air pollution, and heavy metals, is another important source of ROS in the environment. Phthalates are synthetic compounds found in personal care products, plastics, and food packaging materials, among other places. Increased ROS generation, a lack of testicular antioxidants, and a reduction in hormone levels are all likely effects of phthalate exposure.[61] Phthalates can promote LPO, which can raise the level of OS in the testis, resulting in mitochondrial malfunction and sperm function reduction.[62] Air pollution can elevate OS by generating free radicals though damage of sperm lipid membrane, thereby affecting sperm parameters.[63] In addition, heavy metals such as cadmium and lead are considered another source of ROS which may cause testicular OS and subsequently damaging sperm DNA and reduction of sperm parameters (**Figure 1**).[64, 65]

IMPLICATIONS OF OXIDATIVE STRESS ON MALE REPRODUCTION

DNA Damage

Elevated production of ROS coupled with diminished antioxidant levels in sperm lead to sperm DNA fragmentation (SDF).[66] OS has the potential to harm sperm DNA both directly and through the actions of enzymes like sperm caspase and endonuclease. Predominantly, SDF arises from DNA damage during the phase of spermiogenesis due to errors in chromatin compaction. This results in the failure to appropriately transition chromatin structures from histone to protamine. Such damage largely stems from ROS exposure, either during the spermiation process or as sperm cells transition from the seminiferous tubules to the cauda epididymis through the rete testes. This interaction leads to the formation of compounds such as 8-OH-guanine and 8-OH-2'-deoxyguanosine (8-OHdG). Elevated levels of 8-OHdG are intrinsically associated with DNA fragmentation and breaks in DNA strands.[67] Given the dual-stranded nature of DNA, it can experience fragmentation in both single-stranded (ss-) and double-stranded (ds-) configurations.[68, 69] The potential for DNA repair is restricted to specific stages of spermiogenesis, and these repair pathways are not active during the phase of nuclear condensation in the epididymis. Even though the ability to repair SDF declines with advanced maternal age, human oocytes possess the capability to fix ss-DNA breaks, a pivotal process in embryonic development. In contrast, ds-DNA fragmentation, if left unrepaired, can lead to genomic instability and initiate apoptosis. Such adverse effects of SDF plays a role in embryonic development and the eventual outcome of the pregnancy, a phenomenon termed 'late paternal effects'.[70] The prominent phase of embryonic genome expression activation is initiated on the second day of embryonic development (at the 4-cell stage). Consequently, embryogenesis transitions from being primarily dependent on maternal factors to being driven by the embryo's genome. Therefore, sperm cells that exhibit SDF adversely affect post-fertilization events such as blastulation, implantation, and the success rate of pregnancies. Additionally, OS influences embryonic cleavage, referred to as the ‘early paternal effect’.[71] There's an inverse relationship between SDF and successful pregnancy outcomes, but a direct correlation between SDF and miscarriage rates. Given that SDF has been implicated in recurrent pregnancy loss, it is vital to have rigorous monitoring and intervention strategies to minimize associated risks.[66]

Lipid Peroxidation

Sperm cells are highly susceptible to OS due to the high concentration of polyunsaturated fatty acids (PUFAs) with multiple double bonds in their plasma membranes and the relatively low levels of antioxidant enzymes in their cytoplasm. Elevated ROS levels can initiate lipid peroxidation (LPO) in sperm, impairing their function.[55] LPO disrupts the structure and functionality of sperm lipid membranes, leading to increased ROS production.[55] A prominent initiator of LPO is the hydroxyl radical (·OH). The sperm membrane lipids are predominantly unsaturated and have non-conjugated double bonds interspersed with methylene groups. This configuration makes the methylene hydrogen-carbon bonds vulnerable, facilitating hydrogen detachment. The resultant free radicals undergo rearrangements that stabilize them, producing a radical featuring two double bonds separated by a single bond. The abundance of these double bonds makes the lipids highly prone to peroxidation. Lipid peroxyl radicals interact with conjugated radicals, stripping hydrogen from other lipids and forming lipid hydroperoxides.[72] These ROS-mediated events can lead to oxidation of sulfhydryl groups, decreased axonal protein phosphorylation, and diminished sperm motility. Hydrogen peroxide, a ROS type, can permeate the sperm membrane and inhibit specific enzymes, such as glucose-6-phosphate dehydrogenase (G6PD), which governs the entry of glucose into the pentose phosphate pathway. This pathway ordinarily produces NADPH for cellular reductive reactions, but its inhibition leads to

decreased NADPH synthesis in sperm. Glutathione peroxidase, an essential antioxidant enzyme in sperm, utilizes reduced glutathione to neutralize ROS, converting it into oxidized glutathione. Since the regeneration of reduced glutathione requires NADPH, a decrease in NADPH due to G6PD inhibition compromises the antioxidant defense of glutathione peroxidase. Consequently, there's an uptick in phospholipid peroxidation, compromising membrane fluidity and sperm motility. By-product of lipid peroxidation, malondialdehyde (MDA), serves as an indicator of oxidative damage to sperm in various biochemical assays.[55] Furthermore, electron loss from sperm's plasma membrane lipids, induced by ROS, can initiate LPO, yielding highly mutagenic and genotoxic aldehydes, including MDA, 4-hydroxynonenal, and acrolein.[73] Elevated ROS levels can also compromise the integrity of mitochondrial membranes, leading to caspase activation and apoptosis. During this programmed cell death, there's continuous production of cytochrome-c, which escalates ROS levels, potentially intensifying DNA damage and accelerating the apoptotic process.[2] Thus, the sperm plasma membrane emerges as a primary site of ROS attack, which can potentially compromise its genetic integrity via cascade signaling mechanisms (**Figure 1**).

Protein Oxidation

Protein peroxidation involves the oxidative alteration of proteins, influenced by ROS and subsequent reactive carbonyl species. Within the domain of spermatozoa, protein peroxidation profoundly affects sperm functionality, structural soundness, and its inherent ability to engage with and fertilize an ovum.[74] The seminal enzymatic functions and the sperm structural proteins are principally affected by this oxidative process.[75] The enzyme complement in seminal fluid is instrumental in priming the spermatozoa for fertilization events.[76] Enzymes such as hyaluronidase facilitate the dispersion of the cumulus oophorus, while acrosin is essential for the penetration of sperm through the zona pellucida of the oocyte.[76] Oxidative modifications due to peroxidation can significantly transform the enzyme structures, leading to changes in their catalytic properties.[77] Excessive ROS can alter the amino acid structures in these enzymes, potentially deactivating them or diminishing their activity.[78] Specifically, oxidation of thiol groups within cysteine residues, which are integral for many enzymes' catalytic processes, results in the formation of disulfide linkages, modifying the enzyme's active conformation. Such changes undermine the sperm's enzymatic arsenal, reducing its capability to interact with and fertilize the ovum.[77]

The structural proteins provide the scaffolding and movement apparatus for spermatozoa. Oxidative changes due to protein peroxidation can compromise these proteins, primarily impacting the sperm flagellum and its head. The axoneme, an intrinsic structure of the flagellum, is vulnerable to oxidative alterations.[7, 77] Oxidative damage can impede the axonemal motor proteins and dynein components, hindering sperm locomotion. In a similar vein, the acrosomal membrane, which envelops the sperm head, houses proteins vital for the recognition and fusion processes between sperm and egg. Oxidative alterations of these proteins can hinder the ability of the sperm to attach to or penetrate the oocyte.[79]

Consequently, OS-induced protein peroxidation in spermatozoa has detrimental effects on their fertilization capability. The combined impact on both seminal enzymes and structural proteins underscores the importance of preserving the redox equilibrium in the male reproductive system to retain sperm functionality and structural wholeness.[7]

Apoptosis

Apoptosis can be characterized as a non-inflammatory cellular response characterized by specific morphological and biochemical alterations associated with tissue injuries.[27] This process is crucial in eliminating defective sperm, thereby maintaining the supportive capabilities of Sertoli cells.[80] Elevated levels of ROS compromise the integrity of both the inner and outer mitochondrial membranes. This leads to the release of cytochrome-c and activation of caspases, culminating in apoptosis. Mechanisms independent of ROS can also induce apoptosis in sperm. For instance, the cell surface protein, Fas, can trigger apoptosis.[81] Fas is a membrane protein associated with the tumor necrosis factor-nerve growth factor receptor family.[82] Binding of the Fas ligand or an agonistic anti-Fas to Fas initiates apoptosis.[83] Conversely, Bcl-2 acts as an anti-apoptotic gene and offers protection to the cell through ROS production.[84] While the Fas protein induces apoptosis in many cells, some cells labeled with Fas may evade this process due to abortive apoptosis.[85] Given that not all sperm are ejaculated, the prevalence of defective sperm in semen might rise. The persistence of Fas-positive spermatozoa could stem from

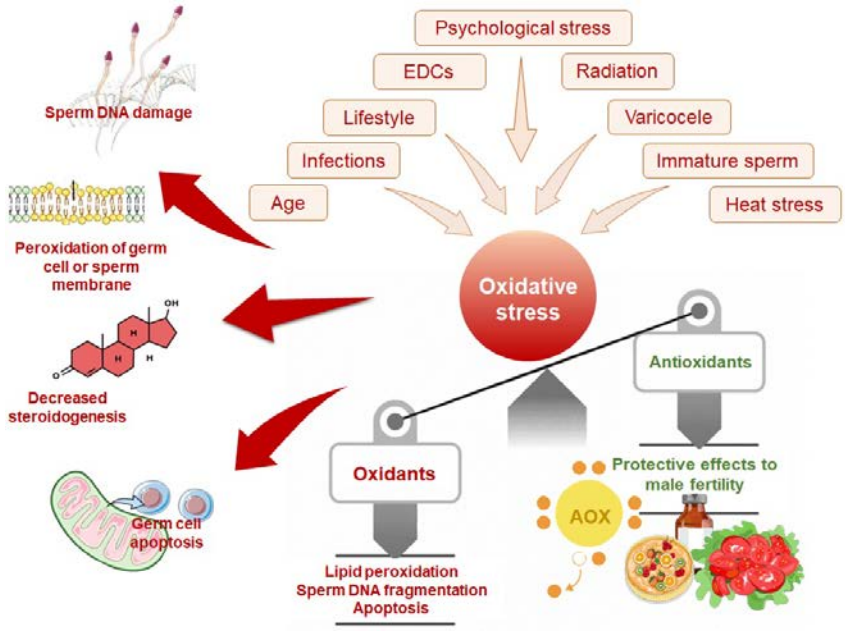


Figure 1. Various causes of generation of oxidative stress and their impacts on male fertility. Endogenous and exogenous antioxidants protect male reproductive system from oxidative damages.

disruptions at various phases. Firstly, in men with hypospermatogenesis, the production of sperm might not reach the threshold required to activate apoptosis, allowing them to bypass the apoptotic cue. Secondly, challenges in initiating Fas-mediated apoptosis can result in the presence of Fas-positive spermatozoa. In such cases, apoptosis is halted, and spermatozoa marked for apoptosis remain uncleared.[85] Upon ROS exposure to mitochondria, the apoptosis-inducing factor (AIF) is generated, which directly binds to DNA, leading to DNA fragmentation.[86]

Impact on Sperm Motility and Function

The sperm cell, specifically its tail (axoneme) and head (acrosome), is a critical cellular structure susceptible to oxidative damage from free radicals.[87] The axoneme, responsible for sperm motility, is chiefly made up of microtubules. These microtubules are at risk from damage by ROS.[87] When ROS concentrations rise, it can result in lipid peroxidation of the membrane enveloping the axoneme, leading to a compromise in its structural soundness. Such disturbances can modify the axonemal functionality, rendering sperm cells less mobile or completely immobile.[88] This reduction in mobility hampers the sperm's journey through the female reproductive system to the oocyte, thereby affecting fertility potential.[88]

Furthermore, the acrosome reaction is modulated by OS. Positioned on the anterior region of spermatozoa, the acrosome is a vesicular structure housing enzymes crucial for breaching the protective barriers of the oocyte. A regulated acrosome reaction is vital for successful fertilization.[89] However, an overabundance of free radicals might either prematurely trigger acrosome reactions or inhibit them entirely.[79] Both situations prevent the sperm from effectively adhering to and penetrating the oocyte. Therefore, while free radicals are integral to certain cellular processes, an imbalance resulting in OS can have severe consequences on sperm functionality. The negative effects on the axoneme and the acrosome reaction can directly diminish male reproductive capacity. This highlights the importance of sustaining an oxidative equilibrium for peak reproductive health.[90]

ROLE OF ANTIOXIDANTS IN MALE REPRODUCTION

Endogenous Antioxidants

Role of enzyatic antioxidants

The male reproductive system is notably susceptible to OS, particularly during spermatogenesis. As already discussed, OS results from either an excess of ROS or an inadequate antioxidant defense mechanism in the body, leading to potential damage to sperm cells and consequently, diminished male fertility. To combat these adverse effects, the body employs enzymatic antioxidants which are instrumental in preserving the redox equilibrium within the male reproductive tract.[8, 91]

Superoxide Dismutase (SOD)

Acting as a primary defense enzyme, SOD facilitates the conversion of the superoxide radical (O_2^-) into either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Two predominant forms of SOD exist in mammalian cells: the cytosolic copper-zinc SOD (CuZnSOD) and the mitochondrial manganese SOD (MnSOD). By transforming superoxide radicals into less aggressive entities, SOD inhibits their reaction with nitric oxide (NO), preventing the formation of peroxynitrite, a highly reactive nitrogen species detrimental to both the sperm membrane and its DNA.[92]

Catalase (CAT)

As a consequence of SOD activity, there is an increase in hydrogen peroxide concentration, necessitating an additional antioxidant response to counteract its buildup. CAT, primarily located in cellular peroxisomes, undertakes the role of converting H_2O_2 into water and molecular oxygen. Through this, CAT impedes H_2O_2 from engaging in the Fenton reaction, which could produce the extremely reactive hydroxyl radical, a potent agent of oxidative harm to DNA, lipids, and proteins.[93, 94]

Glutathione Peroxidase (GPx)

Complementing CAT, GPx provides an alternative route for H_2O_2 elimination. It drives the reduction of H2O2 to water, utilizing two molecules of glutathione (GSH). This reaction results in the oxidation of GSH to glutathione disulfide (GSSG).[95] Notably, GPx is also adept at counteracting lipid hydroperoxides, hence mitigating lipid peroxidation in the sperm membrane. Given the high polyunsaturated fatty acid content in sperm cells, protective function of GPx is of paramount importance.[95]

Therefore, the enzymatic antioxidants - SOD, CAT, and GPx - are quintessential for shielding the male reproductive system from OS. Their synergistic functions ensure efficient neutralization of ROS that could otherwise harm the intricate cellular structures of sperm cells.[96] Disruptions in the activity or levels of these enzymes can culminate in OS, associated with a range of male reproductive complications, encompassing diminished sperm motility, DNA fragmentation, and a lowered fertilization potential. Therefore, safeguarding the peak performance of these antioxidant enzymes is essential for the preservation of male reproductive wellness and fertility.[96]

Role of non-enzymatic antioxidants

The limited cytoplasmic space of the spermatozoa limits the accommodation of antioxidative enzymes. In this context, non-enzymatic antioxidants like vitamin C, vitamin E, and glutathione play an essential protective role for the male reproductive system.[97,98]

Vitamin C (Ascorbic Acid)

Present mainly in seminal plasma, vitamin C is a water-soluble antioxidant that neutralizes ROS, thus mitigating potential harm to sperm DNA, lipids, and proteins.[99] Research indicates that ascorbic acid can thwart lipid peroxidation in sperm cells, a process that compromises the sperm membrane's integrity and its functionality. The capability of vitamin C to rejuvenate vitamin E, another vital antioxidant, further strengthens its protective effect in the male reproductive system. Additionally, vitamin C facilitates collagen synthesis, crucial for maintaining the structural integrity of reproductive tissues, including the vas deferens.[99, 100]

Vitamin E (Alpha-Tocopherol)

Given its lipid-solubility, vitamin E seamlessly embeds itself into cell membranes, including sperm cell membranes.[101] It counteracts ROS, especially peroxyl radicals, inhibiting the onset and continuation of lipid peroxidation in sperm membranes. By conserving membrane integrity, vitamin E ensures the persistence of sperm movement and function.[102] Further research has demonstrated that vitamin E supplementation might improve sperm

quality and can be advantageous for males with unexplained infertility.[101]

Glutathione

Composed of cysteine, glycine, and glutamate, glutathione exists in two forms: reduced (GSH) and oxidized (GSSG). It serves as a foundational element in cellular defense against ROS. Regarding the male reproductive system, glutathione exhibits diverse functionalities.[96] It directly neutralizes ROS, aids other antioxidants, and is pivotal in detoxifying peroxidized lipids, thus preserving the structural and functional integrity of the sperm membrane. Additionally, glutathione is crucial during sperm maturation and capacitation phases.[103]

Therefore, non-enzymatic antioxidants act as vital defenders in upholding male reproductive health. They protect the sensitive sperm cells from oxidative damage, guaranteeing DNA preservation, movement, and overall sperm health. Their combined effort is imperative for male fertility, as supported by research emphasizing the consequences of a lack or imbalance of these antioxidants. A deeper understanding of their functionalities highlights the significance of a well-balanced diet and lifestyle in fostering reproductive health.

EXOGENOUS ANTIOXIDANTS AND DIETARY INTERVENTIONS

Benefits of Exogenous Antioxidants and Dietary Interventions

Improvement of Semen Parameters

Numerous investigations indicate that the addition of certain antioxidants can bolster sperm quality. Specifically, vitamins C and E can shield sperm cells from oxidative harm, leading to enhanced motility.[104, 105]

Enhancement of Fertilization Rates

Nutrients such as coenzyme Q10 and zinc have exhibited capabilities in amplifying the success rates of fertilization during assisted reproduction procedures.[106-109]

DNA Protection

Oxidative disruptions can result in the fragmentation of sperm DNA, potentially causing suboptimal embryo development or miscarriages. Antioxidants can act as a protective barrier, ensuring favorable reproductive results.[6]

Potential Risks of Exogenous Antioxidants and Dietary Interventions

Pro-Oxidative Effect

Despite the conventional understanding of antioxidants as agents that neutralize ROS, overconsumption can paradoxically manifest pro-oxidant properties, escalating oxidative tension and thereby deteriorating sperm health.[110]

Potential Interaction with Medications

Certain antioxidants may show interference with ongoing medicinal treatments, which could attenuate their effectiveness or instigate unexpected adverse reactions.[111]

Unknown Optimal Dosage

The precise beneficial quantities of many antioxidants for enhancing male reproductive health remain elusive. Both insufficient and excessive intakes can be harmful, with the former possibly lacking effect and the latter potentially causing detrimental outcomes.[110, 111]

Clinical Studies on Antioxidant Supplementation for Male Fertility

Exogenous intake of specific micronutrients and substances has been rigorously studied for their potential advantages in mitigating OS within the male reproductive apparatus. OS arises when there's a discrepancy between the generation of ROS and the organism's capacity to neutralize or eliminate their detrimental consequences through antioxidant mechanisms.[112]

L-Carnitine & Acetyl-L-carnitine

These compounds are both derivatives of the lysine amino acid and are instrumental in mitochondrial fatty acid oxidation. Pertaining to male reproduction, they are essential for the morphology and motility of spermatozoa.[113, 114] Elevated ROS levels can compromise sperm functionality. Administering L-carnitine and Acetyl-L-carnitine has proven to ameliorate sperm motility, owing to the diminution of oxidative damage and augmentation of mitochondrial activity.[115, 116]

Zinc and Folic Acid

Zinc is an imperative trace mineral required for numerous physiological operations, such as DNA generation, RNA transcription, and metabolic processes at the cellular level. Folic acid is crucial for DNA creation and mending. Within the male reproductive framework, a shortfall of either of these nutrients can lead to suboptimal sperm quality.[117] Concurrent administration of zinc and folic acid has been evidenced to bolster sperm count in men with reduced fertility, indicating a cooperative effect. This could be attributed in part to their protective function against oxidative degradation of sperm DNA.[118]

Vitamin E and Selenium

Vitamin E, a lipid-soluble antioxidant, and Selenium, a trace element, serve as potent antioxidants. Their insufficiency has been associated with subpar male fertility. These antioxidants safeguard sperm cells from oxidative harm, elevating sperm motility and overall vigor. Joint supplementation manifests superior effectiveness in fortifying male reproductive wellness compared to individual intake of either nutrient.[119, 120]

Coenzyme Q10

CoQ10, an integral part of the mitochondrial electron transport mechanism, is vital for generating energy. It also functions as an antioxidant.[108] Within the male reproductive milieu, its insufficiency can lead to reduced sperm movement due to hindered energy synthesis and augmented oxidative tension. Supplementation has evidenced enhancement in sperm metrics, attributed to the curbing of oxidative detriment and the elevation of energy synthesis in spermatozoa.[107, 121]

Nevertheless, while many studies advocate for the favorable effects, some report negligible or zero enhancements in sperm metrics after antioxidant intake. Such inconsistencies might be attributed to variations in research methodologies, demographic sampled, antioxidant kinds and quantities, and supplementation duration.[111] As potential remedies for male infertility are sought, external antioxidants and nutritional modifications appear to be hopeful. They present a viable means to counteract OS, a notorious factor in diminishing male reproductive wellness. Nevertheless,

judicious implementation is paramount. Holistic, prolonged research is imperative to ascertain the perfect quantities and mixtures of antioxidants. Furthermore, discerning individual responses to these interventions will be pivotal for personalized treatments. The associated risks, albeit marginal in comparison to the possible gains, must be acknowledged, and medical strategies should be firmly rooted in rigorous diagnostic assessments and scientifically validated guidelines.[122]

IMPLICATIONS OF REDUCTIVE STRESS ON MALE REPRODUCTION

Reductive stress emerges from an excessive accumulation or synthesis of reducing agents, primarily nicotinamide adenine dinucleotide phosphate (NADPH) and reduced glutathione (GSH), leading to an overly reduced intracellular milieu. Such an imbalance in the cellular redox state is detrimental for cellular homeostasis.[13] This condition can originate from various sources. For instance, there might be an augmented production of reducing agents through processes like the pentose phosphate pathway or an impaired consumption of these reducing agents in mitochondria due to their dysfunction. Exogenous factors, such as excessive antioxidant intake, might also skew the intracellular environment towards a reductive state.[123]

Impact on Sperm Quality and Male Reproductive Functions

Sperm cells, owing to their minimal cytoplasmic content and suboptimal antioxidant defenses, are exceptionally vulnerable to shifts in redox status. A delicate equilibrium between oxidative and reductive conditions is imperative for various sperm functions, including their movement, DNA stability, and capacity to fuse with an oocyte.

Sperm motility

Sperm movement is predominantly powered by the flagellar activity, which depends on mitochondrial ATP synthesis. Reductive stress might hinder mitochondrial performance, subsequently reducing ATP synthesis, and thereby impairing sperm movement.[13, 124, 125]

DNA integrity

Although oxidation is frequently linked to DNA damage, an intensely reductive milieu can also induce harm. Such stress may weaken the chromatin structure in sperm, rendering it prone to fragmentation and genetic alterations. This jeopardized DNA stability might adversely affect post-fertilization embryonic development and enhance the likelihood of genetic disorder transmission.[126]

Fertilization capacity

In addition to its effect on movement and DNA stability, the redox state also influences the capacitation process that preconditions the sperm for oocyte fusion. An overly reductive environment might inhibit efficient capacitation, hindering the sperm's capacity to penetrate and fertilize the oocyte.[13, 123]

Protective Mechanisms in the Male Reproductive System

To counteract the harmful repercussions of reductive stress, the male reproductive system has developed various protective measures:

Enzymatic defense

Certain enzymes, such as NADPH oxidases (NOXs), play a pivotal role in regulating the redox equilibrium. In the presence of reductive stress, NOXs generate ROS to reestablish the redox balance.[125]

Antioxidant regulation

Although antioxidants chiefly protect against OS, they also participate in sustaining redox equilibrium. In a reductive state, certain antioxidants might undergo oxidation without subsequent regeneration, thereby serving as a balancing agent.[127]

Testicular architecture and the blood-testis barrier

The distinct structuring of the testes, characterized by Sertoli cells forming tight junctions to establish the blood-testis barrier, guarantees a regulated milieu. This barrier offers protection against systemic variations, ensuring a consistent environment for sperm maturation.[128]

Therefore, reductive stress, even though reductive stress is less frequently highlighted in contrast to OS, it holds profound implications for male reproductive well-being. The male reproductive system is equipped with mechanisms to counteract reductive imbalances; however, chronic reductive stress can deteriorate sperm quality and overall male fertility. Delving deeper into reductive stress could yield innovative perspectives on the causes and remedies for male infertility.[13]

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Redox reactions, involving oxidants and antioxidants, are pivotal in the complex domain of male reproductive biology. Spermatogenesis, sperm maturation, and mature sperm functionality are intricately influenced by the redox environment, emphasizing the significance of both oxidative and reductive stressors. An excess of ROS results in OS, leading to detrimental consequences such as DNA fragmentation, lipid peroxidation, and altered protein structures, which compromise sperm functionality. However, in controlled amounts, ROS are integral to certain physiological functions of sperm, showcasing the dual role of oxidants. Antioxidants act as defensive agents, counteracting the adverse impacts of elevated ROS levels and ensuring redox balance. This equilibrium is vital not only for sperm viability but also for the overall stability of the male reproductive system. The nuanced interplay between oxidants and antioxidants emphasizes the imperative for a deeper comprehension and necessitates detailed studies on redox regulation in male reproduction. Prospective research areas encompass the elucidation of molecular pathways governing redox dynamics and the discovery of new antioxidant molecules or approaches. Insights derived from such endeavors could spearhead novel therapeutic methodologies. Such advancements could potentially address issues like male infertility and further enhance the broader comprehension of redox processes in reproductive biology. Delving deeper into this field has the potential to reveal uncharted aspects of reproductive medicine and introduce revolutionary clinical treatment methods.

Conflict of Interest

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

Funding

None

REFERENCES

1. P. Sengupta, S. Roychoudhury, M. Nath, S. Dutta. Oxidative stress and idiopathic male infertility. *Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility-Volume One* **2022**, 181-204.
2. H. Wagner, J.W. Cheng, E.Y. Ko. Role of reactive oxygen species in male infertility: An updated review of literature. *Arab J Urol* **2018**, 16(1), 35-43.
3. E.Y. Ko, E.S. Sabanegh Jr, A. Agarwal. Male infertility testing: reactive oxygen species and antioxidant capacity. *Fertil Steril* **2014**, 102(6), 1518-1527.
4. P. Sengupta, S. Dutta, B.S. Chhikara. Bioorthogonal chemistry in the reproductive medicine. *Chem. Biol. Lett.* **2023**, 10 (3), 545.
5. A. Agarwal, N. Parekh, M.K.P. Selvam, R. Henkel, R. Shah, S.T. Homa, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *World J Men's Health* **2019**, 37(3), 296-312.
6. D. Martin-Hidalgo, M.J. Bragado, A.R. Batista, P.F. Oliveira, M.G. Alves. Antioxidants and male fertility: From molecular studies to clinical evidence. *Antioxidants* **2019**, 8(4), 89.
7. S. Dutta, R. Henkel, P. Sengupta, A. Agarwal. Physiological role of ROS in sperm function. *Male infertility: Contemporary clinical approaches, Andrology, ART and antioxidants* **2020**, 337-345.
8. T. R. Dias, D. Martin-Hidalgo, B. M. Silva, P. F. Oliveira, M. G. Alves. Endogenous and exogenous antioxidants as a tool to ameliorate male infertility induced by reactive oxygen species. *Antiox Redox Sig* **2020**, 33(11), 767-785.
9. P.E. Castleton, J.C. Deluao, D.J. Sharkey, N.O. McPherson. Measuring reactive oxygen species in semen for male preconception care: a scientist perspective. *Antioxidants* **2022**, 11(2), 264.
10. J.P. Cardoso, M. Cocuzza, D. Elterman. Optimizing male fertility: oxidative stress and the use of antioxidants. *World J Urol* **2019**, 37, 1029-1034.
11. A. Agarwal, P. Sengupta. Oxidative stress and its association with male infertility. *Male infertility: contemporary clinical approaches, andrology, ART and antioxidants* **2020**, 57-68.
12. P. Sengupta, S. Dutta, A.T. Alahmar, *Reductive Stress and Male Infertility*, in *Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility Volume II*. 2022, Springer. p. 311-321.
13. N. Sadeghi, G. Boissonneault, M. Tavalaee, M.H. Nasr-Esfahani. Oxidative versus reductive stress: a delicate balance for sperm integrity. *Syst Biol Reprod Med* **2023**, 69(1), 20-31.
14. A. Amaral, B. Lourenço, M. Marques, J. Ramalho-Santos. Mitochondria functionality and sperm quality. *Reproduction* **2013**, 146(5), R163-R174.
15. R. Chianese, R. Pierantoni. Mitochondrial reactive oxygen species (ROS) production alters sperm quality. *Antioxidants* **2021**, 10(1), 92.
16. T.M. Said, A. Agarwal, R.K. Sharma, A.J. Thomas Jr, S.C. Sikka. Impact of sperm morphology on DNA damage caused by oxidative stress induced by β-nicotinamide adenine dinucleotide phosphate. *Fertil Steril* **2005**, 83(1), 95-103.
17. J.D. Lambeth. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. *Curr Opinion Hematol* **2002**, 9(1), 11-17.
18. G. Donà, C. Fiore, A. Andrisani, G. Ambrosini, A. Brunati, E. Ragazzi, et al. Evaluation of correct endogenous reactive oxygen species content for human sperm capacitation and involvement of the NADPH oxidase system. *Hum Reprod* **2011**, 26(12), 3264-3273.
19. S.C. Richer, W. Ford. A critical investigation of NADPH oxidase activity in human spermatozoa. *Mol Hum Reprod* **2001**, 7(3), 237-244.
20. A.O. Adefuye, H.A. Adeola, K.J. Sales, A.A. Katz. Seminal fluid-mediated inflammation in physiology and pathology of the female reproductive tract. *J Immunol Res* **2016**, 2016.
21. R. Aitken, D. Buckingham, D. Harkiss. Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa. *Reproduction* **1993**, 97(2), 441-450.
22. M. Furuhashi. New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity. *Am J Physiol Endocrinol Metab* **2020**.
23. Y.-J. Kim, J.-E. Park, J.-Y. Chung, J.Y. Kim, S.G. Lee, S.-J. Lee, et al. Constitutive expression of cytochrome P450 1B1 endows testicular Leydig cells with susceptibility to 7, 12-dimethylbenzanthracene-induced cell death. *J Toxicol Sci* **2022**, 47(8), 317-326.
24. A.J. Koppers, M.L. Garg, R.J. Aitken. Stimulation of mitochondrial reactive oxygen species production by unesterified, unsaturated fatty acids in defective human spermatozoa. *Free Rad Biol Med* **2010**, 48(1), 112-119.
25. J.S. Aprioku. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. *J Reprod Infertil* **2013**, 14(4), 158.
26. E. Gomez, D.W. Buckingham, J. Brindle, F. Lanzafame, D.S. Irvine, R.J. Aitken. Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: correlation with biochemical markers of the cytoplasmic space, oxidative stress, and sperm function. *J Androl* **1996**, 17(3), 276-287.
27. T.M. Said, A. Agarwal, R.K. Sharma, E. Mascha, S.C. Sikka, A.J. Thomas, Jr. Human sperm superoxide anion generation and correlation with semen quality in patients with male infertility. *Fertil Steril* **2004**, 82(4), 871-877.
28. A. Golas, P. Malek, M. Piasecka, J. Styrna. Sperm mitochondria diaphorase activity--a gene mapping study of recombinant inbred strains of mice. *Int J Dev Biol* **2010**, 54(4), 667-673.
29. P. Sabeti, S. Pourmasumi, T. Rahiminia, F. Akyash, A.R. Talebi. Etiologies of sperm oxidative stress. *Int J Reprod Biomed* **2016**, 14(4), 231-240.
30. T.G. Cooper, E. Noonan, S. von Eckardstein, J. Auger, H.W. Baker, H.M. Behre, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* **2010**, 16(3), 231-245.
31. R.M. Fariello, P.T. Del Giudice, D.M. Spaine, R. Fraietta, R.P. Bertolla, A.P. Cedenho. Effect of leukocytospermia and processing by discontinuous density gradient on sperm nuclear DNA fragmentation and mitochondrial activity. *J Assist Reprod Genet* **2009**, 26(2-3), 151-157.
32. O.C. Theam, S. Dutta, P. Sengupta. Role of leucocytes in reproductive tract infections and male infertility. *Chem Biol Lett* **2020**, 7(2), 124-130.
33. R. Sharma, S. Gupta, R. Henkel. Relevance of leukocytospermia and semen culture and its true place in diagnosing and treating male infertility. **2021**.
34. K. Makker, A. Agarwal, R. Sharma. Oxidative stress & male infertility. *Indian J Med Res* **2009**, 129(4), 357-367.
35. P. Sengupta, S. Dutta, A.T. Alahmar, U.J.A. D'souza. Reproductive tract infection, inflammation and male infertility. *Chem Biol Lett* **2020**, 7(2), 75-84.
36. K. Bhattacharya, P. Sengupta, S. Dutta, I.R. Karkada. Obesity, systemic inflammation and male infertility. *Chem Biol Lett* **2020**, 7(2), 92-98.
37. S. Dutta, P. Sengupta, B.S. Chhikara. Reproductive inflammatory mediators and male infertility. *Chem. Biol. Lett* **2020**, 7(2), 73-74.
38. A. Hamada, A. Agarwal, R. Sharma, D.B. French, A. Ragheb, E.S. Sabanegh, Jr. Empirical treatment of low-level leukocytospermia with doxycycline in male infertility patients. *Urology* **2011**, 78(6), 1320-1325.
39. S. Aboulmaouahib, A. Madkour, I. Kaarouch, O. Sefrioui, B. Saadani, H. Copin, et al. Impact of alcohol and cigarette smoking consumption in male fertility potential: Looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage. *Andrologia* **2018**, 50(3).
40. J.S. Brand, M.F. Chan, M. Dowsett, E. Folkerd, N.J. Wareham, R.N. Luben, et al. Cigarette smoking and endogenous sex hormones in postmenopausal women. *J Clin Endocrinol Metab* **2011**, 96(10), 3184-3192.
41. P. Sengupta. Reviewing reports of semen volume and male aging of last 33 years: From 1980 through 2013. *Asian Pac J Reprod* **2015**, 4(3), 242-246.
42. A. Valavanidis, T. Vlachogianni, K. Fiotakis. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int J Environ Res Public Health* **2009**, 6(2), 445-462.
43. M.A. Ghaffari, M. Rostami. The effect of cigarette smoking on human sperm creatine kinase activity: as an ATP buffering system in sperm. *Int J Fertil Steril* **2013**, 6(4), 258-265.

44. P. Gogol, B. Szcześniak-Fabiańczyk, A. Wierzchoś-Hilczer. The photon emission, ATP level and motility of boar spermatozoa during liquid storage. *Reprod Biol* **2009**, 9(1), 39-49.
45. M.F. Hamad, N. Shelko, S. Kartarius, M. Montenarh, M.E. Hammadeh. Impact of cigarette smoking on histone (H2B) to protamine ratio in human spermatozoa and its relation to sperm parameters. *Andrology* **2014**, 2(5), 666-677.
46. X. Cui, X. Jing, X. Wu, Z. Wang, Q. Li. Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. *Mol Med Reports* **2016**, 14(1), 753-761.
47. B. Guthauser, F. Boitrelle, A. Plat, N. Thiercelin, F. Vialard. Chronic excessive alcohol consumption and male fertility: a case report on reversible azoospermia and a literature review. *Alcohol Alcohol* **2014**, 49(1), 42-44.
48. S. Manzo-Avalos, A. Saavedra-Molina. Cellular and mitochondrial effects of alcohol consumption. *Int J Environ Res Public Health* **2010**, 7(12), 4281-4304.
49. S.M. Bailey, G. Robinson, A. Pinner, L. Chamlee, E. Ulasova, M. Pompilius, et al. S-adenosylmethionine prevents chronic alcohol-induced mitochondrial dysfunction in the rat liver. *Am J Physiol Gastrointest Liver Physiol* **2006**, 291(5), G857-867.
50. S. Dutta, P. Sengupta. Role of nitric oxide on male and female reproduction. *Malays J Med Sci* **2021**.
51. R. Angelopoulou, G. Lavranos, P. Manolakou. ROS in the aging male: model diseases with ROS-related pathophysiology. *Reprod Toxicol* **2009**, 28(2), 167-171.
52. K.K. Kesari, A. Agarwal, R. Henkel. Radiations and male fertility. *Reprod Biol Endocrinol* **2018**, 16(1), 118.
53. R. Gautam, K.V. Singh, J. Nirala, N.N. Murmu, R. Meena, P. Rajamani. Oxidative stress-mediated alterations on sperm parameters in male Wistar rats exposed to 3G mobile phone radiation. *Andrologia* **2019**, 51(3), e13201.
54. N.R. Desai, K.K. Kesari, A. Agarwal. Pathophysiology of cell phone radiation: oxidative stress and carcinogenesis with focus on male reproductive system. *Reprod Biol Endocrinol* **2009**, 7, 114.
55. R.J. Aitken, Z. Gibb, M.A. Baker, J. Drevet, P. Gharagozloo. Causes and consequences of oxidative stress in spermatozoa. *Reprod Fertil Dev* **2016**, 28(1-2), 1-10.
56. P. Chauhan, H.N. Verma, R. Sisodia, K.K. Kesari. Microwave radiation (2.45 GHz)-induced oxidative stress: Whole-body exposure effect on histopathology of Wistar rats. *Electromagn Biol Med* **2017**, 36(1), 20-30.
57. K.K. Kesari, S. Kumar, J. Behari. 900-MHz microwave radiation promotes oxidation in rat brain. *Electromagn Biol Med* **2011**, 30(4), 219-234.
58. C.R. Gracia, M.D. Sammel, C. Coutifaris, D.S. Guzick, K.T. Barnhart. Occupational exposures and male infertility. *Am J Epidemiol* **2005**, 162(8), 729-733.
59. M. Sabés-Alsina, O. Tallo-Parra, M.T. Mogas, J.M. Morrell, M. Lopez-Bejar. Heat stress has an effect on motility and metabolic activity of rabbit spermatozoa. *Anim Reprod Sci* **2016**, 173, 18-23.
60. M. Zhang, M. Jiang, Y. Bi, H. Zhu, Z. Zhou, J. Sha. Autophagy and apoptosis act as partners to induce germ cell death after heat stress in mice. *PLoS One* **2012**, 7(7), e41412.
61. C. Pereira, K. Mapuskar, C.V. Rao. Chronic toxicity of diethyl phthalate in male Wistar rats--a dose-response study. *Regul Toxicol Pharmacol* **2006**, 45(2), 169-177.
62. N. Pant, M. Shukla, D. Kumar Patel, Y. Shukla, N. Mathur, Y. Kumar Gupta, et al. Correlation of phthalate exposures with semen quality. *Toxicol Appl Pharmacol* **2008**, 231(1), 112-116.
63. M. Radwan, J. Jurewicz, K. Polańska, W. Sobala, P. Radwan, M. Bochenek, et al. Exposure to ambient air pollution--does it affect semen quality and the level of reproductive hormones? *Ann Hum Biol* **2016**, 43(1), 50-56.
64. P. Sengupta, R. Banerjee. Environmental toxins: Alarming impacts of pesticides on male fertility. *Hum Exp Toxicol* **2014**, 33(10), 1017-1039.
65. P. Sengupta. Environmental and occupational exposure of metals and their role in male reproductive functions. *Drug Chem Toxicol* **2013**, 36(3), 353-368.
66. T. Takeshima, K. Usui, K. Mori, T. Asai, K. Yasuda, S. Kuroda, et al. Oxidative stress and male infertility. *Reprod Med Biol* **2021**, 20(1), 41-52.
67. J.L. Fernández, L. Muriel, M.T. Rivero, V. Goyanes, R. Vazquez, J.G. Alvarez. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. *J Androl* **2003**, 24(1), 59-66.
68. F. Cariati, S. Jaroudi, S. Alfarawati, A. Raberi, C. Alviggi, R. Pivonello, et al. Investigation of sperm telomere length as a potential marker of paternal genome integrity and semen quality. *Reprod Biomed Online* **2016**, 33(3), 404-411.
69. D. Sakkas, J.G. Alvarez. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* **2010**, 93(4), 1027-1036.
70. E. Greco, M. Iacobelli, L. Rienzi, F. Ubaldi, S. Ferrero, J. Tesarik. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl* **2005**, 26(3), 349-353.
71. S. Kuroda, T. Takeshima, K. Takeshima, K. Usui, K. Yasuda, H. Sanjo, et al. Early and late paternal effects of reactive oxygen species in semen on embryo development after intracytoplasmic sperm injection. *Syst Biol Reprod Med* **2020**, 66(2), 122-128.
72. R.A. Saleh, A. Agarwal. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl* **2002**, 23(6), 737-752.
73. A.D. Bui, R. Sharma, R. Henkel, A. Agarwal. Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia* **2018**, 50(8), e13012.
74. C. O'Flaherty, D. Matsushita-Fournier. Reactive oxygen species and protein modifications in spermatozoa. *Biol Reprod* **2017**, 97(4), 577-585.
75. R. Sharma, A. Agarwal, G. Mohanty, A.J. Hamada, B. Gopalan, B. Willard, et al. Proteomic analysis of human spermatozoa proteins with oxidative stress. *Reprod Biol Endocrinol* **2013**, 11, 1-18.
76. K.M. Mills, U.K. Aryal, T. Sobreira, A.M. Minton, T. Casey, K.R. Stewart. Shotgun proteome analysis of seminal plasma differentiate boars by reproductive performance. *Theriogenology* **2020**, 157, 130-139.
77. R.J. Aitken. Reactive oxygen species as mediators of sperm capacitation and pathological damage. *Mol Reprod Dev* **2017**, 84(10), 1039-1052.
78. J. Griveau, D.L. Lannou. Reactive oxygen species and human spermatozoa: physiology and pathology. *Int J Androl* **1997**, 20(2), 61-69.
79. M.A. El-Taieb, M.A. Ali, E.A. Nada. Oxidative stress and acrosomal morphology: A cause of infertility in patients with normal semen parameters. *Middle East Fertil Soc J* **2015**, 20(2), 79-85.
80. S. Lone, N. Shah, H.P. Yadav, M.A. Wagay, A. Singh, R. Sinha. Sperm DNA damage causes, assessment and relationship with fertility: A review. *Theriogenol Insight* **2017**, 7(1), 13-20.
81. C.H. Chen, S.S. Lee, D.C. Chen, H.H. Chien, I.C. Chen, Y.N. Chu, et al. Apoptosis and kinematics of ejaculated spermatozoa in patients with varicocele. *J Androl* **2004**, 25(3), 348-353.
82. P.H. Krammer, I. Behrmann, P. Daniel, J. Dhein, K.-M. Debatin. Regulation of apoptosis in the immune system. *Curr Opinion Immunol* **1994**, 6(2), 279-289.
83. T. Suda, T. Takahashi, P. Golstein, S. Nagata. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* **1993**, 75(6), 1169-1178.
84. D.J. Kane, T.A. Sarafian, R. Anton, H. Hahn, E.B. Gralla, J.S. Valentine, et al. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* **1993**, 262(5137), 1274-1277.
85. D. Sakkas, E. Mariethoz, J.C.S. John. Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway. *Exp Cell Res* **1999**, 251(2), 350-355.
86. U. Paasch, R.K. Sharma, A.K. Gupta, S. Grunewald, E.J. Mascha, A.J. Thomas Jr, et al. Cryopreservation and thawing is associated with varying extent of activation of apoptotic machinery in subsets of ejaculated human spermatozoa. *Biol Reprod* **2004**, 71(6), 1828-1837.
87. P. Sabeti, S. Pourmasumi, T. Rahiminia, F. Akyash, A.R. Talebi. Etiologies of sperm oxidative stress. *Int J Reprod Biomed* **2016**, 14(4), 231.
88. K. Nowicka-Bauer, B. Nixon. Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants* **2020**, 9(2), 134.
89. T. Dahan, H. Breitbart. Involvement of metabolic pathway in the sperm spontaneous acrosome reaction. *Theriogenology* **2022**, 192, 38-44.

90. S. Dutta, A. Majzoub, A. Agarwal. Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab J Urol* **2019**, 17(2), 87-97.
91. S.A. Sheweita, A.M. Tilmisany, H. Al-Sawaf. Mechanisms of male infertility: role of antioxidants. *Curr Drug Metab* **2005**, 6(5), 495-501.
92. D.D. Mruk, B. Silvestrini, M.-y. Mo, C.Y. Cheng. Antioxidant superoxide dismutase-a review: its function, regulation in the testis, and role in male fertility. *Contraception* **2002**, 65(4), 305-311.
93. K. Sadia, S. Sultan, K. Khan, L.M. Javeres, B. Rumman, S.T. Shah, et al. Antioxidant enzymes and association of CAT SNP-21 A/T (rs7943316) with male infertility. *Mol Reprod Dev* **2021**, 88(9), 598-604.
94. U. Marzec-Wróblewska, P. Kamiński, P. Łakota, M. Szymański, K. Wasilow, G. Ludwikowski, et al. Human sperm characteristics with regard to cobalt, chromium, and lead in semen and activity of catalase in seminal plasma. *Biol Trace Elem Res* **2019**, 188, 251-260.
95. A. Giannattasio, M. De Rosa, R. Smeraglia, S. Zarrilli, A. Cimmino, B. Di Rosario, et al. Glutathione peroxidase (GPX) activity in seminal plasma of healthy and infertile males. *J Endocrinol Invest* **2002**, 25, 983-986.
96. D.I. Martinov, N.P. Ayvazova, E.I. Konova, M.A. Atanasova. Glutathione content and glutathione peroxidase activity of sperm in males with unexplained infertility. *J Biomed Clin Res* **2021**, 14(1), 53-61.
97. E. Moretti, G. Collodel, A.I. Fiaschi, L. Micheli, F. Iacoponi, D. Cerretani. Nitric oxide, malondialdheyde and non-enzymatic antioxidants assessed in viable spermatozoa from selected infertile men. *Reprod Biol* **2017**, 17(4), 370-375.
98. R. Walczak–Jedrzejowska, J.K. Wolski, J. Slowikowska–Hilczer. The role of oxidative stress and antioxidants in male fertility. *Cent Eur J Urol* **2013**, 66(1), 60.
99. M. Al-Mousaw. Ameliorated Effect of Ascorbic Acid and Selenium against the Stress Effect on Sperm Quality of Rats. *Arch Razi Inst* **2021**, 76(4), 1137.
100. M.R. Luck, I. Jeyaseelan, R.A. Scholes. Ascorbic acid and fertility. *Biol Reprod* **1995**, 52(2), 262-266.
101. A. Rasul, S. Mededovic, H. Memmedov, E. Canan Alp Arıcı. Vitamins and male infertility: role of various vitamins versus oxidative stress. *Cent Asian J Med Pharm Sci Innov* **2022**, 2(5), 151-164.
102. Y. Attia, B. Abou-Shehema, A. Abdellah, O. Aly, A.S. El-Naggar. Effect of ascorbic acid and/or alpha-tocopherol fortification on semen quality, metabolic profile, antioxidants status, and DNA of roosters exposed to heat stress. *J Anim Plant Sci* **2020**, 30(2), 325-335.
103. M. Llavanera, Y. Mateo-Otero, S. Bonet, I. Barranco, B. Fernández-Fuertes, M. Yeste. The triple role of glutathione S-transferases in mammalian male fertility. *Cell Mol Life Sci* **2020**, 77, 2331-2342.
104. U.R. Acharya, M. Mishra, J. Patro, M.K. Panda. Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reprod Toxicol* **2008**, 25(1), 84-88.
105. C. Angulo, R. Maldonado, E. Pulgar, H. Mancilla, A. Córdova, F. Villarroel, et al. Vitamin C and oxidative stress in the seminiferous epithelium. *Biol Res* **2011**, 44(2), 169-180.
106. S. Cilio, M. Rienzo, G. Villano, B.F. Mirto, G. Giampaglia, F. Capone, et al. Beneficial effects of antioxidants in male infertility management: A narrative review. *Oxygen* **2022**, 2(1), 1-11.
107. A.T. Alahmar, A.E. Calogero, P. Sengupta, S. Dutta. Coenzyme Q10 improves sperm parameters, oxidative stress markers and sperm DNA fragmentation in infertile patients with idiopathic oligoasthenozoospermia. *World J Men's Health* **2021**, 39(2), 346.
108. A.T. Alahmar, A.E. Calogero, R. Singh, R. Cannarella, P. Sengupta, S. Dutta. Coenzyme Q10, oxidative stress, and male infertility: A review. *Clin Exp Reprod Med* **2021**, 48(2), 97.
109. K. Kerns, M. Zigo, P. Sutovsky. Zinc: A necessary ion for mammalian sperm fertilization competency. *Int J Mol Sci* **2018**, 19(12), 4097.
110. S. Dutta, P. Sengupta, S. Roychoudhury, S. Chakravarthi, C.W. Wang, P. Slama. Antioxidant paradox in male infertility:'A blind eye'on inflammation. *Antioxidants* **2022**, 11(1), 167.
111. M. Ali, M. Martinez, N. Parekh. Are antioxidants a viable treatment option for male infertility? *Andrologia* **2021**, 53(1), e13644.
112. M. Arafa, A. Agarwal, A. Majzoub, M.K. Panner Selvam, S. Baskaran, R. Henkel, et al. Efficacy of antioxidant supplementation on conventional and advanced sperm function tests in patients with idiopathic male infertility. *Antioxidants* **2020**, 9(3), 219.
113. L. Mongioi, A. Calogero, E. Vicari, R. Condorelli, G. Russo, S. Privitera, et al. The role of carnitine in male infertility. *Andrology* **2016**, 4(5), 800-807.
114. X. Zhou, F. Liu, S. Zhai. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. **2007**.
115. M. Costa, D. Canale, M. Filicori, S. D'lddio, A. Lenzi, I.S.G.o. Carnitine, et al. L-carnitine in idiopathic asthenozoospermia: a multicenter study. *Andrologia* **1994**, 26(3), 155-159.
116. S. Micic, N. Lalic, D. Djordjevic, N. Bojanic, N. Bogavac-Stanojevic, G.M. Busetto, et al. Double-blind, randomised, placeb o-controlled trial on the effect of L-carnitine and L- acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. *Andrologia* **2019**, 51(6), e13267.
117. E.F. Schisterman, L.A. Sjaarda, T. Clemons, D.T. Carrell, N.J. Perkins, E. Johnstone, et al. Effect of folic acid and zinc supplementation in men on semen quality and live birth among couples undergoing infertility treatment: a randomized clinical trial. *J Am Med Assoc* **2020**, 323(1), 35-48.
118. X. Li, Y.M. Zeng, J. He, B.W. Luo, X.C. Lu, L.L. Zhu. Effects of folic acid and folic acid plus zinc supplements on the sperm characteristics and pregnancy outcomes of infertile men: A systematic review and meta-analysis. *Heliyon* **2023**.
119. H.A. El-Fadil Ibrahim, S.I. Shalaby, A. Abdelfattah-Hassan, R.M. Hebishy, E.M. Abdel Mohsen Abdel Ghani. Ameliorative effects of vitamin E and selenium on bleomycin-induced male infertility. *Slovenian Veterinary Research* **2023**, 60.
120. R. Bahmyari, A. Ariafar, M. Sayadi, S. Hossieni, S. Azima. The effect of daily intake of selenium, vitamin E and folic acid on sperm parameters in males with idiopathic infertility: a single-blind randomized controlled clinical trial. *Int J Fertil Steril* **2021**, 15(1), 8.
121. A.T. Alahmar, P. Sengupta, S. Dutta, A.E. Calogero. Coenzyme Q10, oxidative stress markers, and sperm DNA damage in men with idiopathic oligoasthenoteratospermia. *Clin Exp Reprod Med* **2021**, 48(2), 150.
122. A. Agarwal, K. Leisegang, A. Majzoub, R. Henkel, R. Finelli, M.K.P. Selvam, et al. Utility of antioxidants in the treatment of male infertility: clinical guidelines based on a systematic review and analysis of evidence. *World J Men's Health* **2021**, 39(2), 233.
123. J.W. Cheng, E.Y. Ko, *Causes of reductive stress in male reproduction*, in *Oxidants, antioxidants and impact of the oxidative status in male reproduction*. 2019, Elsevier. p. 55-64.
124. E. Caroppo, M. Dattilo. Sperm redox biology challenges the role of antioxidants as a treatment for male factor infertility. *F&S Rev* **2022**, 3(1), 90-104.
125. C. O'Flaherty. Redox regulation of mammalian sperm capacitation. *Asian J Androl* **2015**, 17(4), 583.
126. M.K.P. Selvam, A. Agarwal, R. Henkel, R. Finelli, K.A. Robert, C. Iovine, et al. The effect of oxidative and reductive stress on semen parameters and functions of physiologically normal human spermatozoa. *Free Rad Biol Med* **2020**, 152, 375-385.
127. E.N. Symeonidis, E. Evgeni, V. Palapelas, D. Koumasi, N. Pyrgidis, I. Sokolakis, et al. Redox balance in male infertility: Excellence through moderation—"Μέτρον ἄριστον". *Antioxidants* **2021**, 10(10), 1534.
128. L. Zhang, X. Ji, F. Ding, X. Wu, N. Tang, Q. Wu. Apoptosis and blood-testis barrier disruption during male reproductive dysfunction induced by PAHs of different molecular weights. *Env Pollut* **2022**, 300, 118959.