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The effect of oxidative stress on male fertility: A review

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Abstract

Male fertility is critical around the world, including in Nigeria, where infertility rates range between 20% and 30%. Male infertility is caused by a variety of factors, including genetics, lifestyle, and environmental pollutants, which frequently involve oxidative stress and reactive oxygen species (ROS). ROS imbalance is linked to idiopathic male infertility, which has no clear cause. Understanding the complexities of these issues is critical for diagnosis and treatment. Internal (endogenous) and external (exogenous) sources generate reactive oxygen species (ROS). External factors include pollutants, ultraviolet (UV) radiation, heavy metals, and smoking. Sources within the body include mitochondria, nicotinamide adenine dinucleotide phosphate oxidase (NAPDH oxidase; NOX) enzymes, and immune cells. Controlled ROS production serves signaling pathways, but imbalance can damage cellular components, leading to cell death. Reactive oxygen species (ROS) negatively affect sperm via both internal and external pathways. External factors such as psychological stress, exercise, heat stress, smoking, and poor diet increase ROS production. Psychological stress raises norepinephrine and cortisol, intensifying ROS. Exercise, especially vigorous, generates excessive ROS, potentially harming sperm. Heat stress from various sources elevates ROS through hormonal disruptions. Smoking, alcohol, and unhealthy foods also contribute to ROS production, damaging sperm health. Reproductive tract infections and immature spermatozoa, along with conditions like varicocele, leukocytes, inflammation, and cytokines, further exacerbate oxidative damage to sperm internally. In conclusion, oxidative equilibrium is essential for preserving the health of sperm. Male infertility can result from oxidative stress, which drastically reduces the quality of sperm. It has been demonstrated that antioxidant therapy has positive impacts on sperm characteristics and pregnancy outcomes. Maintaining oxidative balance is therefore crucial for male reproductive health, and antioxidant supplementation may be a helpful tactic to enhance sperm quality in situations of male infertility.

Keywords: Oxidative Stress; Male Fertility; Reactive Oxidative Species; Infertility; Antioxidant

1. Introduction

The World Health Organisation describes infertility as couple's inability to conceive after 12 months of frequent unprotected sexual intercourse. Infertility is also described as the inability to bring a pregnancy to term and give birth to a live baby (World Health Organisation, 2023). Therefore, in males, infertility is defined as a man's inability to impregnate a woman after 12 months of consistent and unprotected sexual activity.

The topic of male fertility is critical in the global context of reproductive health, extending to countries such as Nigeria. While infertility is not a life-threatening condition, it can result in a transformational experience accompanied by significant psychological pain (Uadia & Emokpae, 2015). Akinloye and Truter, in their 2011 publication, stated that despite the significant concern of infertility on the public health in Nigeria, with a prevalence rate ranging from 20% to 30%, infertility in Sub-Saharan Africa, especially Nigeria, has, nevertheless, gone largely undetected until recently. They further supplied that the region's high fertility rates contributed to a worldwide focus on population expansion and fertility, diverting attention away from infertility as a serious concern.

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From 1965 to 2015, there was a downward trend in sperm count among African males (Sengupta *et al*., 2017). As supplied by Agarwal *et al*. (2015), it has been estimated that 30 million males worldwide at the minimum are infertile, the highest occurring in Africa and Eastern Europe. Male infertility is still a chronic and difficult issue around the world. Despite significant advances in medical and reproductive sciences, the complex interplay of factors contributing to male infertility remains a challenge. To overcome these obstacles, a thorough understanding of the underlying complexities is required, as well as novel diagnostic and treatment procedures.

One of the most visible concerns is the obvious drop in sperm quality and quantity over the last few decades. Environmental pollutants, sedentary lifestyles, and poor eating habits have all been linked to this decline (Mann *et al*., 2020). A large proportion of male infertility cases are classified as unexplained infertility (Esteves, 2013; Hamada *et al*., 2012). Even with modern diagnostic techniques, a subset of infertile persons faces the frustration of not being able to pinpoint a precise cause for their reproductive troubles (Esteves, 2013).

Recent research underscores the intricate interplay of genetic and epigenetic factors in male infertility (Plaseska-Karanfilska *et al*., 2012). Mutations, deletions, and variations in particular genes can have substantial repercussions on sperm production, motility, and function (Plaseska-Karanfilska *et al*., 2012). Modern lifestyles, encompassing heightened stress levels, sedentary behaviors, and excessive utilization of electronic devices, have the potential to contribute to male infertility (Durairajanayagam, 2018). Furthermore, exposure to endocrine-disrupting substances present in the environment, such as those found in plastics and pesticides, has been associated with detrimental effects on male reproductive health (Balawender & Orkisz, 2020).

According to Hampl *et al*. (2012), oxidative stress (OS) is a condition marked by an imbalance between the systemic presence of reactive oxygen species (ROS) and a biological system's ability to efficiently neutralize these reactive intermediates or repair any damage that results from them. Pro-oxidants and antioxidants maintain homeostasis in a healthy physiological setting. Gonadal cells and mature spermatozoa are protected from oxidative damage by intrinsic antioxidant defense systems present in spermatozoa (Henkel, 2011). However, in pathological conditions, the unchecked production of ROS outpaces seminal plasma's antioxidant powers, resulting in OS manifestation (Henkel, 2011).

Oxidative stress (OS) is notable among the hypothesized causes of idiopathic male infertility. Varicocele, cryptorchidism, hypogonadism, and hereditary effects are among the many causes of male infertility that account for about 40% of instances. However, primary and secondary infertility in about 25% of couples lacks observable causes, defining it as idiopathic infertility (Alahmar, 2017). Reactive oxygen species (ROS) and OS have been proposed as probable causative factors in the range of idiopathic infertility (Alahmar, 2019). This review emphasizes the importance of ROS in relation to male infertility.

2. Oxidative Stress (OS) and Reactive Oxygen Species (ROS)

2.1. Oxidative Stress and ROS Generation

An imbalance between the production of highly reactive molecules like reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS), and the intrinsic ability of the endogenous antioxidant defense mechanisms, occurs when tissues and organs experience oxidative stress (Pham-Huy *et al*., 2008). This imbalance causes cellular dysfunction and impairment, which contributes to a variety of illnesses. As a natural byproduct of metabolic processes, these reactive species are continuously generated within cells at low quantities. Additionally, extrinsic variables like radiation (X-rays and UV), ozone, air pollutants, cigarette smoke, bacteria, viruses, drugs, and more might be blamed for their formation (Pham-Huy *et al*., 2008).

Cellular stress that is either acute or ongoing can produce reactive species. Recognizing that these reactive entities might appear as either free radicals or non-radical oxidants is crucial. Free radicals are naturally unstable because they have unpaired electrons in their outer electron orbit. Free radicals' extraordinary reactivity forces them to interact with other molecules, which results in oxidative reactions. As a result, they cause harm at several cellular levels by interacting with important biological molecules like DNA, lipids, and proteins (Lobo *et al*., 2010). Of particular importance, proteins—which are essential cellular building blocks—emerge as the main targets vulnerable to the effect of free radicals.

2.2. ROS Sources

Although there are many sources for the production of free radicals, they can be divided into exogenous and endogenous sources. Free radicals are said to be produced as a result of intracellular metabolisms as well as external factors like pollution, lifestyle, and smoking, according to reports in literature.

2.2.1. External or Exogenous Influences

Reactive oxygen species (ROS) production within cells can be increased by a variety of environmental factors, such as exposure to toxins, pollutants like insecticides and pesticides, cigarettes (direct or passive smoke), UV radiation, heavy metal ions, allergens, and drugs (Oke *et al*., 2019; Mahajan *et al*., 2018).

Ionizing radiation works by oxidizing molecules like organic radicals, superoxides, and hydroxyl radicals to produce organic hydroperoxides and hydrogen peroxide. These peroxides then engage in redox interactions with cellular metal ions like Fe and Cu, initiating subsequent oxidative processes. Notably, numerous studies have shown that fibroblast exposure to alpha particles causes a spike in intracellular oxygen levels and speeds up the production of peroxide at the cellular level (Spitz & Hauer-Jensen, 2014).

Ultraviolet radiation radiation causes oxidative reactions by stimulating the enzymes NADPH-oxidase, porphyrins, and riboflavin. When the exposure is stopped, this process primarily generates 8-oxo-guanine and decreases intracellular glutathione (GSH) levels, which eventually returns to baseline (Marchitti *et al*., 2011).

The creation of free radicals is a process in which heavy metals are involved, according to extensive research. Through processes like Fenton or Haber-Weiss reactions, heavy metals, which include iron, copper, cadmium, nickel, arsenic, and lead, can start the formation of free radicals (Sciskalska *et al*., 2014). Additionally, they can cause direct interactions between metal ions and cellular components that result in similar outcomes, like the production of radicals of the thiol type (Sciskalska *et al*., 2014).

While also impeding antioxidant enzymes like glutathione-transferase, glutathione-peroxidase, and glutathione-reductase by binding to sulfhydryl groups, arsenic promotes the production of peroxides, superoxides, and nitric oxide (Jan *et al*., 2015). These interactions can produce free radicals that can damage DNA, causing base substitutions such as guanine to cytosine, guanine to thymine, and cytosine to thymine (Jan *et al*., 2015). Due to increased inflammatory infiltrates within the respiratory epithelium, ozone exposure has been linked to lung function impairment, even in healthy individuals (Wu *et al*., 2019).

2.2.2. Endogenous or Internal Influence

Endoplasmic reticulum (ER), peroxisomes, membrane-bound NADPH oxidases (NOX) isoforms 1–5, dual oxidases (Duox) 1 and 2 complexes, and nitric oxide synthases isoforms 1–5 (NOS1-3) are among the main locations within cells where endogenous redox-reactive species, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), are produced (Curi *et al*., 2016; Sabeur & Ball, 2007).

While other internal sources are also present, the mitochondrial ETC is the main endogenous source of ROS. Additional sources of ROS, primarily hydrogen peroxide, include microsomes and peroxisomes. As part of their oxygen-dependent defense mechanisms against encroaching microorganisms via the NOX2 isoform, immune cells like neutrophils and macrophages can also produce ROS (Curi *et al*., 2016).

Since oxidative phosphorylation is the main energy source for aerobic organisms, the production of ROS in mitochondria during aerobic metabolism is closely related to this process (Papa *et al*., 2012).

In addition to being ROS receptors, mitochondria also serve as ROS producers. During or after protein biosynthesis, as well as during protein cleavage or degradation, proteins can undergo covalent and enzymatic modifications that can result in oxidative damage and mitochondrial dysfunction, which can worsen disease. Through the use of free radicals and other messengers, these post-translational modifications also help to control mitochondrial function (Hu and Ren, 2016).

Since oxidative phosphorylation is a leaky process, each cycle of ATP production results in 0.2–5% of electrons passing through the ETC. This results in an incomplete reduction of oxygen (Hamanaka *et al*., 2013).

NADPH oxidases (NOX) and, to a lesser extent, cyclooxygenase (COX) 1/2, lipoxygenase, xanthine oxidoreductase (XOR), and cytochrome P450 are the metabolic enzymes that produce superoxide radicals (Finkel, 2003). Superoxide radicals have a limited ability to diffuse through biological lipid membranes because of their anionic characteristics. They are then reduced within the cells to produce hydrogen peroxide, hydroxyl radicals, peroxyl, alkoxyl, and hypochlorite ions, as well as peroxyl and alkoxyl radicals (Valko *et al*., 2007).

According to numerous studies, human cells have the ability to purposefully produce ROS at low doses as part of signaling pathways that control cell survival, proliferation, and defence against invaders (Sena & Chandel, 2012). Cells specifically produce superoxide radicals for physiological signaling via specific enzymatic systems, such as the NOX family (Bedard & Krause, 2007).

Under typical circumstances, oxygen is reduced to water by passing electrons through the mitochondrial ETC. However, about 1% to 3% of the electrons in this system manage to escape and produce superoxide (Ramsay, 2019). Humans also produce ROS from other internal sources, such as the oxidative bursts that occur when phagocytes kill bacteria and viruses, the metabolism of xanthine oxidoreductase (XOR), the arachidonate pathways, the metabolism of peroxisomes, and the detoxification processes (Birben *et al*., 2012).

Signals can be transmitted by a cell's redox state being modulated by ROS. According to Hussain *et al*. (2016) and Bhattacharyya *et al*. (2014), an imbalance in this defence mechanism can, however, result in damage to cellular molecules like DNA, proteins, and lipids, which in turn causes cell death via necrotic and apoptotic pathways. In phagocytic cells like neutrophils and macrophages during phagocytosis or stimulation with various agents through NADPH oxidase activation, the concept of stimulated ROS production was first described. This phenomenon is known as "the respiratory burst" due to the temporary oxygen consumption (Peake & Suzuki, 2004).

Free radicals and organic peroxides are by-products of cellular oxidative metabolism that are produced by metal-catalyzed oxidation of metabolites and oxidoreductases as well as during mitochondrial electron transport (Hussain *et al*., 2016). Along with reactive species like reactive aldehydes, malondialdehyde (MDA), and 4-hydroxy-2-non-enal, nitric oxide is also produced in a respiratory chain reaction under hypoxic conditions (Hussain *et al*., 2016). According to Bhattacharyya *et al*. (2014) and Hussain *et al*. (2016), an imbalance in ROS can damage cellular molecules and alter the redox status of the cell, which can lead to cell death.

3. Sources of ROS in the Male Reproductive System

Reactive oxygen species (ROS) are produced by both endogenous and external mechanisms in the male reproductive system (Darbandi *et al*., 2018). Examples of endogenous sources of ROS include leukocytes, immature spermatozoa, and varicocele (Das & Roychoudhury, 2022). Leukocytes, particularly neutrophils, contribute significantly to the generation of ROS in the ejaculate together with immature, aberrant, and nonviable spermatozoa (Fatima, 2018). According to Darbandi *et al*. (2018), exogenous factors that can result in ROS include changes in lifestyle, technological improvements, an increase in pollution, alcohol consumption, cigarette smoking, vaping, and physical stress. ROS may be formed during the process of preparing semen for assisted reproduction, which may then activate ROS (Das & Roychoudhury, 2022).

3.1. How ROS is generated in the male reproductive tract

Because they include at least one oxygen atom, reactive oxygen species (ROS), which have a short half-life, are unstable and highly reactive. ROS take electrons from neighboring molecules in order to become stable electrically. This process involves molecules losing electrons, which results in the creation of additional radicals and the beginning of a radical-chain reaction. The generated radical then engages in further interactions with adjacent molecules, maintaining the radical state until two radicals come together to form a stable bond (Gonsalvez *et al*., 2017; Bisht *et al*., 2017).

Human spermatozoa's midpiece contains an especially high number of mitochondria (Ramalho-Santos *et al*., 2009). An NAD(P)H-oxidase in the plasma membrane and a NADH-dependent oxidoreductase in the inner mitochondrial membrane are the main producers of superoxide, a kind of ROS (Gonsalvez *et al*., 2017; Bisht *et al*., 2017). Oxygen generated by oxidative phosphorylation and electron addition to intracellular oxygen between complex I and III of the electron transport chain is the primary ROS produced in human spermatozoa (Vinogradov & Grinennikova, 2005).

A prominent initiator of peroxidative damage to germ cell plasma membranes is hydrogen peroxide (H_2O_2), a membrane-permeable chemical (Agarwal *et al*., 2014). The Haber-Weiss process, which involves the reduction of ferric (Fe^{3+}) to ferrous ion (Fe^{2+}), can produce highly reactive hydroxyl radicals when transition metals like iron (Fe3+) and

copper are present (Kehrer, 2000). The Fenton reaction is a chemical reaction in which Fe^{2+} combines with H_2O_2 to produce Fe^{3+}, hydroxide (OH), and the extremely reactive hydroxyl radical. Additionally, nitric oxide (NO) and peroxynitrite can interact to form the latter, which then aids in necrotic or apoptotic cell death (Blaylock *et al*., 1998).

These previously known pathways can produce ROS inside the male reproductive system. Numerous mitochondria found in the flagellum's midpiece generate the significant energy needed for sperm movement. Due to electron leakage in the electron transport chain brought on by mitochondrial membrane potential disruption, ROS is produced. The acrosomal and midpiece areas of human spermatozoa include the Ca^{2+}-dependent NADPH oxidase, NOX5, which is a significant ROS producer and may cause oxidative stress. NOX5 was first discovered in the human testis. When Ca^{2+} binds to NOX5's cytosolic N-terminal EF-hand domain, NOX5 is activated, which results in conformational changes brought on by oxidative stress (Petrushanko *et al*., 2016).

Cytoplasm is ejected from growing spermatozoa during spermatogenesis. Incomplete extrusion or spermiogenesis disruptions result in the retention of extra cytoplasm surrounding the midpiece. Enzymatic equipment for the formation of ROS is present in this preserved cytoplasm. Increased intrinsic ROS production is sparked by obstructions to the removal of extra cytoplasm, which leads to oxidative damage to the plasma membrane and sperm DNA (Rengan *et al*., 2012).

According to Gharagozloo and Aitken (2011), the prostate and seminal vesicles are significant producers of peroxidase-positive leukocytes, which include macrophages and polymorphonuclear leukocytes. These cells create ROS at a rate that is roughly 100 times higher than usual in inflammatory responses (Lavranos *et al*., 2012). Increased NADPH generation via the hexose monophosphate shunt is what causes the increased ROS production, which is one of the cells' inherent defense mechanisms.

The World Health Organization (WHO, 2023) defined leukocytospermia as semen samples having more than one million peroxidase-positive leukocytes per milliliter of semen, leukocyte involvement in inflammation is directly related to leukocytospermia (Azenabor *et al*., 2015). According to Shiraishi *et al*. (2012) and Agarwal *et al*. (2006), varicocele, which is characterized by aberrant vein dilation in the pampiniform plexus surrounding the spermatic cord, is linked to elevated seminal ROS levels.

4. Importance of Oxidative Balance in Maintaining Sperm Health

For sperm health to be maintained, oxidative equilibrium must be kept in check. The balance between pro-oxidants and antioxidants is maintained in a condition of optimum health. Spermatozoa are capable of reducing oxidative stress and have antioxidant defenses (Agarwal *et al*., 2014). However, oxidative stress can seriously impair the quality of sperm, affecting their count, motility, morphology, and DNA integrity (Agarwal *et al*., 2014). This ultimately contributes to male infertility. According to Takalani *et al*. (2023), oxidative stress refers to the imbalance between various oxygen species, which can affect sperm and seminal fluid and affect male fertility.

In order to protect spermatozoa from the oxidative stress brought on by invading leukocytes, seminal plasma's antioxidant properties are essential (Agarwal *et al*., 2014). According to research, therapeutic antioxidant supplementation can have a positive impact on sperm concentration, motility, morphology, and DNA fragmentation (Mannucci *et al*., 2022). Improvements in sperm redox status and semen parameters were seen in a large majority of clinical trials, specifically nineteen out of twenty that focused on the effects of antioxidant therapy on seminal oxidative stress (Mannucci *et al*., 2022). These improvements showed a notable correlation with the success of pregnancies. It is emphasized that seminal plasma's antioxidant characteristics have a special role in protecting spermatozoa from the oxidative stress caused by invading leukocytes (Agarwal *et al*., 2014).

5. Mechanisms of Oxidative Damage to Sperm

The impact of reactive oxygen species (ROS) can be felt in either endogenous or exogenous ways, as has already been mentioned in the literature. This is comparable to the roles ROS play in the context of male infertility.

5.1. Exogenous Pathways

5.1.1. Psychological Stress

According to several research (Gollenberg *et al*., 2010; Lampiao, 2009), psychological stress is correlated with compromised semen characteristics and has been linked to idiopathic male infertility.

According to Flaherty *et al*. (2017), psychological stress can increase levels of norepinephrine and cortisol in the blood, which raises the quantities of reactive oxygen and nitrogen species (ROS) and reactive nitrogen species (RNS) inside of cells. According to Flaherty *et al*. (2017) and Bakunina *et al*. (2015), this rise causes adverse effects on cellular microstructures as well as the activation of the immunological and inflammatory systems.

According to Hardy *et al*. (2005), psychological stress has a direct effect on Leydig cells, further illuminating the effect it has on male reproductive capabilities. Through the regulation of androgen synthesis and the onset of death in Leydig cells, this impact inhibits the glucocorticoid action on Leydig cells, resulting in a fall in circulating testosterone levels (O'Hara *et al*., 2015). Additionally, stress alters the autonomic catecholaminergic actions during stressful times, which interferes with steroidogenesis. Due to this interference with Leydig cell activities, testosterone synthesis is reduced, and steroidogenic enzyme activities are repressed (Hardy *et al*., 2005).

5.1.2. Exercise

According to research by Adefuye *et al*. (2016), vigorous exercise may cause an excessive amount of reactive oxygen species (ROS) to be produced. Although the precise redox mechanisms are still not entirely understood, it appears that the main endogenous producers of ROS in skeletal muscle are mitochondria, NADPH oxidase (NOX), and xanthine oxidase (XO) (Adefuye *et al*., 2016).

According to several studies, moderate physical exercise can cause levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone to rise, which is associated with an increase in energy and muscle strength (Vaamonde *et al*., 2012; Grandys *et al*., 2009). Contrarily, there is data that suggests intense exercise may lower LH, FSH, and testosterone levels as well as possibly affect semen parameters, according to Safarinejad *et al*. (2009).

5.1.3. Heat Stress

Numerous studies have shown that a variety of factors, such as fever, using a sauna or steam room, sleeping position, spending a lot of time sitting or driving, using a polyester-lined athletic support, using a laptop on one's lap, an electric blanket, have been reported to negatively affect spermatogenesis (Garolla *et al*., 2013). In addition, it has been demonstrated that medical diseases such cryptorchidism, varicocele, and acute febrile illnesses raise testicular temperature and inhibit spermatogenesis (Jung & Schuppe, 2007).

The activation of the hypothalamic-pituitary-adrenal (HPA) axis and the ensuing rise in plasma glucocorticoid concentrations are important variables in the response to heat stress. According to Aggarwal and Upadhyay (2013), heat stress negatively affects male fertility in part through interfering with the normal release of GnRH from the hypothalamus, coupled with LH and FSH from the anterior pituitary gland.

Numerous investigations have shown that testicular heat stress decreases the levels of circulating LH and testosterone while increasing the levels of serum cortisol (Hansen, 2009). Additionally, testicular heat stress reduces testosterone manufacture in adult rats' testes and causes Leydig cells to undergo apoptosis (Li *et al*., 2016).

Additionally, increased testicular temperature has a negative effect on Sertoli cell activity, testicular androgen-binding protein synthesis, spermatogenesis, and semen characteristics. Because of this, elevated heat stress increases the production of reactive oxygen species (ROS) in the male reproductive tract, both directly by changing cellular metabolism (Belhadj *et al*., 2014) and indirectly by impacting stress hormone levels (Megahed *et al*., 2008). In turn, this increase in ROS production damages other endocrine cells as well as testicular germ cells, upsetting the balance of hormones and reducing male fertility (Argawal *et al*., 2014).

5.1.4. Smoking

As mentioned by Meri *et al*. (2013), there is a substantial mechanism involved in the production of male subfertility or infertility as a result of smoking. Due to the disruption of oxygen transport to the testis, this mechanism largely includes the generation of reactive oxygen species (ROS), which in turn impairs the elevated metabolic demands of spermatogenesis (Meri *et al*., 2013; Sheynkin & Gioia, 2013; Tostes *et al*., 2008). Smoking also causes the release of a variety of mutagens and metabolites, such as radioactive polonium, cadmium, benzopyrene, carbon monoxide, tar, naphthalene, and aromatic hydrocarbons, which all interfere with the normal development and operation of male reproductive organs (Meri *et al*., 2013; Sheynkin & Gioia, 2013).

According to Shiels *et al*. (2009), smoking can increase oxidative stress (OS) indirectly by impairing the effectiveness of antioxidant defense mechanisms in addition to directly producing reactive oxygen radicals in cigarette smoke. According

to research, smoking can affect Leydig and Sertoli cells primarily via altering plasma levels of testosterone, prolactin, estradiol, FSH, LH, and sex hormone binding globulin (SHBG) (Shiels *et al*., 2009).

5.1.5. Alcohol

As supplied by Wu and Cederbaum (2003), drinking alcohol promotes the production of reactive oxygen species (ROS) through the liver's metabolic pathway by activating the cytochrome P450 enzymes, changing the body's levels of specific metals (specifically free iron or copper ions), and ultimately lowering antioxidant levels.

As stated by Qureshi *et al*. (2005), any factor that increases the levels of specific metals can also promote ROS production and oxidative stress (OS) because these metals play a crucial role in the creation of hydroxyl radicals. According to research, alcohol not only increases the body's iron stores through the use of iron-rich alcoholic beverages like red wine, but it also improves the body's ability to absorb iron from food sources (Whitfield *et al*., 2001).

There is evidence linking alcohol use and higher estradiol levels in both animal and human research. This finding is significant since estradiol affects beta-endorphin release, which is typically linked to the negative consequences of alcohol intake (Emanuele & Emanuele, 2001). According to Maneesh *et al*. (2006), the effects of chronic alcohol use on the interconnections between the brain and endocrine systems have been linked to lower serum levels of testosterone, LH, and FSH. Kim *et al*. (2003) list other effects of alcohol, including disruption of the gonadotropin-releasing hormone (GnRH) molecule's cleavage from its precursor pre-pro GnRH and suppression of protein kinase C15 movement necessary for GnRH-stimulated LH and FSH release.

5.1.6. Food

According to Lobo *et al*. (2010), many antioxidant substances derived from plants have been proven to be efficient free radical and active oxygen scavengers. According to research, people who consume diets high in fat, caffeine (>800 mg/day), red meat, processed meat, pizza, sugary drinks, and sweets tend to have less favourable semen parameters than those who incorporate a diet rich in fish, fruits, vegetables, legumes, whole grains, and omega-3 and omega-6 fatty acids (Mendiola *et al*., 2009).

Synthetic and natural antioxidants are frequently added to both food and medicine as a way to make up for low vitamin intake through eating.

It is well known that eating meals high in fat and protein on a regular basis causes an increase in the production of reactive oxygen species (ROS) and oxidative stress (OS). According to research by Kolodziej *et al*. (2017) and Kahle *et al*. (2014), this happens through interfering with antioxidant defence mechanisms and mitochondrial metabolism. According to Chakraborty *et al*. (2016), this cascade disrupts hormone levels, which has a detrimental effect on semen quality.

Antioxidant therapy may have a beneficial effect on semen parameters by protecting semen from ROS, reducing oxidative stress, and improving essential sperm properties. Argawal and Sekhon (2010) supplied that these improvements can be linked to the stimulation of testosterone production, release of FSH and LH, rise of inhibin B levels, and augmentation of the androgen profile. According to Ahmadi *et al*. (2016)'s research, selenium, coenzyme Q10 (CoQ10), and N-acetyl-cysteine are significantly important in influencing semen parameters through mechanisms that increase testosterone and inhibin B.

5.2. Endogenous Pathway

5.2.1. Reproductive tract infections

Both under normal circumstances and during times of inflammation, testicular spermatogenic and somatic cells are in charge of releasing a variety of immunoregulatory and pro-inflammatory cytokines (Loveland *et al*., 2017). Notably, non-immune cells like Leydig cells and Sertoli cells, which generally exist as essential components of seminal plasma to maintain proper spermatogenesis, can even produce cytokines like IL-1, IL-6 (Loveland *et al*., 2017).

Reproductive tract infections can be caused by a variety of conditions, including ejaculatory duct inflammation, epididymitis, sexually transmitted infections such as gonorrhea, *Chlamydia trachomatis*, *Escherichia coli*, *Mycobacteria*, and *Ureaplasma urealyticum*, urethritis, testicular torsion, varicocele, and various other causes like chronic prostatitis, orchit (Joki-Korpela *et al*., 2009). Elevated levels of reactive oxygen species (ROS) can appear within the male genital tract, affecting parts like the prostate gland, seminal vesicles, or the epididymis (Azenabor *et al*., 2015). As the

inflammatory damage advances and the antioxidant defense deteriorates in response to the presence of bacterial strains (Joki-Korpela *et al*., 2009).

According to Dejucq and Jegou (2001), reproductive tract infections can alter testicular temperature after episodes of high fever, clog seminiferous tubules due to interstitial edema, or alter testosterone production. These factors all indirectly contribute to germ cell degeneration and the disruption of spermatogenesis.

5.2.2. Immature Spermatozoa

As part of the preparation for fertilization, developing spermatozoa undergo cytoplasmic extrusion throughout the process of spermatogenesis. However, spermiogenesis is interrupted in a portion of injured spermatozoa, resulting in the retention of extra cytoplasm at the midpiece. ERC stands for excess residual cytoplasm, which describes this syndrome. In response to ERC, the NADPH system is activated by the hexose-monophosphate shunt. As supplied by Rengan *et al*. (2012) and Hampl *et al*. (2012), this system acts as a source of electrons for the production of reactive oxygen species (ROS) and probable oxidative stress (OS) within spermatozoa.

5.2.3. Varicocele and Male Factor Infertility

Vascular abnormalities in the pampiniform plexus, which surrounds the spermatic cord, are what give varicocele its name. According to Will *et al*. (2011), this syndrome is present in about 40% of male partners in infertile couples, making it a major cause of male factor infertility. It has been demonstrated that the degree of seminal ROS correlates with the severity of varicocele. Particularly, increased ROS levels are linked to higher-grade varicoceles (Shiriashi *et al*., 2012).

5.2.4. Leukocytes and ROS Production

Polymorphonuclear leukocytes (50–60%) and macrophages (20–30%) are two types of peroxidase-positive leukocytes that fall into this group (Saleh *et al*., 2003). These leukocytes have seminal vesicles and the prostate as their primary sources of origin. Different intracellular or extracellular triggers, such as infection or inflammation, might cause these main sources of ROS to become active. According to Lavranos *et al*. (2012), this activation causes the release of ROS at levels up to 100 times higher than usual and an increase in the generation of NADPH via the hexose monophosphate shunt.

5.2.5. Inflammation, Cytokines, and OS

A respiratory burst can be brought on by elevated levels of proinflammatory cytokines like interleukin (IL)-8 and a decrease in the antioxidant superoxide dismutase (SOD), which in turn causes elevated ROS levels and eventually oxidative stress (OS). The World Health Organization defines seminal leukocyte concentrations above normal levels, as seen in leukocytospermia, as the presence of over one million peroxidase-positive cells per milliliter of semen (Lu *et al*., 2010; World Health Organization, 2010).

6. Beneficial Effects of Antioxidant Therapy on Semen Parameters and Reproductive Outcomes

Numerous studies have shown beneficial effects of antioxidant therapy on different areas of male fertility, including sperm function, live birth rates, sperm parameters, and assisted reproductive technology results (Cyrus *et al*., 2015).

6.1. α-Tocopherol (Vitamin E): Guarding Sperm Against Oxidative Stress

Tocopherol, also referred to as Vitamin E, is a fat-soluble chemical molecule that is essential for scavenging superoxide anions and hydroxyl free radicals. At the level of plasma membranes, this activity successfully suppresses lipid peroxidation caused by reactive oxygen species (ROS). Much research (Ourique *et al*., 2016; Adesiyan *et al*., 2011; Omu *et al*., 1999) have shown that there is a clear association between the level of vitamin E in semen plasma and the proportion of motile sperm present in the ejaculate.

Infertile men's sperm had noticeably decreased quantities of vitamin E, according to a 1999 study by Omu *et al*. Greco *et al*. (2005) also noted a significant decline in the proportion of sperm with lipid peroxidation in patients receiving vitamin E treatment over a six-month period. In cases of asthenozoospermia in particular, this treatment was linked to a potential increase in conception rates (Greco *et al*., 2005). Infertile men's sperm motility has been shown to increase when -tocopherol and selenium are combined (Adesiyan *et al*., 2011). Furthermore, Ourique *et al*. (2016) discovered that in rats given valproic acid treatment, vitamin E had a protective effect on sperm motility and oxidative stress.

6.2. Ascorbic Acid (Vitamin C): Countering Oxidative Damage

According to Majzoub and Agarwal (2018), semen plasma contains substantially more ascorbic acid, also known as Vitamin C, than blood serum does. They supplied that this vitamin is crucial in the neutralization of hydroxyl, superoxide, and peroxide radicals, protecting against endogenous oxidative damage. According to research, adding vitamin C (together with vitamin E) to the semen of people with normozoospermia and asthenozoospermia can lessen the DNA fragmentation brought on by ROS (Nouri *et al*., 2008).

6.3. Carnitines (L-Carnitine and L-Acetyl Carnitine): Nurturing Sperm Maturation

In the epididymis, sperm mature as a result of the action of carnitine. In comparison to blood plasma, the amount of free L-carnitine in the epididymal tail is 2,000 times higher. Through active epithelial pumps that are induced by androgens, this vital substance is delivered from the bloodstream to the epididymis (Arruda *et al*., 2010). Sperm cultivated in conditions containing carnitine exhibit better mobility and vitality in comparison to controls, according to numerous in vitro studies (Banihani *et al*., 2014).

In a study published in 2017, Abd-Elrazek and Ahmed-Farid showed that giving L-carnitine to adult oligospermic rats reduced the cytotoxic effects of busulfan, resulting in better sperm parameters, lessened oxidative stress, and prolonged cellular energy. Furthermore, Nazari *et al*. (2021) shown that 1500 mg of L-carnitine, a potent antioxidant, could improve sperm quality in infertile men by increasing cell concentration and general motility.

6.4. N-Acetylcysteine: Countering Oxidative Stress and DNA Damage

By scavenging hydroxyl and hypochlorous acid radicals, N-acetylcysteine (NAC), an amino acid precursor to glutathione, actively contributes to the direct decrease of oxidative stress. Jannatifar *et al*. (2019) concluded that NAC therapy increased the percentage and number of motile sperm while simultaneously lowering the number of sperm cells with defective morphology and deoxyribonucleic acid (DNA) damage. Barekat *et al*. (2016) demonstrated beneficial benefits of NAC on chromatin integrity and pregnancy rates in the setting of supplementary therapy following varicocelectomy.

6.5. Zinc: Guardianship of Sperm Structure and Function

According to Majzoub and Agarwal (2018), zinc is essential for the metabolism of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), signal transduction, gene expression, and the control of apoptosis. Additionally, studies show that zinc has a significant protective effect on sperm structure. Zinc supplementation has been demonstrated to reduce oxidative stress, apoptosis, and DNA fragmentation in asthenozoospermic patients' sperm (Isaac *et al*., 2017). The addition of zinc to sperm medium protects bull sperm from external oxidative stress and improves their ability to support embryo development, according to studies (Barbato *et al*., 2017).

6.6. Lycopene: Nature's Defender Against Oxidative Stress

According to Majzoub and Agarwal (2018) and Kelkel *et al*. (2011), the carotenoid lycopene, which is naturally generated and found in fruits and vegetables, contributes significantly to the human redox defense system by having strong ROS-quenching properties. According to research by Tvrda *et al*. (2016), lycopene has amazing ROS-scavenging and antioxidant characteristics, which may prevent oxidative stress from damaging sperm and maintain the functionality of male reproductive cells.

7. Conclusion

In conclusion, oxidative equilibrium is essential for preserving the health of sperm. Male infertility can result from oxidative stress, which drastically reduces the quality of sperm. It has been demonstrated that antioxidant therapy has positive impacts on sperm characteristics and pregnancy outcomes. Maintaining oxidative balance is therefore crucial for male reproductive health, and antioxidant supplementation may be a helpful tactic to enhance sperm quality in situations of male infertility. In terms of enhancing sperm function and reproductive outcomes, antioxidant therapy, which includes substances like -tocopherol, ascorbic acid, carnitines, N-acetylcysteine, zinc, and lycopene, holds out a lot of hope. Together, these therapies help to reduce oxidative stress and improve male fertility.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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