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Impact of Oxidative Stress on Male Reproduction: Amelioration by Melatonin and Some Selected Food-Grade Antioxidants

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Abstract

Infertility is a common issue. In India, it has doubled in prevalence, raising health issues in both individuals and communities. Couples who are infertile suffer emotionally, socially, and financially. The Indian Society of Assisted Reproduction estimates that there are up to 27.5 million infertile persons living in India, both men and women. Endocrine disruptors, environmental stress, and abnormalities/imbalances in the body's natural antioxidant defence mechanism all put male fertility at greater risk. Studies on how high altitude affects male fertility are very few. Recent research has shown that oxidative stress from different sources impairs spermatogenesis and causes an imbalance in the level of male hormones. There are two primary causes of oxidative stress: extrinsic and intrinsic, responsible for free-radical generation. There is an internal antioxidant defence mechanism that scavenges the reactive oxygen species, i.e., free radical generation which neutralizes oxidative stress. These antioxidants are important for the protection of cellular integrity. Apart from the antioxidants, various food supplements like melatonin, vitamin C, vitamin E, carotenoids, cysteines, etc., are suitable antioxidants for improving male fertility. Therefore, there is a great need for information on some clinically examined edible phytooxidants, including melatonin, for amelioration of oxidative stress-induced male infertility. This review focuses on the information available as of now about free radical (ROS)-induced reproductive damages in the plains and the high-altitude regions and the role of various antioxidants, including melatonin, in male infertility.

Keywords: Antioxidant, Male Infertility, Melatonin, Oxidative Stress, Spermatozoa.

1. Introduction

Nearly 30 million men nationwide suffer infertility, ranging between 2.5% and 15% globally[1,2]. Males account for about 40% of instances of infertility, and several diseases, including varicocele, cryptorchidism, hypogonadism, and hereditary factors, are known to play a role in the infertility process. Furthermore, numerous psychological, physiological, and sociocultural issues have been connected to infertility[3]. So, it is necessary to understand the mechanism and know the important factors of infertility in males to decipher the key stages and molecules for clinical management. In today's scenario, the terms oxidative stress, oxidative damage, free radicals load, and antioxidants are the most common terminologies in scientific research and have become an integral part of the scientific debate. Various studies revealed that some of the fundamental and common factors causing male infertility are cellular oxidative damage and free radicals mainly Reactive Oxygen Species (ROS). There is plenty of evidence suggesting that free radicals (oxidative stress) are related to male infertility by reducing sperm motility, sperm DNA damage, decreased sperm production, and various genetic disorders. So, it's critical to understand how cellular oxidative damage is linked with male infertility.

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Reproduction is an energy-demanding process[4]. The expense of reproduction stands as a fundamental concept in the realm of evolution and for the perpetuation of the species[4]. No doubt that reproduction requires energy[5], and to fulfill the energy needs, both basal and local metabolic rates must be enhanced. It is, therefore, suggested that the role of stress in reproduction is inversely proportional to the fertility rate of both males and females. Reproduction contributes to several other activities such as an increase in BMR, and a decrease in immunity and carbohydrate metabolism in several species[6-9]. Consequently, reproduction enhances energy expenditure by uplifting basal metabolic rate[10]. The higher rate of metabolism results in increased free radical production. Therefore, one might expect that reproduction increases vulnerability to oxidative stress; and it is also expected that reproduction will strengthen the body's antioxidant defence mechanisms.

The major target in reproduction affected by oxidative stress and free radicals in a variety of species is themembrane of spermatozoa (lipid peroxidation) thus impairing the motility of sperm[11-13]. So, one would expect that the effort of breeding can generate free radicals (oxidative stress) whose efficacy and forte would rely on the effectiveness and accessibility of the redox (antioxidant) defence system[14]. As for additional assessments to be carried out for the management and treatment of male infertility, opinions differ concerning which individuals should undergo testing for OS. Furthermore, there is not much information on an antioxidant-based therapeutic regime for male infertility. Meanwhile, the types, doses, and duration of antioxidant action for patients having extreme levels of free radicals in blood and semen are still controversial. Therefore, the present review aims to provide an update on indications about ROS, i.e., free radical production, and induction of oxidative stress associated with male infertility, its immediate/long-term therapeutic control, etc.

2. Role of Oxidative Stress in Male Reproduction

Due to the fast change in lifestyle opted by many people, dependency on processed cuisine, and exposure to a diverse array of chemicals and drugs, etc., play an important role in inducing oxidative stress. In the metabolic system, steady-state redox balance is maintained at a set value, and deviations from it lead to initiating oxidative stress[15]. In other words, when there is an imbalance between the generation of free radicals (ROS) and the body's antioxidant compounds it leads to the induction of oxidative stress[16,17]. ROS are very erratic and highly reactive molecules, that bind through a diverse group of biomolecules, for example, DNA, proteins, lipids, and a few of the carbohydrates[18-20]. Hence, Leydig cells, spermatozoa, etc., are vulnerable to ROS thereby affecting male reproductive physiology. The body's antioxidant defence system comprises endogenous and exogenous antioxidants that scavenge ROS and diminish their toxic effect on the cellular system[20].

The inflammation in testicular tissues causes infiltration of leukocytes and the production of immature sperm; these are other causes of ROS in human sperm[21,22]. ROS are produced in the process by which leukocyte activation and chemotaxis promote infection and inflammation of the male reproductive organs. The breakdown of pathogens triggers the leukocyte myeloperoxidase system, which produces ROS[23]. The excessive generation of ROS by leukocytes can ultimately result in oxidative stress in semen. These endogenous ROS are the primary factor in the production of abnormal and immature sperm[24]. Furthermore, cytoplasm deposits in the mid-piece fall off to cause cell elongation and compression[24].

There are mainly two different sources of oxidative stress - exogenous and endogenous. Exogenous sources are ionizing radiation, ultraviolet light, processed food, drugs (paracetamol, diclofenac, cisplatin, chlorpromazine, dexamethasone, etc.), various kinds of pollutants, etc., which lead to the generation of various free radicals (reactive molecules) such as ROS and Reactive Nitrogen Species (RNS). NOS, lipoxygenase, NADPH oxidase, mitochondrial Electron Transport Chain (ETC), and other naturally occurring enzymes aid in the production of different free radicals, including ROS and RNS. The mitochondrial ETC, NOS, NADPH oxidase, and lipoxygenase are examples of these endogenous sources (Figure 1).

Mitochondria are also responsible for the generation of many reactive molecules like ROS, besides essential cell organelles for ATP generation (Figure 2). Therefore, mitochondrial health is very important for maintaining spermatozoal motility and their forward progression during fertilization. The primary and major ROS is superoxide which is produced in mitochondria. Superoxide dismutase is an enzyme that helps to

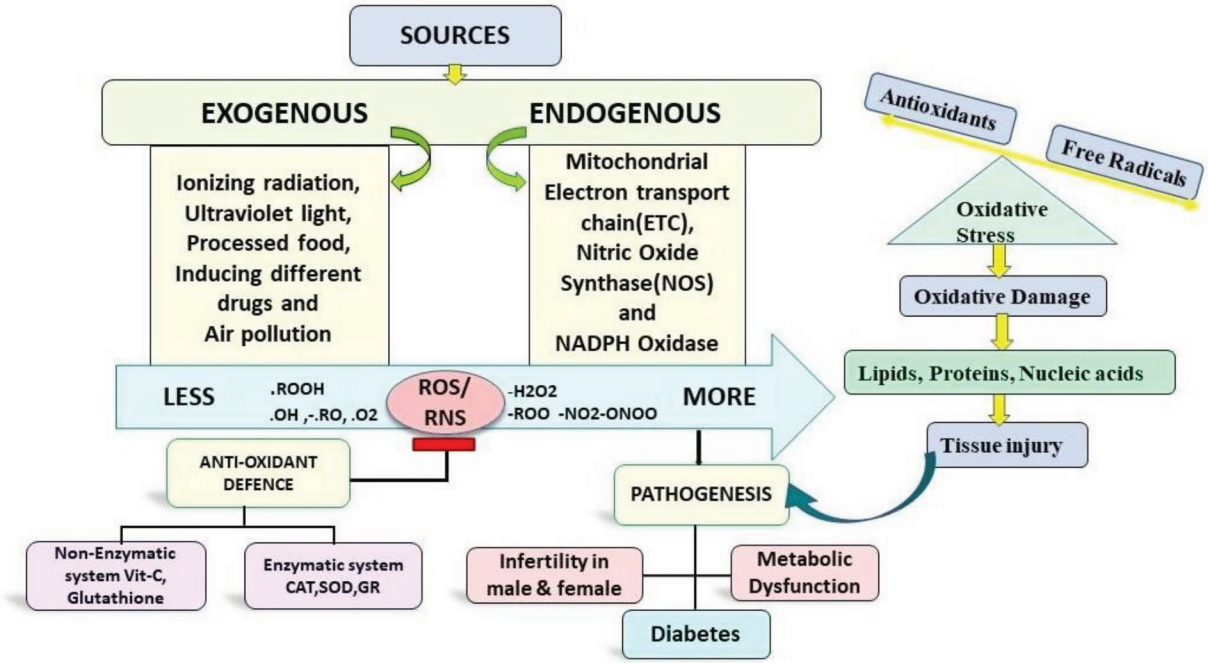

Figure 1. Pathophysiology of oxidative stress-induced male and female infertility.

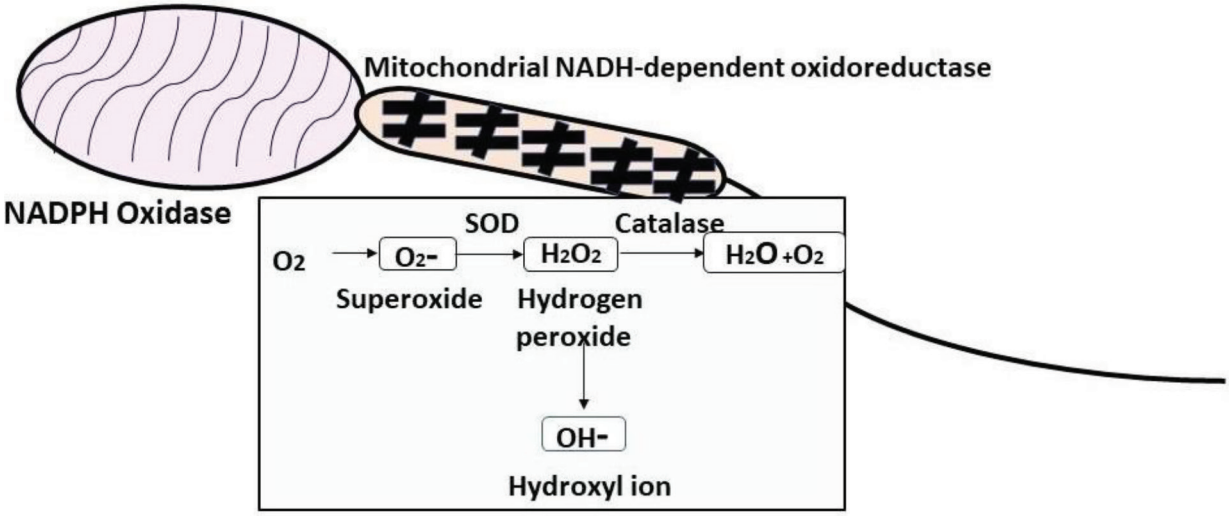

Figure 2. Pathophysiology of oxidative stress-induced mitochondrial dysfunction and spermatozoa damage in testes

convert harmful molecules of superoxide into hydrogen peroxide and molecular oxygen which ameliorates oxidative stress.

In NOS, Nitric Oxide (NO) is a principal factor released from normal endothelial and in inflammation, its production by the vasculature increases significantly and in combination with other ROS, contributes to oxidative stress. The plasma membrane contains the membrane-bound enzyme complex known as Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, which faces extracellular space. One of the main sources of cellular ROS is NADPH oxidases (NOXes), which continue to play a significant role in the production of ROS in normal conditions (Figures 2, 3). Lipid peroxidation is facilitated by lipoxygenases, which also catalyze the deoxygenation of polyunsaturated fatty acids in lipids and brain tissue[25,26]. So, concurring with the present status of knowledge on oxidative stress and male reproduction, we suggest that

a heightened vulnerability to oxidative stress might be a common cause of infertility in males.

3. Oxidative Stress-Mediated Male Infertility in the High-Altitude Environment

Extreme climate, cold stress, high UV-radiation, and hypoxia in high altitude environments cumulatively affect the hypothalamus–hypophysis–gonad axis, and several other cellular and physiological mechanisms associated with maintaining oxygen homeostasis, cardiovascular, respiratory, and reproductive functions in human and animal[27-29]. Furthermore, hypoxia induces ROS generation, which later affects cellular components like protein, carbohydrates, lipids, and DNA[30]. Since, spermatozoal integrity is maintained by cholesterol and lipid structure, which is significantly, affected under hypoxia-induced oxidative stress, spermatozoal damage is very common at high altitudes[27,31,32].

Several human and rodent studies reported imbalances in hormonal secretion, ovulatory activity, male infertility, and structural changes in testicular, ovarian, and prostate tissues due to hypoxia and oxygen deprivation at high altitudes[29-37]. Seminal examination revealed a significant reduction in sperm motility, volume, density, total counts, increased dimorphisms, and head damage in males exposed to hypoxia prevalent at high altitudes[27,28,32-37]. Therefore, the likelihood of becoming pregnant is reduced in both people and animals that live at high elevations. It was reported that reduction in sperm concentration and testicular dysfunction can be the consequences of physical exercise at high altitudes[38]. A recent study reported a decline in semen antioxidant potential in lowlander men presented to high altitude, whereas antioxidant activity was restored to some extent on 70 days onward arrival to the plain region[37]. Another simulation study depicting hypoxia in male rats revealed significant damage to seminiferous tubules, reduction in testicular and epididymis weights, higher apoptosis in germ cells, and low sperm concentration[28]. This damage to gonadal tissues also affects imbalance in reproductive hormones, viz. low levels of FSH, LH, testosterone, and estrogen. In conclusion, a high-altitude environment and hypoxia significantly affect the male reproductive functions. However, there are conflicting reports on the reversal of adverse changes in male reproductive physiology and structural changes due to exposure to high altitude after coming to low altitude and plain areas. This needs to be investigated separately in the pre-pubertal, puberty, and post-pubertal stages considering the growth phase and reproductive stage of males during high-altitude exposure and post-induction. Therefore, environmental and physical stress at high altitudes limit the performance and reproduction in lowlander susceptible animals and human beings, which can be improved through antioxidant supplementation and exposure normoxic environment.

4. Mechanism of Oxidative Stress-Induced Spermatozoa Damage

Since spermatozoa are very dynamic and motile, they require for their activation a continuous supply of energy so that they are ironic in mitochondria[19]. Thus, if the semen has dysfunctional spermatozoa, it significantly raises the production of ROS, and whenever the ROS level surges it affects mitochondrial activity and, later, functions performed by sperm, such as motility. Recent studies have shown that there is leakage of an electron from actively respiring spermatozoa which generates mostly ROS which are free radicals[38]. The ROS, such as hydroxyl (OH), superoxide (O_{2-}), nitric oxide (NO), peroxyl (RO_2), lipid peroxyl (LOO) and Thiol [RS-], and non-radical compounds (ozone [O_3], hydrogen peroxide [H_2O_2], lipid peroxide [LOOH], hypo chloric acid [HOCL], and singlet oxygen [$-1O_2$]), are generated which contain an unpaired electron that makes highly reactant to any biomolecules[18,19,39]. Excessive semen ROS has been found anywhere from 30 to 80 percent of male infertile[24]. Systems that produce ROS have also evidently demonstrated the vulnerability-bound hydrogen peroxide to be the most deadly oxygen metabolite concerning oxidative stress on sperm motility[40].

Within human spermatozoa, the majority of ROS is O_2 – (oxygen radical), and when O_2-reacts with H_2 it leads to the production of H_2O_2, and thereby OH radicals are generated. Due to the disturbance of membrane fluidity, these OH-radicals are the strong initiators of the LPO cascade and can cause sperm function to be lost[41-43]. Hence, sperm cells are prone to lipid peroxidation due to the high levels of unsaturated fatty acids they contain (Figure 3). Since the energy needed to dissociate carbon

and hydrogen is lowest at the mostly methylene position, the latter is more vulnerable to attack by free radicals. As of significance, the hydrogen constructs the process that starts lipid peroxidation get encouraged, producing a lipid radical with a carbon core that, when combined with oxygen, produces peroxyl (ROO•) and alkoxyl (RO•) radicals, which stabilize and eliminate hydrogen atoms from neighbouring carbons. Now, it is generally agreed upon that semen OS generates sperm cell membrane LPO, and male Sperm DNA Fragmentation (SDF), resulting in apoptosis[24]. So, it is clear that in situations where motility is normal, oxidative stress can impair spermatozoa's ability to fertilize[44].

There are two major methods of free radical production in spermatozoa: - (i) Nicotinamide adenine dinucleotide phosphate oxidase system present in the sperm plasma membrane may lead to the production of ROS. (ii) In the sperm mitochondria, the nicotinamide adenine dinucleotide-dependent oxidoreductase reaction may also lead to the generation of reactive molecules (Figures 2, 3).

5. Gene Responsible for Abnormality of Spermatozoa and Male Infertility

The gene *NOX5* in the sperm encodes an enzyme NOX5, a calcium-dependent NADPH oxidase found in the acrosomal and mid-piece regions which facilitates the production of O_2 in spermatozoa[45]. NOX5 was first discovered in the human testis. It is activated by calcium binding to its N-terminal domain, which causes changes in cell shape and leads to oxidative stress. This finding provides further evidence that NOX5 is a major source of ROS generation in human spermatozoa. It is unknown if people with infertility linked to Oxidative Stress (OS) have increased NOX5 in their spermatozoa[41]. The morphologically aberrant, immature spermatozoa generate intracellular nicotinamide adenine dinucleotide phosphate (NADPH) and maintain an excess of the remnant body that contains significant levels of the cytoplasmic glucose-6-phosphate dehydrogenase enzyme. Afterwards, NADPH oxidase NOX5, which is intramembrane-based, converts NADPH to ROS.

Epigenetic modifications include DNA methylation, acetylation, phosphorylation, etc. It has been noted that oxidative stress can affect gene expression despite altering the order of genes, which has ramifications for epigenetics. ROS directly modify DNA, which influences male fertility. It has been demonstrated that disruption of DNA methylation disrupts spermatogenesis, resulting in a drop in the number of sperm as well as an upsurge in infertility[46,47]. In addition, histone methylation dysregulation is linked to breaks in double-stranded

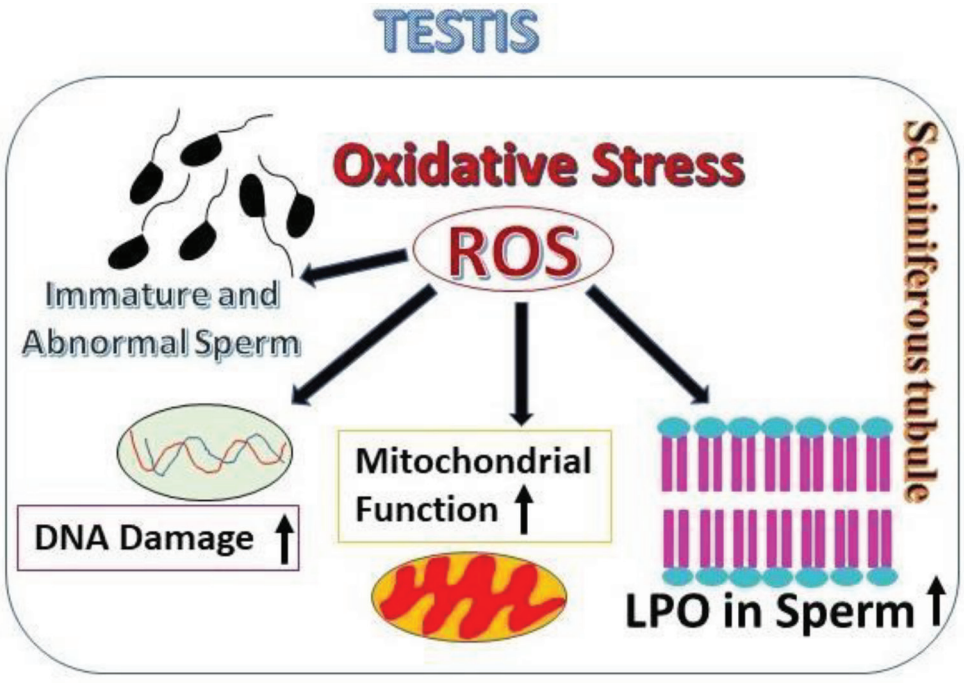

Figure 3. Oxidative stress-mediated spermatozoal dysfunction and male infertility

genetic material, with implications for infertility and the development of the next generation[48]. Lastly, it is believed that microRNAs that control spermatogenesis in an epigenetic manner are critical for preserving fertility. A study was conducted on male patients with infertility in non-obstructive azoospermia, the levels of three specific microRNAs (miR713p, miR429, and miR141) were significantly increased[49]. The epigenetic process linking oxidative stress to infertility is still under investigation. Once revealed, they provide a better understanding of molecular pathology, serve as therapeutic approaches, and afford insights regarding infertility components that are passed down from generation to generation[50].

6. Comparative Aspects of Oxidative Stress in Males and Females

The different results suggest that males might be more vulnerable to an increase in breeding effort, which is due to a decrease in antioxidant defence in comparison to females. ROS are essential for reproductive processes including ovulation, but when levels are too high, they can damage oocytes through oxidative stress and result in infertility. Melatonin's antioxidant action inside follicles may shield oocytes from ROS[51]. Reproductive processes including the growth of follicles, oocyte maturation, ovulation, fertilization, implantation, and the development of embryos are all impacted by ROS. In conjunction with the stimulation of vascularization within follicles, vascular endothelial cells and macrophages produce a significant quantity of ROS during the ovulation process following the Luteinizing Hormone (LH) surge[51]. The oxidative stress that ROS produced in follicles during ovulation causes in the oocyte and granulosa cells deteriorations of the integrity of the oocytes and may potentially lead to infertility[51]. The administration of melatonin may develop into a potential therapeutic approach to stop the aging-related decline in oocyte quantity and quality.

7. The Role of Food-Grade Antioxidants and Melatonin in Male Infertility

From various studies, it has been found that in a healthy male, two main mechanisms protect sperm DNA from OS. In the beginning, the genetic material is densely coiled and packed with the histone proteins known as chromatin to facilitate that the genetic materials are slightly bare to free radicals[52]. Secondly, there are many natural antioxidants present in spermatozoa and seminal plasma which leads to minimizing ROS production and maintenance to its normal levels[38]. These antioxidants prevent free radical formation by reacting with and then neutralizing them, which prevents OS onset and preserves the spermatozoa[53]. Antioxidants including coenzyme Q10 and lactoferrin are also present in sperm cells[54]. However, in a few infertile men, an insufficient number of antioxidants interferes with OS's complex stability and ends up in OS[38].

The body's antioxidant defence balance is crucial to keep the equilibrium among free radicals in addition to antioxidants; when it fails, the exogenous antioxidant supplements can play a preventive role. The foundation of oral antioxidant treatment is the understanding that initial oxidative damage develops because of increased ROS production and reduced seminal antioxidant status. Diet plays a very crucial role in day-to-day health benefits. So, diets rich in antioxidant contents can prevent the formation of oxidative stress in the testes so it must be consumed[54]. The exogenous antioxidant category includes a large number of oral antioxidants, which the body receives from food sources, e.g., Vitamin C, Vitamin E, Coenzyme Q10, N acetylcysteine, carnitines, zinc, selenium, and pentoxifylline. Numerous research reports have been carried out to evaluate the efficacy of oral intake of antioxidants as a male infertility treatment.

Numerous physiological functions that are essential to our bodies, such as breathing, digestion, metabolizing alcohol, drug exposure, heat, environmental damage, pollutants, and metallic substances play a role in the development of OS, so all these have been shown to contribute to OS. It is reported that oxidative stress is associated with the development of numerous metabolic, and chronic disorders or cancers[17,18,55]. It is also recognized, in addition, that activities that may cause a rise in the internal body temperature of the scrotal area such as lengthy, prolonged work hours, hot baths, gyms, and prolonged driving should be avoided. Lifestyle modifications can help to reduce the generation of ROS and perhaps address the disparities that lead to a state of oxidative stress.

7.1 Vitamin C and Carotenoids

Vitamin C and carotenoids are natural and essential antioxidants. They preserve the integrity of cell membranes

and control the process of spermatogenesis. They also play a role in regulating spermatogenesis. Citrus fruits and effervescent berries are good sources of water-soluble vitamin C, which has antioxidant qualities. Several studies have examined the effects of vitamin C consumption on spermatozoa and found noteworthy antioxidant benefits[56]. Furthermore, decreased Vitamin C and expanded ROS ranges had been found in the reproductive fluid (seminal fluid) of males having azoospermia[57]. Vegetable hues such as orange, red, yellow, and purple naturally include substances called carotenoids. These substances function as predecessors of vitamin A, of which retinol is a necessary element. Carotenoid deficiency can result in reduced sperm motility and male infertility[58].

7.2 Vitamin E

Vitamin E (alpha-tocopherol) is a biological compound that is fat-soluble and frequently distributed across cell membranes (Table 1). Its antioxidant activity is based primarily on suppressing ROS-induced peroxidation of lipids and removing the hydroxyl extra electron-containing molecule (free radicals) and superoxide. Therefore, alpha-tocopherol primarily offers protection from free radical damage in the components of the sperm membrane and, to a lesser extent, reduces ROS production. Therefore, Vitamin E primarily protects sperm membrane components from damage and, to a lesser extent, reduces ROS production. Its oral administration significantly increases sperm motility by reducing the malondialdehyde an end product of lipid peroxidation at the time of sperm production and is, therefore, a secondary marker of the intensity of intracellular processes[59].

7.3 Carnitine

Carnitine or levocarnitine is a naturally occurring amino acid that plays a role in mitochondrial transfer and oxidation of long-chain fatty acids, its chemical structure was established in 1927 as (3-hydroxy-4- trimethylamine butyrate) [60,61]. It serves as a vital substance that helps with the transportation of long-chain fatty acids within the mitochondrial matrix. This process facilitates cellular energy production through oxidation[62,63]. An important aspect here is the high levels of carnitine in the male reproductive tract, particularly the epididymis. This suggests that it plays an important function in the consumption of energy and maturation of sperm[64,65].

7.4 Melatonin

Oxidative stress-induced endocrine disruption has a deleterious effect on pineal gland secretion which affects the reduction in melatonin levels in the body[66]. Thereafter, it has a chain of effects on the biological clock and metabolic function. Melatonin, a neurohormone, is an excellent free-radical scavenger. It scavenges/neutralizes free radicals (ROS) which are produced by oxidative stress. Melatonin also activates the major enzymatic antioxidant mechanisms of body tissues[67,68] and is also found in human sperm[69]. Melatonin, with its antioxidant and anti-apoptotic effects, up-regulates antioxidant defence mechanisms and inhibits apoptosis, thus preventing dexamethasone-

Table 1. Physiological and ameliorative role of some food-grade natural antioxidants in male reproduction and fertility

Sl. No.	Name of Antioxidant	Role and their action	Effects
1	Vitamin C	Counterbalance free radicals	Protects viability as well as motility.
2	Vitamin E	Neutralizes free radicals	Enhances the function /activity of many antioxidants and reduces the oxidation of lipids.
3	Cysteines	Reduced glutathione (GSH) formation is increased by cysteines	Prevent lipid peroxidation.
4	Pentoxifylline	An important second messenger cAMP breakdown is prevented and it conquers the formation of pro-inflammatory factors	Reduces the peroxidation of lipids
5	Carotenoids	Quenche singlet molecular oxygen	Limit the oxidation of lipids
6	Carnitine	An energy source and balance between free radicals and antioxidants by reducing reactive molecules (free radicals)	Prevents lipid peroxidation and also protects from damage to the DNA
7	Bilirubin	Extracellular antioxidants	

induced testicular damage[70]. In addition to eliminating the very harmful hydroxyl radical (OH)[71,72], it has been reported that melatonin also eliminates many reactive oxygen and nitrogen species such as singlet oxygen ($1O_2$;)[73], nitric oxide (NO)[74], peroxynitrite anion (ONOO) and/or its metabolites[74], and hydrogen peroxide (H_2O_2;)[75]. In addition to these, melatonin changes the enzyme activities which metabolize free radicals[76-78] and alters membrane fluidity[79,80]. This reduces the ability of damaging species to attack macromolecules in this structure.

8. Conclusion

Oxidative stress and free radicals are known to be detrimental to the health of a healthy individual. This review indicates that oxidative stress is central to structural and DNA damage of spermatozoa and induces male infertility in plain and high-altitude regions. Therefore, the evaluation of antioxidant enzymes, oxidative stress parameters, and body antioxidant levels are necessary for new diagnostic methods for diagnosing diseases related to reproduction. In addition to assessing sperm oxidative stress, blood redox status, and leukocyte ROS level, this also opens new possibilities and small applications for specialists to test sperm quality and monitor fertility. There are various antioxidant supplements available, including melatonin, which may be used in male infertility to ameliorate free radicals-mediated/induced male infertility. Therefore, dealing with the redox parameters may help reduce systemic oxidative stress as an aspect of male infertility. It can help develop novel treatment strategies by taking antioxidant supplements to improve the detection along management of male infertility.

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