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Role of Antioxidants in Male Reproduction: Review

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To counter oxidative stress, cells constitutively express enzymes that detoxify the reactive oxygen species and repair the damage. An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The antioxidant enzymes are major cell defence against acute oxygen toxicity. The functions of these antioxidant enzymes are to protect the membrane and cytosolic components against damage caused by free radicals. Glutathione peroxidase, Superoxide dismutase, glutathione-s-transferase, catalase, xanthine oxidase. Amongst a variety of antioxidants; vitamin E antioxidant use is essential because it travels through the body in molecules called lipoproteins and protect them from oxidation. For many years, vitamin E considered as an anti-sterility factor. In the male reproductive system, vitamin C is known to protect spermatogenesis, and it plays a major role in semen integrity and fertility in men. It increases testosterone levels and prevents sperm agglutination. It is an important chain-breaking antioxidant, contributing up to 65 % of the total antioxidant capacity of seminal plasma found intracellularly and extracellularly. N-Acetyl cysteine reacts with highly oxidizing radicals such as $^{\bullet}OH$, $^{\bullet}NO_2$, $CO_3^{\bullet-}$, and also bind redox-active metal ions. Thiosl can also afford radioprotection through the donation of reducing equivalents.

Keywords: *Antioxidants; Male fertility; Sperm quality.*

Introduction

To counter oxidative stress, cells constitutively express enzymes that detoxify the reactive oxygen species and repair the damage caused by them [1]. The defenses are inadequate, however, if the rates of intracellular $O_2^{\cdot-}$and H_2O_2 formation are accelerated. Fortunately, the human body makes several antioxidants. The most important is ubiquinol and glutathione. Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase also destroy free radicals [2].

Antioxidants act at different levels in the oxidative process by scavenging initiation of free radicals, binding metal ions, scavenging peroxyl radicals, and removing oxidatively damaged bio-chemicals [3]. Some antioxidants must be provided as micronutrients; they include ascorbic acid, beta-carotene, and vitamin E and trace metals such as selenium.

An antioxidant is any substance that when present at low concentrations compared to those of an

oxidizable substrate significantly delays or prevents oxidation of that substrate [4]. The term oxidizable substrate includes almost everything found in living cells, including proteins, carbohydrates, lipids, and DNA. Antioxidants are compounds capable of providing free radicals with the electrons they are missing while remaining stable themselves, thus preventing a cascade of interactions that could create even more free radicals [5].

Mode of action of antioxidants

Chain breaking reaction, e.g. a-tocopherol, By reducing the concentration of reactive oxygen species, e.g. Glutathione.

Chelation of transition metal catalyst. In this way, transferring, lactoferrin and ferritin function to keep iron-induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrates [6-9*].*

Endogenous Antioxidant Defense: The antioxidant enzymes are dominant cell defense against acute oxygen toxicity. The functions of these antioxidant enzymes are to protect the membrane and cytosolic components against damage caused by free radicals. Glutathione peroxidase, Superoxide dismutase, glutathione-s-transferase, catalase, xanthine oxidase are some of the more essential antioxidants.

Enzyme antioxidants

Superoxide dismutase [10]: The SOD catalyzes the alteration of the superoxide radical into hydrogen peroxides. Superoxide dismutase (SOD) is found in all oxygen-consuming organisms, and some aero-tolerant anaerobes [10]. SOD trims down the transition metal ions, such as iron and it gets converted to most reactive radical - the hydroxyl radical. Thus, elimination of hyperoxide radical can attenuate the formation of hydroxyl radical.

SODs are of three types depending on some obligate anaerobes.

The superoxide dismutase catalisze the dismutation of superoxide to hydrogen peroxide

$O_2^- + O_2^- + 2H^+ \; H_2O_2 + O_2$

The hydrogen peroxide must then be removed by catalase or glutathione peroxidase, as described above. There are three forms of SOD in mammalian tissues, each with a specific subcellular location and different tissue distribution.

Copper-zinc superoxide dismutase (CuZn-SOD):

Manganese superoxide dismutase (Mn SOD):

Extracellular superoxide dismutase (ECSOD):

Catalase [11]: Catalase was the first antioxidant enzyme to be characterized and catalyzes the two-stage conversion of hydrogen peroxide to water and oxygen.

Catalase – Fe (III) + H_2O_2 compound I

Compound I + H_2O_2 catalase–Fe(III)+$2H_2O$+ O_2

Catalase, a heme-containing protein located in the peroxisomes of most tissues, removes hydrogen peroxide within the cells by catalyzing the breakdown of hydrogen peroxide.

Catalase is present in all major body organs, is primarily concentrated in liver and erythrocytes. Catalase consists of four protein subunits, each of which contains one molecule of NADPH bound to it, which helps to stabilize the enzyme [12]. In erythrocytes, it exists in a soluble state. It plays an important dual role: firstly, an actual catalytic role in the decomposition of hydrogen peroxide to water and oxygen and secondly, a peroxidic role in which the peroxide is utilized to oxidize a range of H donors (AH_2) such as methanol, ethanol, and formate. In each case, an active enzyme-hydrogen peroxide complex is formed initially followed by an exceedingly rapid second state in which the second molecule of hydrogen peroxide serves as H^+ donor for the enzyme-hydrogen peroxide complex. The catalase enzyme is mostly localized in microbodies of liver and kidney and found in other cells in much smaller aggregates [13].

Other Antioxidant Enzymes: GPx and GST are amongst the remaining enzymes. GPx is tetramers with selenium (generally known as se-GPx) per molecule. GPx catalysis the reaction of reduced glutathione (GSH) to the oxidized form of glutathione at the expense of hydrogen peroxides [11].

GPx is present in all animal tissues, especially very much active in the liver and moderately active in heart, lung and brain and very low activity in muscle. Non-se-GPx is generally present in cytosol has been demonstrated in mitochondria [14]. GST is thought to play a physiological role in initiating the detoxification of potential alkylating agents [15]. These compounds catalyze the reaction of such compounds with the –SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water soluble. The cytosolic GSTs are mainly divided into four subunits: a, m, p and q. [16].

Glutathione (GSH): The tripeptide glutathione (g-glutamyl-cysteinyl-glycine) is involved in many critical cellular functions, ranging from the control of physicochemical properties of cellular proteins and peptides to the detoxification of xenobiotics and free radicals [17]. It also protects cells against the toxic effects of oxygen, by reacting directly or enzymatically with reactive oxygen species (intermediates), and less directly, by maintaining other compounds which have antioxidant activity, such as ascorbate and a-tocopherol, in reduced form.

The oxidation-reduction state of the reduced glutathione oxidized glutathione couple (GSH/GSSG) is of significant importance in cellular metabolism since it is the most extensive mobile thiol redox system of the cell [18]. Cellular transhydrogenase serves to maintain NADH and NADPH in equilibrium. The NADPH generating systems regulate the cellular concentration of GSH.

Exogenous Antioxidants: This is not produced by the body, but supplied externally and remains in body for a much longer time and show antioxidant action.

Vitamin E (a-Tocopherol): A wide range of literature suggests the usefulness of vitamin E. Amongst the variety of usage; its antioxidant use is essential because vitamin E travels through the body in molecules called lipoproteins and protects them from oxidation. For Many years, vitamin E considered as an anti-sterility factor [19].

It breaks the chain reactions of lipid peroxidation. This is important because lipid material plays a critical role in membranes, low-density lipoproteins, hormones, and many tissues, including nerves.

Lipids are very susceptible to oxidation from free radical attack [20-22]. Vitamin E refers to a group of eight naturally occurring tocopherols: alpha, beta, gamma and delta and four tocotrienols with the same first Greek names. Alpha-tocopherol is the most abundant of the eight, and it is by far the most effective in supporting reproduction in its antioxidant effects. The other members of the family are also important. Tocotrienols retard the activity of liver enzymes that play a vital role in the synthesis of cholesterol. Gamma-tocopherol is effective in fighting nitrogen-based free radicals.

Vitamin E is well accepted as the first line of defense against lipid peroxidation, protecting polyunsaturated fatty acids in cell membranes through its free radical quenching activity in

biomembranes at an early stage of a free racist attack. MDA concentration was prevented by treatment with vitamin E; it may help in the prevention of against production of free radicals [22].

Vitamin E influences the cellular response to OS through modulation of single transduction pathways [23]. Insufficient intake of vitamin E has been reported to produce harmful effects on the process of healthy sperms [2].

Vitamin E effect on male reproduction [24-27]:

Vitamin E was considered as an anti-sterility vitamin, and it associated with the normal function of the male reproductive system [2].

Vitamin E supplementation could potentially attenuate the adverse effects of stress on testicular physiology and endocrinology [25].

It maintains the membrane integrity that safeguards of cellular function.

Reduces the TBARS production maximum under control and stress conditions [24].

It can inhibit reactive oxygen species by terminating lipid peroxidation and stabilize the molecular composition of cellular membranes; therefore, it prevents the harmful effects of ROS on cells [2].

Vitamin E activates the development of smooth surfaced ER in Leydig cells, suggesting the enhancement of steroidogenesis. It plays a vital role in testosterone biosynthesis [28].

Testicular mitochondrial membranes contain abundant polyunsaturated lipids, which are highly susceptible to oxidation injury, resulting in gonadal dysfunction. Vitamin E supplementation reduces oxidative tissue injury in testis [19].

Vitamin E ameliorates oxidative stress in spermatozoa helping to maintain optimum fertilizing ability [24,27].

It mainly regulates the oxidation process in the body as it acts as a powerful antioxidant preventing membrane damage mediated by free radicals [2].

It enhances spermatogenesis (total sperm count, quality, concentration), sperm membrane fluidity, and lowers the incidence of abnormal sperm production by inhibition of lipid peroxidation [24, 27,29].

Increase reproductive performance, the viability of sperm [24].

It protects spermatozoa’s from oxidative damage, as well as the loss of motility, and increase sperm function [26].

It protects spermatozoa by preventing from endogenous oxidative DNA and membrane damages.

Vitamin C (Ascorbic acid) [30,31]: Vitamin C is an important antioxidant substance in biological systems. It is a water-soluble micronutrient, well absorbed by the gastrointestinal tract, required for multiple biological functions and biochemical reactions in humans and animals. It is an important element for the body. It is an essential nutrient for the biosynthesis of collagen, L-carnitine, and norepinephrine as humans are unable to synthesize vitamin C, so humans need small amount this vitamin in their diet. Thus, prolonged deprivation of vitamin C generates defects in the posttranslational modification of collagen, followed by illness and eventually death.

In the male reproductive system, vitamin C is known to protect spermatogenesis, and it plays a

significant role in semen integrity and fertility both in men and animals. It increases testosterone levels and prevents sperm agglutination. It is a critical chain-breaking antioxidant, contributing up to 65 % of the total antioxidant capacity of seminal plasma found intracellularly and extracellularly.

The hydrophilic ascorbic acid located in the aqueous phase cannot scavenge lipophilic radicals within the lipid reagents of the membranes and lipoproteins. Ascorbic acid reacts rapidly with O_2^-, $HO_2^{\cdot}$ and even more quickly with $OH^{\cdot\cdot}$ to give semidehydrous ascorbate.

Ascorbic acid acts as a synergist with tocopherol and functions as an antioxidant even when the oxidation proceeds also proposed that ascorbic acid plays a crucial role in restoring 'vitamin E pool' of the system by reacting with tocopherol radical. Vitamin C and vitamin E reacts rapidly with free radicals, and it is widely accepted that the antioxidant properties of these compounds are responsible in part of their biological activity [32].

Vitamin E is considerably lipophilic than vitamin C and in biomembranes is the more potent antioxidants, particularly concerning lipid peroxidation; penetration to a precise site in the membrane may be an essential feature of the protection against highly reactive radicals. It is known these two vitamins act synergistically, vitamin E reacting as the primary antioxidant and the resulting vitamin E radicals, then reacting with vitamin C to regenerate vitamin E [33]. Enzymatic systems exist in vivo to reduces the NADP dehydroascorbate to ascorbate, which was in agreement with findings by [34].

In certain circumstances ascorbic acid acts as a pro-oxidant rather than as an antioxidant, In fact, the iron ascorbate mixture has been used as an initiating system in in-vitro experiments summarized the antioxidant roles of vitamin E and vitamin C as under.

Effect of Vitamin c reproductive function:

It acts on the hypothalamic-pituitary-testicular axis, cause elevation of testosterone levels, protects the cells from sperm oxidative stress, and increases sperm concentration, and decreases the loss of sperm motility [35,36].

β- Carotene: Carotenoid pigments are widely distributed in nature where they play an essential role in protecting cell and organisms. Vitamin A or b- Carotene quenches singlet oxygen. It may act as an unusual kind of chain-breaking antioxidant.

N-Acetyl Cysteine(NAC) [14, 37]: N-Acetyl Cysteine(also known as NAC, N-Acetyl Cysteine, N-Acetyl-L-Cysteine) is the amino acidL-Cysteineplus anacetyl(-CO-CH_3) group attached to the amino (NH_2) group.

Amino acids which contain a sul-group haveproperties. The acetyl group is responsible for more water-soluble and functions to speed absorption and distribution on orally ingested cysteine. The acetyl group also reduces the reactivity of the thiol(**-SH**), making NAC less toxic and less susceptible to oxidation than cysteine. NAC has relatively low toxicity and is associated with mild side effects such as nausea, vomiting, rhinorrhea, pruritis, and tachycardia. NAC is safe, even in large doses, and is a better source of cysteine than cysteine itself. The hydrogen atom in the sulfhydryl(**-SH**) group of many sulfur-containing anti-oxidant molecules(**thiols**) can act as an electron for neutralizing free-radicals.

Historical use of N-acetyl* cysteine:** *NAC* has been used as an antioxidant precursor to glutathione (γ-glutamylcysteinylglycine; GSH) in the treatment of paracetamol overdose for more than 30 years. The NAC clinical applications have also broadened.N***-acetyl cysteine is now widely used as a mucolytic and in the treatment of HIV, and it has reported efficacy in COPD, contrast-induced nephropathy and patients with Alzheimer disease.

NAC as an antioxidant [14]: NAC reacts with highly oxidizing radicals such as ˙OH, ˙NO_2, CO_3˙⁻, and also bind redox-active metal ions. Thiols can even afford radioprotection through the donation of reducing equivalents i.e.., the carbon-centered radicals formed on DNA backbone or protein by ˙OH attack can be restituted via hydrogen donation from RSH.

Biological activities of NAC [38]

Regulation of cell cycle and apoptosis

It boosts natural cellular antioxidant systems such as glutathione

Carcinogenesis

Tumor progression

Mutagenesis

Gene expression and signal transduction

Immune modulation

Cytoskeleton and trafficking

Mitochondrial functions

NAC reduced the levels of Pro-inflammatory cytokines TNF-α, IL-β, IL-6, IL-10, and NF-kB in rodents [38].

Mechanism: It primary mechanism involves serving as a precursor of cysteine and replenishing cellular GSH levels, Additional mechanism includes scavenging of ˙OH, ˙NO_2, CO_3˙⁻, and thiyl radicals as well as detoxification of semiquinones, HOCl, HNO, and heavy metals. Importantly under physiological conditions, NAC does not react with NO, SOD, H_20_2, and peroxynitrite.

Insignificant routes under physiological conditions, NAC's potent direct antioxidant effects, ability to reduce viscosity in body secretions provide an additional asset. These unique futures of NAC contribute overall boosting effect on sperm and semen quality. As an antioxidant, NAC has been shown to decrease the concentrations of destructive ROS in human semen, contributing to improvements in motility. The same**600 mg/day**reduces semen viscosity, makes the sperm more motile to move forward and reach their goal [39].

CONCLUSION

Antioxidants been shown to decrease the concentrations of destructive ROS in human semen, contributing to improvements in the quality of sperm by acting at different stages of defense mechanism. Antioxidants are associated with the normal function of the male reproductive system

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