THE EFFECT OF OXIDATIVE STRESS ON SPERMATOZOA

D. A. L. Munasinghe

ABSTRACT

Free radicals are atoms, molecules or ions with unpaired electrons. As they have unpaired electron, they try to attack biomolecules and steal electrons to be paired and stable. Thus, free radicals are highly reactive. The objective of this literature survey is to find out the way of synthesis of body free radicals and their effect on spermatozoa. the information regarding the topic was gathered by searching the relevant data bases during the period of December 2023 to March 2024. Free radicals are produced in normal body metabolic mechanisms such as ATP synthesis and phagocytosis in macrophage. In addition, they are generated due to exposure the body to environmental factors such as radiations, pollutants, smokes. Ingestion of certain medications and foods also create free radicals in the body. Free radicals have both beneficial and adverse effects. Thus, the regulation of body free radical level is a must. Antioxidants are the molecules which neutralize free radicals**.** This includes a system of enzymes, vitamins and biomolecules which capable of breakdown of free radicals or neutralizing them by donating electrons. When free radical level exceeds its reference level, imbalance of free radical is occurred and the condition is known as oxidative stress. This is harmful to the body tissues as free radicles damage them. Oxidative stress can cause damage to spermatozoa also. They attack unsaturated fatty acids of the plasma membrane of sperm cells. Further, radicals attack nitrogen bases of DNA, hence fragmatization of DNA. Thus, sperm cells get destroyed and altered morphologically. The mortality of the cells is also reduced. These all may cause male infertility. Anyway, free radicals cause stimulation of acrosome rection of sperm which required for the fertilization of sperm with eggs/ovum. Finally, the outcome of the review study could be considered as a platform for launching for further studies on the topic and to consider the management of oxidative stress with regard to male fertility.

Keywords: oxidative stress, reactive oxygen species, spermatozoa, male fertility,

INTRODUCTION

Free radicals are atoms, molecules or ions with unpaired electrons. The presence of an unpaired electron may give rise to certain common properties to free radicals such as high unstableness and high reactiveness. They can either donate or accept an electron from other molecules for the purpose of stabilization. Therefore, they can behave as oxidants or reductants. Most of the biological free radicals contains oxygen (Ming *et al.,* 2010). Hydrogen peroxide, hydroxyl radical, superoxide anion radical, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical are the example for them.

As it is mentioned, the high reactiveness and unstableness of free radicals may result in them to attack other biomolecules, cellular membranes and tissues of the body to steal electrons form them. This may cause to damage of those tissues or organs. Damage of biomolecules such as DNA, proteins, enzymes, carbohydrates, and lipids is detrimental to the relevant tissue. Not only that it may cause to homeostatic disruption in the body also. Anyway, a level of free radical is required to the body for its normal healthy functions. Free radicals help fight off pathogens that may lead to infection. However, free radicals themselves are also required in minor amount for sperm in regulating certain biological functions such as sperm capacitation, the acrosome reaction, sperm hyper activation, binding to zona peelucidae and sperm-oocyte fusion (Lamirande *et al.*, 1997).

Free radicals and other Reactive Oxygen Species (ROS) are derived either from normal enzymatic and non-enzymatic metabolic pathways which occurs in the human body or from external sources. Enzymatic metabolic pathways such as respiratory chain in mitochondria, phagocytosis in neutrophils and macrophage, prostaglandin synthesis in tissues, and cytochrome P-450 system occurs in liver, produce considerable number of free radicals. Additionally, non enzymatic pathways such as oxidation (reactions of oxygen with organic compounds) and ionizing rection also contribute free radicals to the body (Lobo *et a.,l* 2010). Exposure to X-rays, ozone, cigarette smoking, air pollutants, medicine, food and industrial chemicals are the external sources/procedures which make free radicles. Electrons (e-) released form the hydrogens of NADH/FADH2 in the electron transport system which occurs in the inner mitochondrial membrane, jumps along the enzymatic chain which is located in the inner membrane. this jumping electron may push the H+ from matrix of mitochondria in to intermembrane space and create H+ gradient either side of the inner membrane (between intermembrane space and matrix). Along this gradient H+ comes back to the matrix from intermembrane space through ATP synthase enzyme (locate in the inner membrane) and it's coupled with the synthesis of ATP. The jumped electron finally leaked out from the chain and is accepted by oxygen to make either water with extra H+ or free radicals (superoxide radicals (O_2•-). Sometime through a series of reactions the superoxide radicals generate hydroxyl (OH•) radicals. Anyhow, the perfusion stage after an ischemic condition makes more free radicals via these pathways rather than normal physiological stage.

In the process of phagocytosis in macrophage, reactive oxygen species such as superoxide ($O_2^{-\bullet}$) are made. The NADPH oxidases in macrophage transfer one electron from the cytosolic donor NADPH to a molecule of extracellular oxygen, producing $O_2^{-\bullet}$ (superoxide) these are highly reactive and are utilized by the macrophage to attack bacteria or viruses who were phagocytosed

(Schneider & Oliveira, 2004). During inflammation, prostaglandins are made in tissues via cyclogeneses pathways from arachidonic acid (fatty acid). This pathway also releases certain reactive oxygen species/free radicals. Cytochrome P-450 enzymatic system which catabolize drugs, toxins and chemicals may also release oxygen free radicals in the metabolism of them. In the catabolism of nucleic acid via xanthine oxidase enzyme also produce free radicals. heavy exercise too induces free radicles in the body. Cells which are exposed to abnormal environments such as hyperoxia (high oxygen level in blood), could generate reactive oxygen species. Ionizing radiation is well known to generate oxygen radicals (mostly the singlet oxygen). Further the high heat, light (ultraviolet) can also produce ROS in body tissues. The damaging effects of radiation are higher in well oxygenated tissues than the tissues deficient in oxygen. Radiolysis of water can give rise to hydroxyl radicals. External toxins such as cigarette smoke and air pollution are huge sources for generating free radicals (Mathies, 2014). Pesticide which could be accumulated in certain food and water can act as free radicles itself (Mathies, 2014). Consumption of excessive amounts of alcohol also triggers the substantial production of free radical in the body. Stress and inflammation are the other causes for free radical production (Horward, 2014). The consumption of some specific foods such as high glycemic foods, processed meats like salami, sausages, and bacon, pawa beans also give rise to free radicals to the body

Antioxidants, these may be the molecules that can neutralize free radicals by accepting or donating electron(s) to eliminate the unpaired condition of the radical. Many antioxidants have aromatic ring structures and they are able to delocalize the unpaired electron. Further, the antioxidants down regulate the expression of enzymes which are responsible for free radical synthesis. Thus, the enzymes such as cyclooxygenase, NADPH oxidase, xanthine oxidase, cytochrome P-450, are controlled by antioxidants. In addition to that antioxidants upregulate the enzymes mentioned below which control free radicals by neutralizing them (Figure 1).

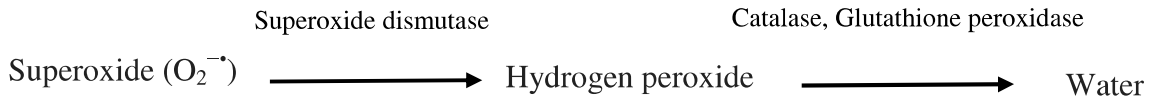

Figure 1: Neutralization of free radicals by enzymes

Though free radicals are harmful in certain amount or situation, they are inevitable to the human body. There is a balance of synthesis and neuralization of free radical in the body. Maintenance of this balance is done by body antioxidant system. When the free radical exceeds this balance, imbalance of free radicals is occurred and it's called as oxidative stress. this may cause damageable effect (Schneider & Oliveira, 2004). Scientists define oxidative stress as "an excess production of reactive oxygen species (ROS) relative to antioxidant defense."

Harmful effect of oxidative stress. are;

- diabetes

- cardiovascular problems like heart disease, hardening of the blood vessels, and high blood pressure
- inflammatory conditions
- Parkinson's disease
- Alzheimer's disease
- various types of cancer
- ageing
- fertility issues (male and female)

METHODSS AND MATERIALS

This literature review study was made basically on the articles pertaining to oxidative and male infertility. Thus, to gather the information on the topic; online journals, online magazines, relevant websites from databases (National Library of Medicine, Springer, Research Gate) and published books (Library of the Faculty indigenous Medicine, University of Colombo) and book chapters were searched and screened during the period of December 2023 to March 2024. All the gathered information was reviewed and analyzed to find the effect of male oxidative stress on sperm cells.

RESULT AND DISCUSSION

In the male, oxidative stress affects pathologically and physiologically on male reproductive cells/spermatozoa (Legacy 2022).

The spermatozoa itself synthesizes the ROS in two locations such as plasma membrane and mitochondria.

- Plasma membrane synthesis.

The cellular oxygen is converted into superoxide by the enzyme NADPH oxidase (locate in plasma membrane) amidst NADPH which provides the reducing power. NADPH is generated from glucose via hexose monophosphate shunt by the action of cytosolic enzyme, glucose-6-phosphate-dehydrogenase (G6PD). The superoxide produced in this way is utilized for the germ-killing purposes of the cell.

- Mitochondrial synthesis

Electron transport system which occurs inside of the inner membrane of mitochondria is the major source of spontaneous superoxide radicals (Agarwal & Saleh, 2002).

In addition to the spermatic contribution, following cells and situations also contribute the ROS to semen.

- Leukocytes

Peroxidase positive neutrophils and macrophages, which are mainly contributed by prostate and seminal vesicle are the major sources of production of ROS in semen (Agarwal & Saleh, 2002). The capacity of production of ROS in them depends on the stimuli such as inflammation, infections which are likely to accelerate the activity of certain ROS relevant enzymes such as G6PD, NADPH oxidase, myeloperoxidase in leukocytes. Further, it has been studied that these leukocytes themselves lure spermatozoa to generate ROS

- Differentiation of precursor germ cells

Male germ cells produce a little amount of ROS at their stages of differentiation (Sharma & Agarwal, 1996).

- Abnormal spermatozoa

When spermatogenesis is impaired, immature spermatozoa with extra cellular plasma is origin. These cells are functionally defective and contribute for excessive generation of ROS due to their excess enzymes and other relative raw materials (Agarwal & Saleh, 2002). Normally hydrogen peroxide is the main ROS generated in human spermatozoa (Agarwal & Saleh, 2002). The cells that contribute ROS (Widlansky & Gutterman, 2011) are as follows;

- Endothelial cells
- Fibroblasts
- Mesangial cells

The situations that contribute ROS (Headman, 2006)

- Varicocele
- Vasectomy reversal
- Urogenital tract infections
- Diabetes
- Physical exercises

Seminal plasma contains enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase/reductase, and catalase. Moreover, non-enzymatic antioxidants such as ascorbate/vitamin C, alpha tocopherol/vitamin E (Agarwal & Saleh, 2002), L-carnitine, acetyl-carnitine, coenzyme $Q_{10,}$ Vitamin B_{12}, uric acid, glutathione, taurine, hypotaurine, and albumin are also available in the plasma (Tremellen, 2008). However, compared to other body cells spermatozoa has low concentration of antioxidant enzymes and they can't protect the plasma membrane that surrounds the acrosome and tail area sufficiently. Thus, sperms are more prone to be damaged by ROS.

The ROS found in semen can affect negatively spermatozoa on various ways.

- Lipid peroxidation

The plasma membrane which surrounds the sperm composes of polyunsaturated fatty acids (PUFA) which are liable to be peroxidized by ROS. This may lose membrane fluidity and cause unable to fuse with oocyte properly. When the reaction occurs in tail area the motility of sperm is affected (Tremellen, 2008). Further, certain aldehyde end products of peroxidation such as 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE), and 2-propenal (Acrolein) can form adducts with sperm proteins, which may induce apoptosis and deleterious effects on human sperm function (Sanchez *et al.*, 2014). Further, cross linking of protein also may lead to impairment of acrosomal reaction (Tremellen, 2008).

- Decrease of phosphorylation of axonemal protein

ROS effects on sperm motility via phosphorylation of membrane protein (Agarwal & Saleh, 2002). Additionally, hydrogen peroxide which infiltrate into the sperm from the semen inhibits the activity of G6PDH and reduces the cellular amount of NADPH. Thus, the reduction of oxidized glutathione is impaired and cellular free radical damage is enhanced. This may accelerate the peroxidation of membrane phospholipids further more.

- DNA damage

Tight packaging and antioxidant system of sperm save its DNA from ROS attack. Exposure of DNA to ROS could modify nitrogenous bases, make base free sites, make deletions, make frame shifts, make cross links of strand, and make chromosomal rearrangements and breakage of the strand as well. Further, very labile hydrogen that can be abstracted and the prevalence of double bonds between base pairs also make DNA vulnerable for free radical attack (Nimse & Dilipkumar, 2015).

- Apoptosis

ROS may promote apoptosis, a process in which the body removes old and senescent cells (Agarwal & Saleh, 2002). Thus, the process could lead to decrease the sperm concentration.

Evidence suggests that ROS mediated damage to sperm is significant and its prevalence is around 30-80 % among infertile men (Tremellen, 2008). Higher concentrations of ROS can pathologically affect sperm metabolism, motility as well as viability (Tremellen, 2008). However, this doesn't sound that the ROS has a complete negative effect on sperm physiology due to the fact, that a low level of ROS is required for the maturation of sperm (Sanocka & Kurpisz, 2004). Thus, the scientific studies on the facts of true pathological and physiological relationship between free radicals and male infertility are required timely in the modern society (Gutteridge, 1994; Agarwal & Saleh, 2002). The ROS found in semen can be due to spermatic as well as non spermatic contribution.

How to avoid exposure to exogenous factors to regulate oxidative stress? (Poljsak, 2011)

- Quit smoking, if you are a smoker.
- Wearing a mask in areas with high levels of air pollution.

- Include a lot of greens in your diet and avoid added sugars and trans-fat, and reduce the number of processed foods you ingest. Try the Mediterranean diet.
- Apply sunscreen.
- Acknowledge about the medications you obtain.
- Have a good night's sleep (chronic sleep loss can lead to oxidative stress).
- Treat for medical condition which lead to free radicals (e.g. varicocele)
- Consider consumption of antioxidants (coenzyme Q10, vitamin C, or vitamin E, blueberries, strawberries, raspberries, kale, spinach, beets, and beans)

CONCLUSION

Free radicals/reactive oxygen species (ROS) are made in the body during metabolic reactions. They have both advantages as well as disadvantages of the body. Disadvantages are seen when free racial exceed the balance. Most of the body cells, tissues, biomolecules are attacked by the free radical to steel electron to get them stabilized. This may cause cell damage. The spermatozoa, the important cell of the male reproductive system, is also affected in various ways by ROS. The positive impact is, the radicals help stimulate acrosome reaction to facilitate the fertilization. Negative impacts are attacking them on DNA, sperm plasma membrane to cause cellular damage and alteration of morphology (teratozoospermia) and motility (asthenozoospermia). To minimize the harmful effect of free radicals, they should be regulated. The human being has an antioxidant system to achieve this function. This survey study could be a good platform for more studies on the topic and perform a meaningful work for the society with regard to fertility issues.

REFERENCES

1. Agarwal, A., Saleh, R. A. (2002) Role of oxidants in male infertility: rationale, significance, and treatment, *The Urology Clinic of North America*. PubMed. (Online) 29 (4), 817. http://www.ncbi.nlm.nih.gov/pubmed/12516754 (Accessed 12th September 2023)
2. Gutteridge, J. M. (1994) Biological origin of free radicals and mechanisms of antioxidant protection, Chem. Biol. Interact (PubMed), 91 (2), 133-140. http://www.ncbi.nlm.nih.gov/pubmed/8194129 (Accessed 12th December 2014)
3. Horward, D. (2014) what is a free radical, International Dermal Institute (postgraduate education in skin) IDI Articles. (Online) http://www.dermalinstitute.com/us/library/22_article_What_Is_A_Free_Radical_.html (Accessed 12th August 2016)
4. Jian-Ming, L.U., Peter, H.L., Qizhi, Y., Changyi, C (2010) Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems, *Journal of cellular and molecular medicine,* Volume 14 (4): pp 840–860. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2927345/
5. Legacy (2022) Oxidative stress and male fertility https://www.givelegacy.com/resources/oxidative-stress-and-male-fertility/
6. Lobo, V., Patil, A., Phatak, A., Chanfra, N. (2010) Free radicals, antioxidants and functional foods: Impact on human health, Pharmacognancy Rev, Volume 4(8): pp118–126. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249911/
7. Lamirande, E., Jiang, H., Zini, A., Kodama, H. & Gagnon, C. (1997) Reviews of reproduction, Reactive oxygen species and sperm physiology. (Online) 2, 48-54. http://www.reproduction-online.org/content/revreprod/2/1/48.full.pdf (Accessed 5th November 2015)
8. Mathies, C. G. (2014) how antioxidants work, Webmed (Online) http://www.webmd.com/food-recipes/features/how-antioxidants-work1 (Accessed on 15th November 2016)
9. Nimse, S. B. & Dilipkumar, P. (2015) Free radicals, natural antioxidants, and their reaction mechanisms, Royal society of chemistry. (Online) 5. http://pubs.rsc.org/en/content/articlehtml/2015/ra/c4ra13315c (Accessed on 18th July 2016)
10. Poljsak, B (2011) Strategies for Reducing or Preventing the Generation of Oxidative Stress, Oxidative medicine and cellular longevity, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3236599/
11. Sharma, R. K. & Agarwal, A. (1996) Role of reactive oxygen species in male infertility. (Online) 48 (6), 835. http://www.ncbi.nlm.nih.gov/pubmed/8973665 (Accessed 4th September 2014)
12. Schneider, C. D. & Oliveira, A. R. (2004) Oxygen free radicals and exercise: mechanisms of synthesis and adaptation to the physical training. Rev Bras Med Esporte. (Online) 10 (4), 1-5. http://www.scielo.br/pdf/rbme/v10n4/en_22047.pdf (Accessed 24th June 2016)
13. Sanocka, D. & Kurpisz, M. (2004) Reactive oxygen species and sperm cells, *Reproductive Biology and Endocrinology.* (Online) *2 (12),* 1186. http://www.rbej.com/content/2/1/12 (Accessed 4th June 2015)
14. Tremellen, K. (2008) Oxidative stress and male infertility- a clinical perspective. *Oxford Journals.* (Online) 14 (3), 243-258. http://humupd.oxfordjournals.org/content/14/3/243.full (Accessed 4th September 2016)
15. Widlansky, M. E. & Gutterman, D. D. (2011) Regulation of Endothelial Function by Mitochondrial Reactive Oxygen Species, Antioxid Redox Signal (Online) 15 (6), 1517–1530. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3151425/ (Accessed 7th September 2016)