



Role of Oxidative Stress in Male Infertility

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Abstract:

Male factors has been considered a major contributory factor to infertility, along with conventional causes of male infertility such as varicocele, cryptorchidism, infections obstructive lesion cystic fibrosis, trauma a new, yet important cause has been identified, oxidative stress(OS). The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa, leukocytes can cause damage to sperm. Mammalian spermatozoa membrane is very sensitive to ROS attack as they are rich in polyunsaturated fatty acids. ROS attacks the fluidity of sperm membrane, damages mitochondria & DNA integrity.

OS has been defined as one of the etiological factor in male infertility. In this article, we discuss the need of ROS in normal sperm physiology, the mechanism of ROS production, lipid peroxidation & its implications on sperm function. We also highlight the depletion of antioxidant system due to overproduction of ROS & need to evaluate oxidative stress as it can be an additional adjunct to routine semen analysis.

Keywords: male infertility, ROS, LPO,OS, Antioxidants

Infertility is defined as the inability to conceive after 1 year of unprotected intercourse by couples of reproductive age^[1]. In males it is inability to impregnate in a year after unprotected intercourse & in case of females; it is inability to conceive in a year after unprotected intercourse. Male factor has been considered a major contributory factor to infertility.

During some recent decade's interest in the study & treatment of causes of male infertility decreased substantially due to the introduction of effective method of laboratory treatment as in-vitro fertilization (IVF) and in-vitro fertilization – intracytoplasmic sperm injection (IVF-ICSI). In 1978 the first IVF baby in the world was born. A major breakthrough in the management of severe male infertility occurred in 1992 with the birth of first baby conceived with the assistance of IVF-ICSI.

As a result, the trend has been bypassing evaluation a male partner of a infertile couple and directly proceeding with IVF or ICSI. In majority of the clinics worldwide the diagnosis of male infertility relies solely depends upon the findings of semen analysis. The result of semen analysis is mainly interpreted by laboratory scientist (generally biologist) or gynecologist who knows little about the clinical history or clinical finding of the real man. Changes in the semen analyses among the infertile patients are significant and may give clue to the cause of male infertility.

There are a number of common causes of infertility in men. There is good evidence that diet, lifestyle and nutritional supplementation can have impact on man's fertility. The pathophysiological causes are hormonal and gonadal disorders, medical conditions such as cystic fibrosis and sickle cell anemia, renal

diseases and urological conditions such as cryptorchidism, varicoceles, testicular cancer, and reproductive tract trauma and/or obstruction. These factors and disorders can temporarily or permanently cause impaired spermatogenesis, diminish sperm migration in the male and female reproductive tracts due to defective motility, result in poor morphology and affect sperm function (acrosome reaction, sperm oocyte fusion), all of which can prevent conception. Many of these conditions increase the production of reactive oxygen/nitrogen species causing oxidative stress, which leads to spermatozoal dysfunction and infertility^[2].

Oxidative stress (OS) has been attributed to affect the fertility status and thus, it has been studied extensively in recent years. Spermatozoa, like any other aerobic cell is constantly facing the 'oxygen-paradox'^[3]. Oxygen is essential to sustain life as physiological levels of reactive oxygen species (ROS) are necessary to maintain normal cell function. Conversely, its breakdown products such as ROS can prove to be detrimental to cell function and survival^[4]. OS has also been implicated in the pathogenesis of many other human diseases such as atherosclerosis, cancer, diabetes, liver damage, rheumatoid arthritis, cataracts, AIDS, inflammatory bowel disease, Parkinson disease, motor neuron disease, and conditions associated with premature birth^[5].

ROS are highly reactive oxidizing agents, with one or more unpaired electrons belongs to the group of free radicals. ROS have a tendency toward chain reaction, in such a manner that "radical begets radical". Most common of those having potential implications in reproductive biology include superoxide (O₂⁻) anion, hydrogen peroxide (H₂O₂),

peroxyl (ROO[•]) radical and the very reactive hydroxyl (OH[•]) radical.

Excessive generation of ROS in semen may be associated with reduced sperm fertilizing potentials. Spermatozoa are rendered dysfunctional by lipid peroxidation and altered membrane function, together with impaired metabolism, morphology, and motility^[6]. Excessive generation of ROS in semen, mainly by neutrophils but also by abnormal spermatozoa, could be linked with infertility. Lipid peroxidation triggers the loss of membrane integrity, causing increased cell permeability, enzyme inactivation, structural damage to DNA, and cell death^[7]. Polyunsaturated fatty acid in the phospholipids of the human spermatozoa is highly susceptible to peroxidation. Oxygen free radicals generated by spermatozoa may be involved in the production of spermicidal cytotoxic end products^[8]. Malondialdehyde (MDA), an end product of lipid peroxidation (LPO), represented the level of lipid peroxidation. High lipid peroxidation as represented by MDA levels may cause changes in the sperm and diminish fertility. Human spermatozoa possess the defense enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) which might be useful in the prediction of sperm fertilizing potentials^[9].

Sources of ROS in male reproductive system

1. Leukocytes, particularly neutrophils and macrophages, have been associated with excessive ROS production, and they ultimately cause sperm dysfunction^[10].
2. Another important source of ROS is immature and morphologically abnormal spermatozoa^[11].
3. The production of ROS is also increased by lifestyle factors such as smoking and pollutions. Smoking increases ROS production, causing sperm DNA damage, and suppresses antioxidants in both semen and serum^[12].

Reactive oxygen species production by spermatozoa

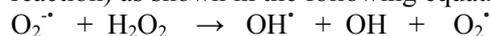
Clear evidence suggests that human spermatozoa can produce ROS^{[13][14][15]}. Levels of ROS production by sperm correlate negatively with quality of sperm in the original semen^[16]. The link between poor sperm quality and increased ROS generation lies in the retention of excess residual cytoplasm (cytoplasmic droplets) in abnormal spermatozoa. When spermatogenesis is impaired, the cytoplasmic extrusion mechanisms are defective. Spermatozoa released from the germinal epithelium carrying surplus residual cytoplasm are thought to be immature and functionally defective^[17].

Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose-6-phosphate-dehydrogenase (G6PD). This enzyme controls the rate of glucose flux through the hexose monophosphate shunt, which, in turn, controls the intracellular availability of nicotinamide adenine dinucleotide phosphate (NADPH) (Fig.1). The latter is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH-oxidase^[18].

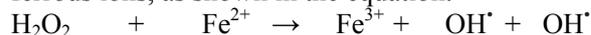
Spermatozoa may generate ROS in two ways:

1. As a result of the NADPH-oxidase system at the level of the sperm plasma membrane.
2. As a result of the NADH-dependent oxidoreductase (diphorase) at the level of mitochondria^[13].

The mitochondrial system is the major source of ROS in spermatozoa from infertile men. The primary ROS generated in human spermatozoa is the superoxide anion (O₂^{•-}). This one-electron reduction product of O₂ secondarily reacts with itself in a dismutation reaction, which is greatly accelerated by superoxide dismutase, to generate hydrogen peroxide (H₂O₂). In the presence of transition metals such as iron and copper, H₂O₂ and O₂^{•-} can interact to generate the extremely pernicious hydroxyl radical (OH[•]) (Haber-Weiss reaction) as shown in the following equation:



Alternatively, the hydroxyl radical can be produced from hydrogen peroxide (Fenton reaction), which requires a reducing agent such as ascorbate or ferrous ions, as shown in the equation:



The hydroxyl radical is thought to be an extremely powerful initiator of the lipid peroxidation cascade and can precipitate loss of sperm functions.

Reactive oxygen species production by leukocytes

Peroxidase-positive leukocytes are the major source of ROS in semen^{[19][20][21]}. Peroxidase-positive leukocytes include polymorphonuclear leukocytes, which represent 50% to 60% of all seminal leukocytes, and macrophages, which represent 20% to 30% of all seminal leukocytes^{[22][23][24]}. Peroxidase-positive leukocytes in semen are contributed largely by the prostate and seminal vesicles^[25]. The capacity of leukocytes to generate ROS depends on their activation, which may occur in response to a variety of stimuli, including inflammation and infection^[26]. During activation, NADPH production is increased, and the

myeloperoxidase system of leukocytes is activated, leading to a respiratory burst with subsequent release of high levels of ROS^[27]. Such an oxidative burst is thought to be an effective early defense that kills the microbes in cases of infection^[28].

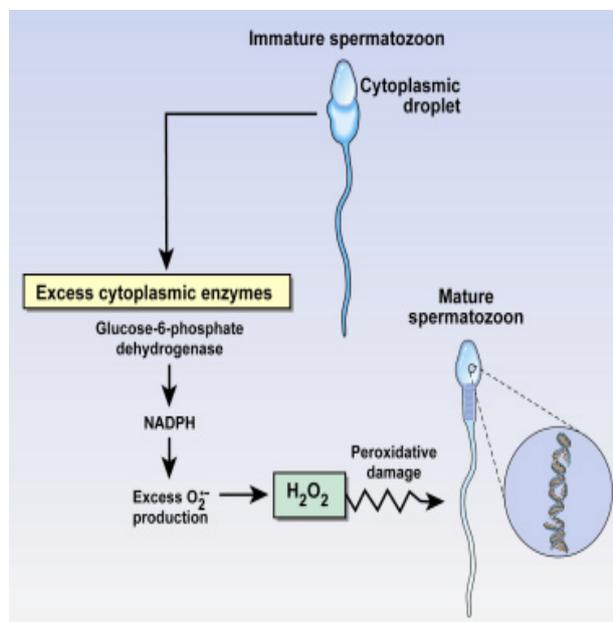


Figure:1 Mechanism for the link between oxidative stress and sperm DNA damage. Immature spermatozoa characterized with cytoplasmic droplets have deranged redox metabolic activity and greater ability to produce ROS. In turn, oxidative stress leads to damage of the sperm DNA damage.

Effect of ROS on sperm function

A. ROS and sperm function

Until recently, ROS were exclusively considered toxic to the human spermatozoa. However a strong body of evidence suggests that small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities^{[28][29][30]}. The ultimate goal of spermatozoa is the successful fertilization of ovum resulting in normal conception. In order to achieve this, the spermatozoa after spermiation must mature within the male genital tract, travel through the female reproductive system, undergo capacitation and acrosome reaction, bind to and penetrate the zona pellucidae of the ova as well as the oolemma, and finally fuse with the female pronucleus. Low levels of ROS can enhance the ability of human spermatozoa to bind with zona pellucida. Other studies have found that incubating spermatozoa with low concentrations of hydrogen peroxide stimulates sperm capacitation, hyper activation, acrosome reaction and oocytes fusion^[31]. Reactive oxygen

species other than hydrogen peroxide such as nitric oxide and superoxide anion have also been shown to promote sperm capacitation and acrosome reaction^[32]. Theoretically, cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities i.e. positive oxidative stress status (OSS), a situation in which there is a shift towards prooxidants, because of either excess ROS or diminished antioxidants. Levels of antioxidants in seminal plasma from infertile men are significantly low^[33]. However pathological levels of ROS detected in semen from infertile men are more likely a result of increased ROS production rather than reduced antioxidant capacity of the seminal plasma^[34]. Mammalian spermatozoa are rich in polyunsaturated fatty acids and, thus, are very susceptible to ROS attack which results in a decreased sperm motility, presumably by a rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability, and increased midpiece morphology defects with deleterious effects on sperm capacitation and acrosome reaction^[35]. Lipid peroxidation of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to infertility^[36].

B. Lipid peroxidation of spermatozoa and its biological implications

Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen activation in biology. LPO is broadly defined as "oxidative deterioration of PUFA" which are fatty acids that contain more than two carbon carbon double bonds^[33]. The most common types of LPO are: (a) nonenzymatic membrane LPO, and (b) enzymatic (NADPH and ADP dependent) LPO. The enzymatic reaction involves NADPH cytochrome P-450 reductase and proceeds via an ADP-Fe⁺³ O₂⁻ (perferryl) complex^[37]. In spermatozoa, production of malondialdehyde (MDA), an end product of LPO, which can be assayed by the thiobarbituric acid (TBA) reaction, which is a simple and useful diagnostic tool for the measurement of LPO for in vitro and in vivo systems^[38]. In general, the most significant effect of LPO in all cells is the perturbation of membrane (cellular and organellar) structure and function (transport processes, maintenance of ion and metabolite gradients, receptor mediated signal transduction, etc.). Spermatozoa, unlike other cells, are unique in structure, function, and susceptibility to damage by LPO^[39]. Spermatozoa are unable to repair the damage induced by excessive ROS because they

lack the cytoplasmic enzyme systems that are required to accomplish this repair. This is one of the features that make spermatozoa unique in their susceptibility to oxidative insult^[40].

C. Impairment of sperm motility

The increased formation of ROS has been correlated with a reduction of sperm motility^{[33][41]}. The link between ROS and reduced motility may be due to a cascade of events that results in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity that is necessary for sperm oocyte fusion^[42]. Another hypothesis is that H₂O₂ can diffuse across the membranes into the cells and inhibit the activity of some enzymes such as glucose 6-phosphate dehydrogenase (G6PD). Inhibition of glucose-6 phosphate dehydrogenase (G6PD) leads to a decrease in the availability of NADPH and a concomitant accumulation of oxidized glutathione, which in turn can reduce the antioxidant defenses of the spermatozoa and peroxidation of membrane lipids^[43].

D. Oxidative stress induced DNA damage

Increased ROS formation and lipid peroxidation results in oxidative stress which can damage mitochondrial DNA. The oxidative damage to mitochondrial DNA is well known to occur in all aerobic cells, which are rich in mitochondria and this, may include spermatozoa. ROS induces DNA damage in the form of modification of all bases (primarily guanine via lipid peroxy or alkoxy radicals), production of base-free sites, deletions, frame shifts, DNA cross-links through covalent binding to MDA, and chromosomal rearrangements^[44]. ROS can also induce oxidation of critical -SH groups in proteins and DNA, which will alter structure and function of spermatozoa with an increased susceptibility to attack by macrophages^[45]. In addition, the redox status of human spermatozoa is likely to affect phosphorylation and ATP generation with a profound influence on its fertilizing potential^[46]. It is recently showed that stimulation of endogenous NADPH-dependent ROS generation in human sperm appears to regulate acrosome reaction^[47] via cAMP mediated, tyrosine phosphorylation^[48]. In general, the oxidizing conditions increase tyrosine phosphorylation with enhanced sperm function while reducing conditions have the opposite effect. However, this has been debated for a long time, and it is still not clear whether sperm have a NADPH-dependent

oxygenase system. Nonetheless, how these mitochondrial DNA or membrane changes regulate specific sperm functions in association with altered tyrosine phosphorylation is an interesting area for further investigation. These studies may open a new series of diagnostic tool in clinical infertility to assess sperm function and damage.

Antioxidants

Antioxidants Studies have shown that antioxidants protect spermatozoa from ROS producing abnormal spermatozoa, scavenge ROS produced by leukocytes, prevent DNA fragmentation, improve semen quality in smokers, reduce cryodamage to spermatozoa, block premature sperm maturation and stimulate spermatozoa and improve ART outcome. Seminal plasma contains superoxide dismutase, catalase, and glutathione peroxidase / glutathione reductase in addition to non-enzymatic antioxidants such as ascorbate, urate, vitamin E, pyruvate, glutathione, albumin, vitamin A, ubiquinol, taurine, and hypotaurine.

Enzymatic Antioxidants

Enzymatic antioxidants are also known as natural antioxidants; they neutralize excess ROS and prevent it from damaging the cellular structure.

Superoxide dismutases: These(SOD, EC1.15.1.1) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidants defense in nearly all cells exposed to oxygen.

There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally the Ni type which binds nickel.

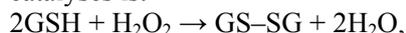
In humans (as in all other mammals and most chordates), three forms of superoxide dismutase are present. SOD-1 is located in the cytoplasm, SOD-2 in the mitochondria and SOD-3 is extracellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD-2 has manganese in its reactive centre. The genes are located on chromosomes 21, 6 and 4, respectively (21q22.1, 6q25.3 and 4p15.3-p15.1).

Catalase: It is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen^[49]. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second.

Human catalase works at an optimum temperature of 37°C, which is approximately the temperature of the human body. Catalase is usually located in a cellular organelle called the peroxisome.

Glutathione peroxidase: This (EC 1.11.1.9) is the general name of an enzymes family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxidase to their corresponding alcohols and to reduce free hydrogen peroxide to water.

An example reaction that glutathione peroxidase catalyses is:



where GSH represents reduced monomeric glutathione, and GS-SG represents glutathione disulfide. glutathione reductase then reduces the oxidized glutathione to complete the cycle:



There are several isozymes encoded by different genes, which vary in cellular location and substrate specificity. Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels. Glutathione peroxidase 2 is an intestinal and extracellular enzyme, while glutathione peroxidase 3 is extracellular, especially abundant in plasma. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans.

Non-enzymatic Antioxidants

Three non-enzymatic antioxidants of particular importance are:

Vitamin E: It is the major lipid-soluble antioxidant, and plays a vital role in protecting membranes from oxidative damage. Its primary activity is to trap peroxy radicals in cellular membranes.

Vitamin C or ascorbic acid: It is a water-soluble antioxidant that can reduce radicals from a variety of sources. It also appears to participate in recycling vitamin E radicals. Interestingly, vitamin C also functions as a pro-oxidant under certain circumstances.

Glutathione: This is the most important intracellular defense against damage by reactive oxygen species. It is a tripeptide (glutamyl-cysteinyl-glycine). The cysteine provides an exposed free sulphhydryl group (SH) that is very

reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione, but the reduced form is regenerated in a redox cycle involving glutathione reductase and the electron acceptor NADPH.

In addition to these "big three", there are numerous small molecules that function as antioxidants. Examples include bilirubin, uric acid, flavonoids and carotenoids.

CONCLUSION

Production of very low amounts of ROS in semen appears to play a physiological role in regulating normal sperm functions, whereas high levels of ROS endanger sperm

function and viability. Oxidative stress due to excessive production of ROS, impaired antioxidant defense mechanisms, or both precipitates a range of pathologies that are currently believed to negatively affect the male reproductive function. Oxidative stress-induced damage to sperm may be mediated by lipid peroxidation of the sperm plasma membrane, reduction of sperm motility, and damage to the DNA in the sperm nucleus. Thus there is established role of OS in the pathogenesis of male infertility. One important reason for the inability to utilize the OS test in clinical practice is related to the lack of a standard protocol for assessment of seminal OS. It can accurately discriminate between fertile and infertile men and identify patients with a clinical diagnosis of male-factor infertility that are likely to initiate a pregnancy when followed over a period of time. Hence testing of OS can help select subgroups of infertile men in whom OS is a significant factor and who might benefit from antioxidant supplementation. We strongly believe that incorporating such a test into the routine andrology workup is an important step for the future of the male infertility practice.

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