

## Oxidative Stress in Male Infertility : Role of Anti-Oxidants

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**Abstract :** The medical treatment of the infertile male has been largely empirical and unrewarding. The cost and complexity of sophisticated techniques don't represent a final solution to the infertile male. Oxidative stress (OS) precipitates a range of pathologies that affect the male reproduction. The development of rational approaches to treatment must be based on this cellular pathology that results in defective spermatozoa. Cellular damage in the semen is the result of positive oxidative stress status (OSS) because of either excess ROS or diminished anti-oxidants. The generation of ROS has become a real concern because of their potential toxic effects at high levels on sperm quality and function. The administration of antioxidants in patients with 'male factor' infertility has begun to attract considerable interest. Unfortunately, the clinical use of antioxidants is still in its infancy. Adequate randomized controlled trials to base any firm recommendations for clinical practice of antioxidants are lacking.

### INTRODUCTION

In general, the medical treatment of the infertile male has been largely empirical and unrewarding, while surgical treatment has limited applicability, with the possible exception of varicocele ligation or embolization. In contrast, the past two years have seen remarkable achievements in the application of techniques of assisted reproduction in the treatment of couples with 'male factor' infertility. The pragmatic clinical application of the sophisticated techniques of micro-assisted fertilization has yielded clinically outstanding results. Unfortunately, its cost and complexity is such that it cannot represent a final solution to the problem of the infertile male.

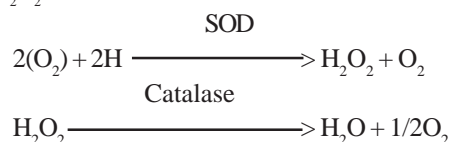
The development of rational approaches to diagnosis and treatment must be based upon an understanding of the cellular pathologies that result in the production of defective spermatozoa. "Oxidative stress" (OS) precipitates a range of pathologies that currently are thought to afflict the reproductive function<sup>1</sup>. OS is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (ROS). 25% to 40% of infertile men had high levels of ROS in semen samples. The generation of ROS has become a real concern because of their potential toxic effects at high levels on sperm quality and function.

### ROS AND ANTIOXIDANTS

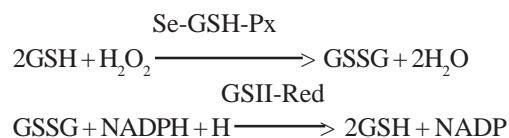
ROS are highly reactive oxidizing agents belonging to the group of free radicals. They have a tendency toward chain reaction. Most common of those having potential implications in reproductive biology include superoxide ( $O_2^-$ ) anion, hydrogen peroxide ( $H_2O_2$ ), peroxy radical (ROO) radical and the very reactive hydroxyl (OH) radical. The nitrogen derived free radical nitric oxide (NO) and peroxynitrite anion (ONOO) also appear to play a significant role in the reproduction and fertilization.

Seminal plasma is endowed with an array of antioxidants to

continuously inactivate ROS to keep only a small amount necessary to maintain normal cell function. Among the well-known biological antioxidants, SOD and its two isozymes and catalase have a significant role in seminal plasma. SOD spontaneously dismutates ( $O_2^-$ ) anion to form  $O_2$  and  $H_2O_2$  while catalase converts  $H_2O_2$  to  $O_2$  and  $H_2O$ .



SOD protects spermatozoa against spontaneous  $O_2^-$  toxicity and lipid peroxidation (LPO)<sup>2</sup>. SOD and catalase also remove ( $O_2^-$ ) generated by NADPH-oxidase in neutrophils and may play an important role in decreasing LPO and protecting spermatozoa during genitourinary inflammation. Glutathione peroxidase (Se-GSH-Px) with glutathione (GSH) as the electron donor removes peroxy (ROO) radicals from various peroxides including  $H_2O_2$ <sup>3</sup>. Glutathione reductase (GSH-Red) then regenerates reduced GSH from GSSG as shown in the following equation :



A selenium-associated polypeptide, presumably Se-GSH-Px, has been demonstrated in rat sperm mitochondria, which plays a significant role in this peroxy scavenging mechanism and in maintaining sperm mortality. In addition, Se-GSH-Px and GSH-Red directly act as antioxidant enzymes involved in the inhibition of sperm lipid peroxidation (LPO)<sup>3</sup>. GSH has a likely role in sperm nucleus decondensation and may alter spindle microtubule formation in the ovum, thus affecting the outcome of pregnancy. A high GSH/GSSG ratio will help spermatozoa to combat oxidative insult.

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## ROS AND SPERM FUNCTION

Small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities<sup>4</sup>. Low levels of ROS can enhance the ability of human spermatozoa to bind with zona pellucida. Incubating spermatozoa with low concentrations of hydrogen peroxide stimulates sperm capacitation, hyper activation, acrosome reaction and oocytes fusion<sup>5</sup>. ROS other than hydrogen peroxide such as nitric oxide and superoxide anion promote sperm capacitation and acrosome reaction<sup>5</sup>. Cellular damage in the semen is the result of positive oxidative stress status (OSS), a situation in which there is a shift towards pro-oxidants, because of either excess ROS or diminished anti-oxidants. Pathological levels of ROS detected in semen from infertile men are more likely a result of increased ROS production rather than reduced antioxidant capacity. Mammalian spermatozoa are rich in polyunsaturated fatty acids and, thus, are very susceptible to ROS attack. Spermatozoa, unlike other cells, are unique in structure, function and susceptibility to damage by LPO<sup>2</sup>. 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<sup>5</sup>.

LPO of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to infertility. The most significant effect of LPO is the perturbation of membrane (cellular and organellar) structure and function (transport processes, maintenance of ion and metabolite gradients, receptor mediated signal transduction, etc.). It results in decreased sperm motility, presumably by a rapid loss on intracellular ATP leading to axonemal damage, decreased sperm viability and increased midpiece morphology defects with deleterious effects on sperm capacitation and acrosome reaction<sup>6</sup>.

The increased formation of ROS has been correlated with a reduction of sperm motility<sup>6</sup> due to decrease in axonemal protein phosphorylation and sperm immobilization, both of which results in reduced membrane fluidity necessary for sperm oocyte fusion. H<sub>2</sub>O<sub>2</sub> can diffuse cross 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 less NADPH, a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH oxidase and a concomitant accumulation of oxidized glutathione, which in turn can reduce the antioxidant defenses of the spermatozoa and peroxidation of membrane lipids<sup>7</sup>. 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. Sperm DNA is protected from oxidative insult by its characteristic tight packing and the antioxidants<sup>8</sup>. ROS induces DNA damage in the form of base modifications, production of base-free sites, deletions, frameshifts, DNA cross-links through covalent binding to malondialdehyde (MDA), chromosomal rearrangements, single and double DNA strand breaks and 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<sup>9,10</sup>.

Redox status affects phosphorylation and ATP generation of human

spermatozoa with a profound influence on its fertilizing potential<sup>9</sup>. The oxidizing conditions increase tyrosine phosphorylation with enhanced sperm function while reducing conditions have the opposite effect. High levels of ROS disrupt the inner and outer mitochondrial membranes resulting in release of cytochrome-C protein from the mitochondria that activates the caspases and induces apoptosis<sup>10</sup>.

## ANTIOXIDANT THERAPY

Antioxidants, in general, are compounds and reactions which dispose, scavenge and suppress the formation of ROS, or oppose their actions. They provide protection against the toxic effects of ROS. They must reach their target at the right time and in the right concentration. Also, the scavenger radicals formed during the interaction of the scavenger with toxic radical intermediate should be less reactive (i.e. have a long half-life) than the radical they attack. Antioxidants attack in different phases of LPO. They may :

- \* *Inhibit the initiation process* (abstracting the allylic hydrogen from the alpha-methylene carbon atom), e.g. vitamin E.
- \* *Inhibit the formation of hydro peroxides* (chain breakers like vitamin F and C).
- \* *Degrade the hydro peroxides* formed without producing radicals (e.g. glutathione peroxidase, thiols).
- \* *Act as metal chelating substances* (inhibiting the metal catalysis accelerating the decomposition of hydro peroxides), e.g. D-penicillamine.
- \* *Remove the free radical* (scavenger activity), e.g. vitamin E and A.

Vitamin C acts as prooxidant (facilitates LPO) in low concentrations in the presence of trace amounts of transition metals (iron, copper) either free or chelates because it reduces the metals, thereby promoting the metallic catalysis of LPO. In high concentrations, when there is sufficient number of metal binding sites, it act as antioxidant. It acts by interrupting the chain reaction: losing hydrogen, reacting with the peroxy radicals and stable monohydroascorbate is produced. In addition, it also has direct O<sub>2</sub> and OH scavenger action. When vitamin C interacts with reactive oxygen intermediates, the product is a mixture of monodehydroascorbate and dehydroascorbic acid<sup>11</sup>. Concentration of vitamin C in seminal plasma directly reflects dietary intake, and its lower levels leads to infertility and increased damage to the sperm's genetic material. Supplementation of vitamin C improves the fertility of men<sup>12</sup>. It protects sperm from oxidative damage, sperm agglutination and improves the quality of sperm and steroidogenesis<sup>13</sup>.

Vitamin E, a chain breaking antioxidant inhibits LPO in membranes by scavenging peroxy (RO) and alkoxy (ROO) radicals. The main lipid phase antioxidant activity of vitamin E in the cell membrane occurs via hydrogen donation to hydro peroxides, thus formation of hydro peroxides is prevented, the chain reaction is terminated and the extension of the pathological free radical reduction in the plasma membrane depends on the recycling of vitamin E by external reducing agents such as ascorbate or thiols. In this way, it is able to function again as a free radical chain breaking antioxidant, even

though its concentration is low. Oral supplementation of vitamin E significantly decreased LPO and improved sperm motility. Kessopoulou et al<sup>14</sup>, found that the zona binding test, a sperm function assay, was significantly improved with vitamin E administration. Its combination with selenium significantly increased sperm motility and the overall percentage of normal spermatozoa. Vitamin E supplementation improves fertility in humans and animals by decreasing free-radical damage to sperm cells.

Glutathione is vital to sperm antioxidant defenses and has demonstrated a positive effect on sperm motility<sup>15</sup>. Selenium and glutathione are essential to the formation of phospholipid hydroperoxide glutathione peroxidase, an enzyme present in spermatids which becomes a structural protein comprising over 50 percent of the mitochondrial capsule in the mid-piece of mature spermatozoa. Deficiencies of either substance can lead to instability of the mid-piece, resulting in defective motility<sup>16</sup>. Glutathione demonstrated a statistically significant effect on sperm motility, especially increasing the percentage of forward motility and on sperm morphology improving quality<sup>17</sup>. Treatment with selenium significantly improved sperm motility; however, sperm density was unaffected. Treatment with GSH resulted in significant increase in the observed content of long chain polyunsaturated fatty acids. This appeared to be associated with a fall in the lipid peroxidation potential of the spermatozoa of the treated infertile subjects, and an increase in sperm motility<sup>18</sup>. The findings of these studies indicate that glutathione therapy could represent a possible therapeutical tool in cases where ROS or exposure to toxins is the probable cause of infertility.

In sperm cells, coenzyme Q10 (CoQ10) in the mitochondrial mid-piece involved in energy production functions as an antioxidant, preventing lipid peroxidation of sperm membranes. It is a nutrient used by the body in the production of energy. While its exact role in the formation of sperm is unknown, there is evidence that as little as 10 mg per day (over a two-week period) will increase sperm count and motility. CoQ10 significantly increased sperm motility and count<sup>19</sup>. Clearly additional studies will be needed to evaluate the possible role of coenzyme Q10 in the treatment of male infertility.

## CONCLUSION

Oxygen toxicity is an inherent challenge to aerobic life forms, including the spermatozoa. Increased oxidative damage to sperm membranes (indicated by increased LPO), proteins and DNA is associated with alterations in signal transduction mechanisms that affect fertility. Spermatozoa possess an inherent but limited capacity to generate ROS which may help the fertilization process. A variety of defense mechanisms encompassing antioxidant enzymes (SOD, catalase, glutathione peroxidase and reductase), vitamins (E,C and carotenoids) and biomolecules (glutathione and ubiquinol) are involved in biological systems. A balance between the benefits and

risks from ROS and antioxidants appears to be necessary for the survival and normal functioning of spermatozoa. The administration of antioxidants, like vitamin C, vitamin E, glutathione and CoQ10 showed improvements in the sperm physiology. Unfortunately, the clinical use of antioxidants is still in its infancy. Adequate randomized controlled trials to base any firm recommendations for clinical practice of antioxidants are lacking.

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