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Oxidative Stress Induced Infertility in Varicocele

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Abstract

Oxygen toxicity is an intrinsic threat to aerobic life of spermatozoa, the actively motile gametes responsible for propagation of the species. Reactive oxygen species (ROS) in the semen have both physiological and pathological role in male fertility, by causing damage to sperm membranes, proteins, and DNA. Seminal plasma is endowed with an array of free radical scavengers called antioxidants that protect the spermatozoa against ROS. Hence, antioxidants are essential for the survival and functioning of spermatozoa. Oxidative stress, a disturbance caused by the imbalance between excessive production of reactive oxygen species and decreased antioxidant defense mechanism is now being considered to play a major contributory role in causing male factor infertility. Varicocele, vascular lesions of the pampiniform plexus, is one of the most controversial issues in the field of andrology, regarding its diagnosis and management. Earlier studies suggest a link between oxidative stress and impaired sperm function in male reproductive disorders like varicocele. Varicocele has been found to be associated with increased production of ROS in the semen, with decreased antioxidant levels in the seminal plasma, suggesting that spermatogenic dysfunction in males with varicocele maybe in part related to oxidative stress. However, the pathophysiology of oxidative stress induced infertility in varicocele has still not been completely understood. Seminal oxidative stress is thus emerging as a very important step in the diagnosis, prognosis and treatment of males approaching infertility clinics for evaluation.

Keywords Varicocele; Oxidative stress; Reactive oxygen species; Total antioxidant capacity; Infertility

Abbreviations:

ADP: Adenine Dinucleotide Phosphate; **cAMP:** Cyclic Adenosine Monophosphate; **EN:** Eosin Nigrosin; **FasL:** Fas Ligand; **GSH:** Glutathione; **G_6PDH:** Glucose-6-Phosphate Dehydrogenase; **LPO:** Lipid Peroxidation; **MPF:** Maturation Promoting Factor; **NADPH:** Nicotinamide adenine dinucleotide phosphate; **OS**: Oxidative Stress; **PKA**: Protein Kinase A; **PUFA**: Polyunstaurated Fatty Acid; **ROO:** Peroxyl; **ROS:** Reactive Oxygen Species; **SOD:** Superoxide Dismutase; **TAC:** Total Antioxidant Capacity; **TNF-alpha:** Tumor Necrosis Factor Alpha; **VAR:** Varicocele; **WBC:** White Blood Cells; **WHO:** World Health Organization

Background

During the 1st century A.C, Celsus, a Greek physician, described varicocele as "twisted veins over the testicle, causing it to swell" [1]. Varicocele, mostly found within the left spermatic vein, is seen as tortuous and dilated pampiniform plexus. Nearly fifteen to twenty percent of the general population with varicocele suffers from either primary and secondary infertility [2,3]. The etiology of varicocele still remains controversial, with involvement of factors like congenital/anatomical abnormalities, reflux of spermatic venous blood and presence of anti-sperm antibodies [4,5].

Oxidative stress produced as a result of increased production of reactive oxygen species, has emerged as a major concern in causing male factor infertility, regardless of its source of origin-testicular or seminal. Elevated ROS levels have been implicated in reducing fertility in patients with varicocele [6-9]. ROS is involved physiologically in regulation of spermatozoal function, but has toxic effects on fertility as well [10]. The pathophysiology of varicocele has been studied extensively to clarify the underlying mechanisms of varicocele induced male infertility. A large number of antioxidant defense mechanisms in the seminal plasma can readily reduce any negative impact of ROS by scavenger mechanisms [11]. However, although the seminal plasma is richly endowed with antioxidants that protect the spermatozoa against oxidative stress and DNA damage, their major role in vivo is still debatable [12]. Several studies have been conducted to see the association between oxidative stress and infertility in varicocele. Using different methodologies, various studies have measured oxidative stress markers in semen of varicocele men and compared them with levels of these markers in healthy fertile men or infertile men with idiopathic infertility [13]. The speculation that varicocele causes male infertility is based on the fact that varicocele has an increased incidence, its correlation with decreased semen parameters and reduced testicular size and finally from the fact that correction of varicocele leads to an improvement of semen parameters and pregnancy rates [1].

Etiology and pathophysiology of varicocele

The pathophysiology of varicocele still remains controversial involving factors like altered testicular thermo-dynamics, changes in testicular blood flow and venous pressure, leydig cell dysfunction and presence of autoantibodies against spermatozoa [2,14] (Figure 1). Reflux of warm blood from the abdominal cavity into the scrotum, resulting from malfunctioning of valves in spermatic and cremasteric veins attributes to the raised intra-testicular temperature [15]. Several theories have been proposed for the mechanism involved in defective spermatogenesis because of altered testicular thermodynamics.

According to one theory, anatomical disparity between the right and left spermatic veins leads to increased hydrostatic pressure in the left spermatic vein causing dilatation of the pampiniform plexus. Another theory suggests that dysfunctional valves of the internal spermatic veins cause regression of blood.

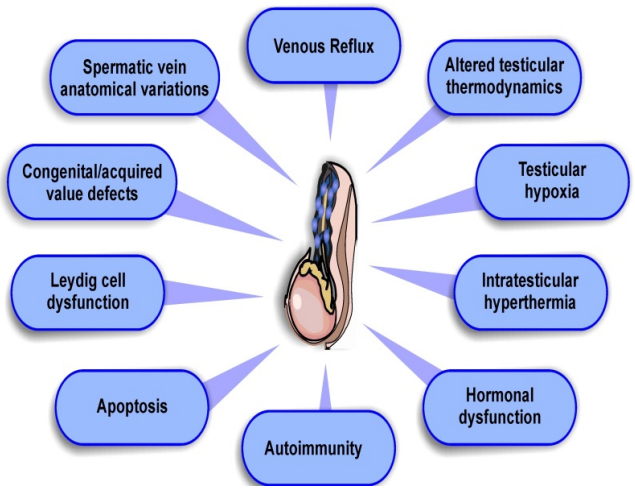

Figure 1: Etiology and pathophysiology of varicocele.

Naughton et al. proposed that compression of the left renal vein between the superior mesenteric artery and aorta causes partial obstruction of the left spermatic vein leading to varicocele formation. It was also suggested that spermatogenic dysfunction seen in varicocele was the result of thermal damage to spermatozoal proteins and DNA within the seminiferous tubules [4]. The normal anatomical asymmetry and valvular dysfunction causes pooling of blood more in the left spermatic vein [16]. This phenomenon called the "nutcracker effect" seen as increased compression of the left renal vein between superior mesenteric artery and descending aorta causes retrograde flow of blood down the cremasteric and internal spermatic veins [14]. The right varicocele is considered rare but with the use of modern diagnostic techniques e.g., colored Doppler ultrasound, increased frequency of bilateral localization of varicocele has now been documented in recent studies [1,2,17].

Free radicals

Free radicals maybe defined as chemical molecules or highly reactive ions or atoms having one or more unpaired electrons. They begin a chain reaction by oxidative modification of stable biomolecules by stealing their electrons and making them become free radicals themselves [18,19] (Figure 2). This reduction-oxidation process of free radicals plays a vital role in acquisition of fertilizing ability of the spermatozoa [9,20]. Presence of an increased content of polyunsaturated fatty acids (PUFA) within the spermatozoal plasma membrane makes them very susceptible to attack by the reactive oxygen species [21,22]. To protect the spermatozoa against free radical toxicity, the seminal plasma possess an antioxidant system comprising of high and low molecular weight factors [23]. An indicator of oxidative stress is the imbalance between total antioxidant capacity (TAC) and production of ROS in the seminal fluid and is correlated with male infertility [8]. Infertile men have an impaired seminal plasma antioxidant capacity than fertile men, suggesting an association between male infertility and total antioxidant capacity [9,24].

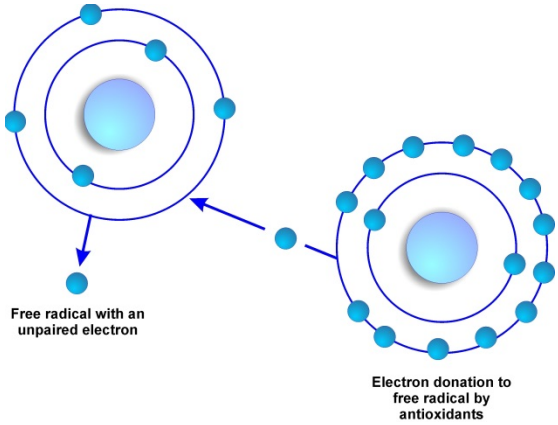

Figure 2: Cellular redox homeostasis by ROS scavenging action of antioxidants.

Generation of reactive oxygen species in human semen - sources of origin

Immature or morphologically defective spermatozoa and leukocytes are the main source of the reactive oxygen species [25]. Generation of ROS in the semen may also be from other different sources like, epithelial cells, but abnormal spermatozoa and leukocytes are the two major sources of production [26,27]. ROS includes both radical (hydroxyl ion, nitric oxide and superoxide) and non-radical (hydrogen peroxide, lipid peroxide, singlet oxygen and ozone) oxygen derivatives [28]. All these markers are involved in a series of reactions forming free radicals causing disruption of living cells later [18] (Figure 3).

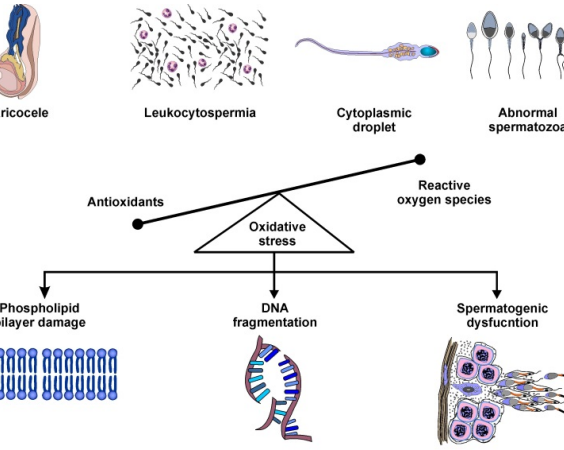

Figure 3: Etiological factors involved in oxidative stress generation and its effect at cellular level.

Cytoplasmic droplet

Existence of a negative correlation between spermatozoal quality and ROS production clearly indicates production of oxidants by the

human spermatozoa [29,30]. The main link between increased ROS production and poor semen quality is the presence of cytoplasmic droplet within the sperm head. During spermiation, spermatozoa released from the germinal epithelium that carry surplus residual cytoplasm, are immature and defective [31]. Cytosolic glucose-6-phosphate de-hydrogenase (G_6PD) controls the intracellular availability of nicotinamide adenine dinucleotide phosphate (NADPH) and mediates ROS production by the residual cytoplasm. NADPH acts as a source of electrons for the production of ROS within the spermatozoa [32]. ROS generation by the spermatozoa occurs through two pathways (i) at the sperm plasma membrane level through the NADPH-oxidase system and (ii) at the level of the mitochondria through NADH-dependent oxidoreductase (diphorase) system [33]. The main source of ROS production, mainly the superoxide anion (O_2^-) in infertile males is the spermatozoal mitochondria system. This superoxide anion leads to generation of hydroxyl radicals in the presence of transition metals e.g., copper and iron and enzyme, superoxide dismutase. These hydroxyl radicals cause deterioration of spermatozoal functions through initiation of a lipid peroxidation cascade [34,35].

Polymorphonuclear leukocytes

According to the World Health Organization, presence of peroxidase positive leukocytes in concentrations greater than 1×10^6/mL of semen is defined as Leukocytospermia [36]. Polymorphonuclear leukocytes (PMNL) represent about 50-60% of cells present in the semen and are the main source of ROS production [37,38]. Activation of leukocytes by stimuli, like inflammations and infections leads to increased production of NADPH. NADPH, in turn, triggers activation of the leukocyte myeloperoxidase system, further leading to a respiratory burst, subsequently resulting in increased production of ROS [8].

Pathophysiological role of ROS on male fertility

At physiological levels, ROS production by human spermatozoa promotes tyrosine phosphorylation in association with sperm capacitation. Enhancement of tyrosine phosphorylation events by ROS in human spermatozoa depends upon suppression of tyrosine phosphatase activity by hydrogen peroxide as well as stimulation of cyclic adenosine monophosphate (cAMP) generation by adenylyl cyclase. cAMP generation by this mechanism causes stimulation of tyrosine phosphorylation via a Protein Kinase A (PKA) dependent mechanism and an intermediary tyrosine kinase [39-41]. This whole process of redox control of tyrosine phosphorylation during sperm capacitation maintains a low and steady state of ROS production. However, factors such as infiltration of leukocytes within the seminal plasma disturbs the physiological rate of ROS production, inducing a state of oxidative stress [42]. Reactive oxygen species are now believed to be involved in various biological processes, regulating spermatozoal physiology and function. Pathology arises as a result of lipid peroxidation by ROS causing alterations in the permeability of the plasma membrane of the spermatozoa [11,43]. It was believed earlier that ROS has deleterious effects on spermatozoal functions, but increasing evidence has now proven that only very low ROS concentrations play a role in signal transduction mechanisms [44]. Physiologically, ROS plays a very important role in fertilization, acrosomal reaction, hyper-activation, capacitation and spermatozoal motility [45,46]. An increase in levels of cAMP occurs because of increased levels of intracellular calcium, tyrosine kinase and ROS, resulting in hyper-activation while the sperm is capacitating. These capacitated hyperactive motile spermatozoa undergo physiological acrosome reaction, thereby acquiring the ability to fertilize [27,47]. Reactive oxygen species like hydrogen peroxide, nitric oxide and superoxide anion, all promote sperm capacitation and acrosome reaction [48,49]. In the recent years, oxidative stress (OS) has been extensively studied as one of the most important marker involved in causation of infertility in males [50]. Similar to any other living cell, spermatozoa also face the "oxygen-paradox" [50] i.e., where oxygen is necessary to support life, its metabolites, e.g., ROS, can alter cellular functions endangering survival of the cell [51]. John Macleod, a Scottish scientist, demonstrated that the enzyme catalase had a role in motility of human spermatozoa, hence demonstrating a possible role of oxidative stress in male factor infertility [52,53]. OS induced as a result of overproduction of ROS influences the fertilization process by having harmful effects on the spermatozoa [54,55].

Leukocytospermia

An association exists between presence of leukocytes in the semen [56,57]. Leukocytes produced during infection and inflammation in the prostatic and seminal vesicle secretions are the extracellular source of ROS.

Leukocytes stimulate production of ROS through direct cell-cell contact of spermatozoa or by release of soluble products [34,37]. Abnormally high concentration of leukocytes in the semen or removal of seminal plasma for sperm preparation during assisted reproductive procedures is a major source of ROS [58,59]. Leukocytes cause an increased production of pro-inflammatory chemokines leading to significantly elevated oxidative stress in semen of infertile males [54]. Presence of increased interleukin-8 and decreased superoxide dismutase activity in semen depicts an association between a defective ROS scavenging mechanism modulated by pro-inflammatory cytokines, suggesting an active pro-inflammatory response [60].

Lipid peroxidation of spermatozoa

Of the spermatozoal cell components affected by oxidative stress, the most vulnerable are the polyunsaturated fatty acids (PUFA) having two carbon-carbon double bonds within the plasma membrane [61]. Oxidative deterioration of PUFA is called lipid peroxidation [62]. Lipid peroxidation of the spermatozoal plasma membrane causes morphological defects of the mid-piece with deleterious effects on the acrosome reaction and sperm capacitation [63,64]. Lipid Peroxidation (LPO) is the key mechanism involved in ROS-induced spermatozoal damage leading to infertility [65]. This manifestation of oxygen activation is commonly of two types (a) enzymatic LPO (NADPH and ADP dependent) and (b) non-enzymatic LPO [54]. LPO causes perturbations in cell signaling mechanisms and all other cellular transport processes, as well as ionic and metabolite imbalance. Increased activity of glutathione peroxidase causes removal of LPO metabolites further affecting the calcium homeostasis within the spermatozoal cell [66].

Reactive oxygen species and sperm toxicity in varicocele

Spermatozoal dysfunction induced by oxidative damage is attributed to the presence of leukocytes and morphologically abnormal spermatozoa in the seminal plasma [67]. Apoptosis plays a very important in elimination of abnormal spermatozoa [68]. A decreased

sperm count is seen as a consequence of increased ROS levels [69]. ROS attacks the PUFA of spermatozoal plasma membrane damaging the axonemes, depletion of ATP, protein phosphorylation of axonemes and decreased percentage spermatozoal motility [63,64]. Impaired spermatozoal motility may also be a result of decreased antioxidant defense mechanism because of inhibition of the enzyme glucose 6 phosphate dehydrogenase, allowing diffusion of hydrogen peroxide across the plasma membrane [48]. Oxidative stress in the varicocele maybe the result of altered testicular microenvironment and hemodynamics. Spermatogenic dysfunction could be the result of various compensatory mechanisms trying to maintain spermatogenesis in varicocele patients. These compensatory mechanisms may cause up regulation or down regulation of various molecular mechanisms and pathways involved in generation of free radicals [14]. An inherent decreased expression of antioxidant enzymes or increased oxidative stress because of increased ROS production cause increased lipid peroxidation, affecting sperm viability, and decreasing sperm motility [54]. Although it has already been suggested that oxidative stress in varicocele leads to impairment of semen parameters, yet the etiology of raised oxidative stress levels still remains unclear [70]. Researchers are debating on the extent of varicoceles effect on sperm characteristics, as earlier studies have shown that sperm parameters seen in varicocele patients vary from normozoospermia to mild or moderate asthenozoospermia, teratozoospermia and asthenoteratzoospermia. Sperm concentration is not affected initially, but later all three sperm parameters-concentration, motility and morphology deteriorate, even causing azoospermia in a few cases [71].

Mechanism to combat ROS - Antioxidants

It is a well-documented fact now, that ROS generation in the spermatozoa play a vital role in the pathophysiology of male factor infertility. It is the abundance of PUFA in spermatozoal plasma membrane which makes it more vulnerable to oxidative damage [72,73]. A very effective protective environment against oxidative stress is provided by an antioxidant system in the seminal plasma [42]. Antioxidants are compounds and reactions which oppose or suppress the actions of ROS. The enzymatic antioxidants, catalase and superoxide dismutase (SOD) with its two isozymes play a very important role in scavenging the ROS. SOD protects spermatozoa by neutralizing lipid peroxidation and spontaneous oxygen toxicity [65]. SOD and catalase decrease lipid peroxidation by removing the superoxide anion generated by NADPH-oxidase in neutrophils [74]. Another most important enzymatic antioxidant is glutathione peroxidase which removes peroxyl (ROO) from hydrogen peroxide as well as other peroxides [75]. The non-enzymatic antioxidants present in the seminal plasma are Vitamin E, pyruvate, urate, ascorbate, glutathione, Vitamin A, albumin, ubiquitol, taurine and hypotaurine. Vitamin C and E, beta-carotenes, carotenoids and flavonoids constitute the dietary antioxidants [76-78]. Vitamin E and C protect spermatozoa against plasma membrane damage while carotenoids (beta carotene) and ubiquinols decrease lipid derived free radicals by quenching the singlet oxygen, thus reducing the detrimental effects on sperm lipid peroxidation [55]. Antioxidants play a very crucial role in maintaining normal sperm function: they prevent sperm DNA damage, protect normal spermatozoa from ROS generated by abnormal spermatozoa, prevent damage to spermatozoa because of cryopreservation, block premature sperm maturation, improve sperm quality in smokers and play a vital role in outcome of assisted reproductive techniques [76,79-81]. The sperm plasma membrane integrity is also maintained by metal chelators like cerulopasmin, lactoferrin and transferrin [82]. It is the lack of a strong defense mechanism against ROS which renders the spermatozoa susceptible to peroxidative damage [83]. The total antioxidant capacity (TAC) of seminal plasma is the sum of non-enzymatic (e.g., taurine, hypotaurine, pyruvate, ascorbate, urate, glutathione and vitamin E) and enzymatic (e.g., superoxide dismutase, glutathione peroxidase and catalase) molecules [21,84,85]. Decreased levels of the total antioxidants in the seminal plasma play a pivotal role in disrupting fertility status in males [24,83,86]. It is important to accurately measure the seminal TAC so that it becomes a reliable and easy to use tool for diagnosis and evaluation of male infertility [87].

Conclusion

Till to this day, oxidative stress is considered to be a very important parameter in causing varicocele-induced infertility. However, its pathophysiology still remains incompletely understood, possibly due to insufficient evidence from lack of well-conducted studies. Assessment of oxidative stress profile might prove to be a valuable tool for evaluation of the fertility status of a male. For now, it seems that assessment of ROS and TAC alone is not sufficient enough to assess if varicocele is the underlying cause of testicular damage. Various assays that have been introduced for evaluation of oxidative stress are now being considered an important and valuable tool for infertility evaluation, especially in varicocele patients. Further studies including specific markers involved in ROS-induced testicular damage, therapy with antioxidants may prove to be more conclusive.

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