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The Incriminating Role of Reactive Oxygen Species in Idiopathic Male Infertility: An Evidence Based Evaluation

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Abstract: The male factor is considered a major contributory factor to infertility. Apart from the conventional causes for male infertility such as varicocoele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma and tumours, a new and important cause has been identified as being responsible for the so-called idiopathic male infertility: oxidative stress. Oxidative Stress (OS) is a condition that occurs when the production of Reactive Oxygen Species (ROS) overwhelms the antioxidant defense produced against them. In male reproductive pathological conditions, the OS significantly impairs spermatogenesis and sperm function, which may lead to male infertility. Reactive Oxygen Species (ROS) known as free radicals are oxidizing agents generated as a result of metabolism of oxygen and have at least one unpaired electron that make them very reactive species. Spermatozoa generate Reactive Oxygen Species (ROS) in physiological amounts, which play a role in sperm functions during sperm capacitation, Acrosome Reaction (AR) and oocyte fusion, but they need to be controlled and their concentrations maintained at a level that is not deleterious to the cells. Administration of antioxidants in patients with 'male factor' infertility has begun to attract considerable interest. The main difficulty of such an approach is our incomplete understanding of the role of free radicals in normal and abnormal sperm function leading to male infertility. The purpose of the present review is to address the relationship between ROS and idiopathic male factor infertility.

Key words: Infertility, reactive oxygen species, oxidative stress

INTRODUCTION

Since the first appearance of humans on earth, infertility has been one of the most controversial medical and social issues. Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year (World Health Organization, 2000). Infertility is a major clinical problem, affecting people medically and psychosocially. Infertility affects approximately 15% of all couples trying to conceive. Male causes for infertility are found in 50% of involuntarily childless couples (Rowe, 2006). Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the genital tract, increased scrotal temperature (varicocele), endocrine disturbances, genetic abnormalities and immunological factors (World Health Organization, 2000). No causal factor is found in 60-75% of cases (idiopathic male infertility). Infertility is the main complication arising in male patients with high levels of OS, apoptosis or sperm DNA damage. Although techniques like Intracytoplasmic Sperm Injection (ICSI) offer considerable promise to such male factor patients, the indiscriminate use of such assisted fertility treatments, especially when the etiology of sperm dysfunction is poorly understood is not warranted. Idiopathic male factor infertility has been linked with oxidative stress by several research groups (Said *et al*., 2004; Kao *et al*., 2007; Joffe, 2010). Oxidative stress originates from the excessive generation of Reactive Oxygen Species (ROS) by the spermatozoa and results in the peroxidation of unsaturated fatty acids in the sperm plasma membrane. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease (Christen, 2000; Nunomura *et al*., 2006), Parkinson's disease (Wood-Kaczmar *et al*., 2006), the pathologies caused by diabetes (Davi *et al*., 2005), rheumatoid arthritis (Hitchon, 2004), neurodegeneration in motor neurone disease (Cookson and Shaw, 1999; Rao and Balachandran, 2002), cancer (Valko *et al*., 2004, 2007; Nakabeppu *et al*., 2006), obesity (Vincent, 2007; Gomez-Cabera, 2008) and cataract (Berthood and Beyer, 2009).

Several forms of sperm DNA damage are caused by ROS, e.g., chromatin cross-linking, chromosome deletion, DNA strand breaks and base oxidation. Moreover, ROS are important in mediating apoptosis by inducing cytochrome c and caspases 9 and 3, which in turn result in a high frequency of single- and doublestranded DNA strand breaks (Said *et al*., 2004). Human spermatozoa

exhibit a capacity to generate ROS and initiate peroxidation of the unsaturated fatty acids in the sperm plasma membrane, which plays a key role in the etiology of male infertility. The short half-life and limited diffusion of these molecules is consistent with their physiologic role in key biological events such as acrosome reaction and hyperactivation. The intrinsic reactivity of these metabolites in peroxidative damage induced by ROS, particularly H_2O_2 and the superoxide anion, has been proposed as a major cause of defective sperm function in cases of male infertility. Understanding of how such conditions affect sperm function will help in designing new and effective treatment strategies.

Idiopathatic infertility: Men with idiopathic infertility generally present with significantly higher seminal ROS levels and lower antioxidant properties than healthy controls (Pasqualotto *et al*., 2001). Therefore, it appears that the presence of OS in infertile normozoospermic men may be the cause behind previously unexplained cases of infertility. Similarly, sperm DNA damage analysis may reveal hidden sperm DNA abnormalities in infertile men with normal standard sperm values who were diagnosed with idiopathic infertility. The increase in sperm DNA damage in these patients may be partly related to high levels of seminal OS. Finally and importantly, some conditions may pass unnoticed but still affect the sperm genomic integrity.

Free radicals: origin and oxidative stress: By definition, a free radical is any chemical compound with one or more unpaired electrons. The assumption that free radicals can influence male fertility has received substantial scientific support (Saalu *et al*., 2009a, b, 2010). The free radicals that have been associated with infertility are oxygen and oxygen-derived oxidants, namely, the superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO-) and hydroxyl radicals (OH^-) (Agarwal *et al*., 2005). These oxidants are widely known as Reactive Oxygen Species (ROS) and, due to unpaired electron(s) tend to strongly react with other chemical compounds (Attaran *et al*., 2000). The nitrogen derived free radical Nitric Oxide (NO.) and peroxynitrite anion (ONOO-) also appear to play a significant role in the reproduction and fertilization. The ultimate effects of (NO.) depend upon its concentration and interactions with hydrogen peroxide. Peroxynitrite (oxoperoxonitrate) amion may be formed *in vivo* from superoxide and nitric oxide and actively reacts with glutathione, cysteine, deoxyribose and other thiols/thioethers (Koppenol *et al*., 1992). This can form a strongly nitrating species in the presence of metal ions or complexes.

Free radicals seek to participate in chemical reactions that relieve them of their unpaired electron, resulting in the oxidation of lipids in membranes, amino acids in proteins and carbohydrates within nucleic acids (Ochsendorf, 1999). The terms free radical and ROS are commonly used in an interchangeable manner, despite the fact that not all ROS are free radicals (Cheeseman and Slater, 1993). An expanding body of evidence now supports a role for oxidative stress as a significant cause of male infertility. However, despite being a common pathology in infertile men, oxidative stress is ignored by many infertility practitioners. The currently popular response of resorting to mechanical techniques such as IVF-ICSI in all cases of male factor infertility is unlikely to be 'best practice' since ROS damaged paternal DNA will result in poor quality blastocysts, less than optimal pregnancy rates and an increase in miscarriage. At present there are over 30 assays of oxidative stress (Ochsendorf, 1999), broadly divided into three different types (Direct methods, Indirect methods and routine semen analysis). In many complex biological systems including semen, the true ROS status leading to oxidative stress reflects a relative balance between the ROS-generated and ROS-scavenged. The measurement of the rate of ROS generation by luminal induced chemiluminescence has been the most common method for quantitating ROS. Although this rate measurement is dynamic, it may not accurately reflect the status of potential sperm damaging ROS. For such evaluations, the amount of ROS-detected, rather than the ROS-generated will represent a more physiological assessment of oxidative stress (Iwasaki and Gagnon, 1992).

Sources of ROS: There are several sources of reactive oxygen species in the human body. Production of superoxide in mitochondria is a by-product of the function of the respiratory chain (Balaban *et al*., 2005). The first known example of regulated generation of Reactive Oxygen Species (ROS) in mammalian cells was through the respiratory burst of phagocytic cells by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase. This enzyme complex uses electrons derived from intracellular NADPH to generate superoxide amion, which is further processed to form hydrogen peroxide and other ROS-providing host defense against bacterial and fungal pathogens (Quinn and Gauss, 2004). The essential role of the phagocytic oxidase in host defense is well illustrated by the serious phenotype of Chronic Granulomatous Disease (CGD), in which susceptibility to infections develops in the absence of a functional phagocytic oxidase (Geiszt *et al*., 2001). Virtually every human ejaculate is considered to be contaminated with potential

sources of ROS as human semen is known to contain different types of cells, such as mature and immature spermatozoa, round cells from different stages of spermatogenesis, leukocytes and epithelial cells. Of these different cell types, leukocytes and spermatozoa have been shown to be the two main sources of ROS (Garrido *et al*., 2004).

Physiological role of ROS: Low levels of ROS have been shown to be essential for fertilization, acrosome reaction, hyperactivation, motility and capacitation (Griveau and Le Lannou, 1997; Agarwal *et al*., 2004). Capacitation has been shown to occur in the female genital tract, a process carried out to prepare the spermatozoa for interaction with the oocyte. During this process, the levels of intracellular calcium, ROS and tyrosine kinase all increase, leading to an increase in cyclic adenosine monophosphate (cAMP). This increase in cAMP facilitates hyperactivation of spermatozoa, a condition in which they are highly motile (Aitken, 1995). However, only capacitated spermatozoa exhibit hyperactivated motility and undergo a physiological acrosome reaction, thereby acquiring the ability to fertilize (De Lamirande and Gagnon, 1995; De Lamirande *et al*., 1997, 1998).

Consequences of excessive generation of ROS: Excessive ROS production by immature, morphologically abnormal spermatozoa with cytoplasmicresidues such as those confronted in teratozoospermic semen specimens may induce oxidative damage of mature spermatozoa during sperm migration from the seminiferous tubules to the epididymis and may be an important cause of male infertility (Sikka, 2004). ROS is involved in many physiological functions of human spermatozoa, their excess production in semen especially during leukocytospermia can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma resulting in oxidative stress. Spermatozoa are also particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA), which readily experience lipid peroxidation by ROS, resulting in a loss of membrane integrity (Halliwell, 1990; Buettner, 1993).

Oxidative stress and apoptosis: Apoptosis is a non-inflammatory response to tissue damage characterized by a series of morphological and biochemical changes (Wyllie *et al*., 1980; Sinha-Hikim and Swerdloff, 1999; Sakkas *et al*., 1999a, b, 2002; Shen *et al*., 2002; Said *et al*., 2004; Grunewald *et al*., 2005). Apoptosis during spermatogenesis has been assessed, discussed and supported in several studies (Hikim *et al*., 1998; Print and Loveland, 2000) and has been associated with male infertility (Lin *et al*., 1997; Hikim *et al*., 1998; Jurisicova *et al*., 1999).

Apoptosis appears to be strictly regulated by extrinsic and intrinsic factors and can be triggered by a wide variety of stimuli. Apoptosis in sperm may also be initiated by ROS-independent pathways involving the cell surface receptor called as Fas or CD 95. Fas is a type I membrane protein that mediates apoptosis. When Fas ligand or anti-Fas antibody binds to Fas, apoptosis occurs (Lee *et al*., 1999). Moustafa *et al*. (2004) determined that infertile patients had high ROS levels in their seminal plasma and higher percentage of apoptosis than normal healthy donors. When the molecular framework of apoptosis is identified, specific apoptotic inhibitors may have a role in promoting germ-cell survival.

Lipid peroxidation: 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 double bonds (Halliwell, 1990). Most membrane PUFA contain unconjugated double bonds that are separated by methylene groups. The presence of a double bond adjacent to a methylene group makes the methylene carbon-hydrogen bond weaker and as a result, the hydrogen is more susceptible to abstraction. Once this abstraction has occurred, the radical produced is stabilized by the rearrangement of double bonds. The PUFA rearranges to form a conjugated diene radical that subsequently can be oxidized (Aitken *et al*., 1989, 1993; Aitken and Fisher, 1994; Aitken, 1995; Alvarez and Storey, 1995; Griveau *et al*., 1995; Kodama *et al*., 1996; Ochsendorf, 1999). The most common types of LPO are: (1) nonenzymatic membrane LPO and (2) enzymatic (NADPH and ADP dependent) LPO. The enzymatic reaction involves NADPH cytochrome P-450 reductase and proceeds via an ADP-$Fe^{3+}O^{2-}$ (perferryl) complex (Ernster, 1993; Oborna *et al*., 2010). Mammalian spermatozoa membranes are very sensitive to free radical induced damage mediated by lipid peroxidation, as they are rich in polyunsaturated fatty acids. In spermatozoa, production of malondialdehyde (MDA), an end product of LPO induced by ferrous ion promoters, has been reported by Darley-Usmar *et al*. (1995). Formation of MDA 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 (Taourel *et al*., 1992). Lipid peroxidation caused by low levels of ROS leads to modification of the plasma membrane, thus facilitating sperm-oocyte adhesion (Kodama *et al*., 1996).

ROS and motility: Sperm count and sperm motility are the first and most important predictors of fertility potential.

The underlying pathology behind free radicals ability to reduce sperm motility was first reported by Jones *et al*.

(1979). They reported that ROS-induced peroxidation of the sperm membrane decreasing its flexibility and therefore tail motion. Sperm membranes are vulnerable to this type of damage as they contain large amounts of unsaturated fatty acids. Direct ROS damage to mitochondria, decreasing energy availability, may also impede sperm motility (Lamirande and Gagnon, 1992; Lamirande *et al*., 1997, 1998). 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 (Lamirande and Gagnon, 1995). The mechanism behind this effect is ROS induced lipid peroxidation of sperm plasma membrane (Aitken and Baker, 2006), which affects membrane fluidity and mobility. In addition ROS may also affect the sperm axoneme, inhibit mitochondrial function and affect the synthesis of DNA, RNA and proteins (Lamirande and Gagnon, 1992). Sperm cell dysfunction, a result of ROS damage, is dependent on the nature, amount and duration of exposure to ROS. The extent of ROS damage is also dependent upon surrounding environmental factors such as oxygen tension and temperature as well as the concentrations of molecular components such as ions, proteins and ROS scavengers (Agarwal and Saleh, 2002).

OS and DNA damage: Sperm genetic material is structured in a special manner that keeps the nuclear chromatin highly stable and compact. The normal DNA structure is capable of decondensation at appropriate time transferring the packaged genetic information to the egg without defects in the fertilization process. The cause of DNA damage in sperm can be attributed to various pathological conditions including cancer (O'Donovan, 2005), varicocele (Saleh *et al*., 2003), high prolonged fever (Evenson *et al*., 2000), advanced age (Singh *et al*., 2003) or leukocytospermia (Erenpreiss *et al*., 2002). Also a variety of environmental conditions can be involved as radiation (Aitken *et al*., 2005), air pollution, smoking (Said *et al*., 2005), pesticides, chemicals, heat and ART prep protocols (Potts *et al*., 1999; Aitken *et al*., 2005; Bennetts and Aitken, 2005; Aitken and De Iuliis, 2010). Abnormal sperm morphology has been reported to be associated with high sperm DNA fragmentation in infertile men (Nicopoullos *et al*., 2008). 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. Two factors protect the sperm deoxyribonucleic acid (DNA) from oxidative insult: the characteristic tight packing of the DNA and the antioxidants present in the seminal plasma (Twigg *et al*., 1998). ROS attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. ROS induced DNA damage accelerate the germ cell apoptosis. Unfortunately spermatozoa are unable to repair the damage induced by excessive ROS as they lack the cytoplasmic enzymes required to accomplish the repair. Free radicals have the ability to directly damage sperm DNA by attacking the purine and pyrimidine bases and the deoxyribose backbone. Normally, sperm DNA is tightly packaged by protamines protecting it from free radical attack. Oxidative stress also is associated with high frequencies of single- and double-strand DNA breaks (Duru *et al*., 2000; Aitken and Krause, 2001). ROS also can cause various types of gene mutations such as point mutations and polymorphism, resulting in decreased semen quality (Spiropoulos *et al*., 2002; Sharma *et al*., 2004; Thomson *et al*., 2009). However, infertile men often exhibit deficient protamination, leaving the sperm DNA particularly vulnerable to ROS attack (Oliva, 2006). Early detection and prompt antioxidant therapy can prevent ROS induced DNA damage. This has far reaching impact if such men opt for assisted reproductive technology (ART)/*in vitro* fertilization. The assessment of sperm DNA damage appears to be a potential tool for evaluating semen samples prior to their use in ART as besides impairment of fertility DNA damage is likely to increase the transmission of genetic diseases during the assisted reproductive procedures. In addition, the redox status of human spermatozoa is likely to affect phosphorylation and ATP generation with a profound influence on its fertilizing potential (Aitken and Baker, 2003).

Smoking, oxidative stress and infertility: Tobacco smoke consists of approximately 4,000 compounds such as alkaloids, nitrosamines and inorganic molecules and many of these substances are reactive oxygen or nitrogen species. Significant positive association has been reported between active smoking and sperm DNA fragmentation (Sun *et al*., 1997), as well as axonemal damage (Zoas *et al*., 1998) and decreased sperm count (Vine *et al*., 1996).

In a prospective study, Saleh *et al*. (2002) compared infertile men who smoked cigarettes with nonsmoker infertile men. Smoking was associated with a significant increase (approximately 48%) in seminal leukocyte concentrations, a 107% ROS level increase and a 10 point decrease in ROS-TAC score. The authors concluded that infertile men who smoke cigarettes present higher seminal OS levels than infertile nonsmokers, possibly due to significant increase in leukocyte concentration in their semen. The link between cigarette smoking and high seminal ROS can be attributed in part to the associated

increase in seminal leukocytes. Indeed, smoking can increase leukocyte concentrations by as much as 48% (Saleh *et al*., 2002). Smokers have decreased levels of seminal plasma antioxidants such as Vitamin E (Fraga *et al*., 1996) and Vitamin C (Mostafa *et al*., 2006), placing their sperm at additional risk of oxidative damage. This has been confirmed by the finding of a significant increase in levels of 8-OHdG within smoker's seminal plasma (Fraga *et al*., 1996).

In a study carried out on 655 smokers and 1131 non smokers, cigarette smoking was associated with a significant decrease in sperm density (-15.3%), total sperm count (-17.5%) and total number of motile sperm (-16.6%) (Künzle *et al*., 2003). Thus, smoking does, in fact, affect the quality and quantity of sperm present within a male.

Varicocele and ROS: Varicocele has long been implicated as a major cause of male infertility (Schlesinger *et al*., 1994; Benoff and Gilbert, 2001; Hauser *et al*., 2001), but the pathophysiology remains unclear. Clinical varicocele is found in about 15% of the general population including adolescents and adults: in 35% of men with primary infertility and in up to 80% of men with secondary infertility (World Health Organization, 1992; Belloli *et al*., 1993; Pfeiffer *et al*., 2006). Oxidative stress is now widely believed to be the principal underlying pathology linking varicocele with male infertility (Barbieri *et al*., 1999; Hendin *et al*., 1999; Saleh *et al*., 2003; Nallella *et al*., 2004; Smith *et al*., 2006; Ishikawa *et al*., 2007; Smith *et al*., 2007; Saalu *et al*., 2008). It appears that infertile men with varicocele have significantly greater spermatozoal DNA damage, which can be related to high levels of OS in semen (Saleh *et al*., 2003). Levels of ROS positively correlate with the degree of varicocele and are expected to decrease after varicocelectomy (Barbieri *et al*., 1999). According to one metaanalysis, varicocele patients as compared with normal sperm donors have significantly increased oxidative stress parameters such as ROS and lipid peroxidation as well as significantly decreased antioxidant concentrations. The exact pathways by which a varicocele damages spermatogenesis and sperm quality remain poorly understood.

Role of ROS in assisted reproductive technique: By means of *in vitro* fertilization (IVF), men with very low sperm counts can be given a reasonable chance of paternity. However, this also increases the possibility of passing genetic abnormalities on to the next generation because the sperm of infertile men shows an increase in aneuploidy, other genetic abnormalities and DNA damage. Although there are prospects for screening of sperm (Griffin and Finch, 2005), current routine clinical practice is based on the screening of peripheral blood samples. The use of Assisted Reproductive Technologies (ART) has the potential to exacerbate sperm oxidative stress. During IVF and intrauterine insemination (IUI) treatment semen is centrifuged to separate sperm from seminal plasma. This exacerbates oxidative stress as centrifugation increases sperm ROS production many fold (Iwasaki and Gagnon, 1992; Shekarriz *et al*., 1995a, b) Spermatozoa selected for ART usually originate from an environment experiencing oxidative stress and a high percentage of these sperm may have damaged DNA (Kodama *et al*., 1997). When IUI or IVF is used; such damage may not be a cause of concern because the collateral peroxidative damage to the sperm plasma membrane ensures that fertilization cannot occur with a DNA-damaged sperm.

When Intracytoplasmic Sperm Injection (ICSI) is used, this natural selection barrier is bypassed and a spermatozoon with damaged DNA is directly injected into the oocyte (Lamirande and Gagnon, 1992; Aitken, 1999). Sperm DNA damage is critical in the context of success of assisted reproductive techniques (Sakkas *et al*., 2003; Sharma *et al*., 2004). Advances in ART have helped in improving treatment of male factor infertility (Alvarez, 2003). Currently, ICSI is the most common ART method, although it is associated with the highest number of miscarriages. One of the explanations can be the poor selection of sperm that are possibly damaged by free radicals during ART procedures. Thus evaluation of seminal ROS levels and extent of sperm DNA damage especially in an infertile male may help develop new therapeutic strategies and improve success of ART.

ROS and antioxidants: Antioxidants, in general, are compounds and reactions which dispose, scavenge and suppress the formation of ROS, or oppose their actions. A variety of biological and chemical antioxidants that attack ROS and LPO are presently under investigation. Among the well known biological antioxidants, superoxide dismutase (SOD) and its two isozymes and catalase have a significant role. SOD spontaneously dismutates (O^{2-}). anion to form O_2 and H_2O_2, while catalase converts H_2O_2 to H_2O and O_2:

$$H_2O_2 \text{ to } O_2 \text{ and } H_2O_2\ (O^{2-}). + 2H+ \text{ SOD } H_2O_2 + O_2$$

$$H_2O_2 \text{ Catalase } H_2O + 1/2\ O_2$$

The most common antioxidants, that protect spermatozoa from excess concentrations of ROS and

OS-induced damage and altogether represent the Total Antioxidant Capacity (TAC) of seminal plasma are SOD (Nissen and Kreysel, 1983; Aitken and Krause, 2001), catalase (CAT) (Jeulin *et al*., 1989), the glutathione (GSH) peroxidase system selenium and selenoproteins such as the phospholipids hydroperoxide glutathione peroxidase (PHGPx) and the glutathione reductase system (Li, 1975; Alvarez and Storey, 1989), vitamins A, C (Niki, 1991) and E (Chow, 1991), glutathione (Kidd, 1997), spermin, thiols, urate (Ronquist and Niklasson, 1984; Gavella *et al*., 1997), albumin, taurine and hypotaurine (Alvarez and Storey, 1983), L-carnitine and zinc. Antioxidants contained within seminal plasma are obviously helpful for preventing sperm oxidative attack following ejaculation. However, during spermatogenesis and epididymal storage, the sperm are not in contact with seminal plasma antioxidants and must rely on epididymal/testicular antioxidants and their own intrinsic antioxidant capacity for protection. Sperm are therefore vulnerable to oxidative damage during epididymal transit, especially when there is epididymal inflammation such as male genital tract infection. A study of 46 alcoholic men of reproductive age has suggested the presence of oxidative stress within the testicle by reporting a significant reduction in plasma testosterone, increase in serum lipid peroxidation byproducts and a drop in antioxidants (Maneesh *et al*., 2006). However, no study to date has directly examined the link between alcohol intake and sperm oxidative damage.

CONCLUSION

The role of physiological and pathological levels of ROS in male fertility is yet to be clearly established. While ROS have been associated with the pathology of numerous diseases including male infertility, Small controlled amounts of ROS are vital for spermatozoa to develop into normal spermatozoa capable of fertilization structures. Since spermatogenesis is a complex process involving various stages and different type of cells, mutations in mitochondrial genome, as a result of excessive ROS could disturb the formation of morphologically and functionally mature spermatozoa thus leading to infertility. Recently, a phenomenal growth has occurred in our knowledge of male reproduction, sperm function and development of diagnostic tools and treatment modalities for male infertility. In addition, knowledge regarding oxidative stress has given rise to several new treatment modalities that are now being tried to improve male infertility. However a wide gap still remains in our knowledge and future multicentric studies with larger samples are needed to help gain a better insight into this essential problem.

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