Review Article

Free radicals: Their beneficial and detrimental effects on sperm function

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Free radicals are molecules with one or more unpaired electron(s) commonly found in seminal plasma. Physiologically, free radicals control sperm maturation, capacitation and hyperactivation, the acrosome reaction, and sperm-oocyte fusion. Pathologically, free radicals induce lipid peroxidation, DNA damage and apoptosis of spermatozoa. The present review deals with both the beneficial and detrimental effects of free radicals on sperm function.

Keywords: Apoptosis, Acrosome reaction, Capacitation, DNA damage, Free radical, Sperm function

Free radicals are molecules with one or more unpaired electron(s)[1]. These highly reactive molecules attack the nearest stable molecule to obtain an electron. Subsequently, the targeted molecule becomes a free radical itself and initiates a cascade of events that can ultimately lead to cellular damage[2]. However, at physiological levels, free radicals also help preserve homeostasis by acting as signal transducers[3].

There are two common forms of free radicals namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS). Examples of ROS include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the extremely reactive hydroxyl radical ($OH^.$) and the peroxyl radical (HO_2^-)[4]. RNS are often considered to be a subclass of ROS [4] and include nitric oxide (NO), nitrous oxide (N_2O), peroxynitrite (NO_3^-), nitroxyl anion (HNO) and peroxynitrous acid (HNO_3)[1,5].

Antioxidants act as free radical scavengers that help to keep free radicals at homeostatic levels in order to maintain physiologic function and prevent pathological effects due to development of oxidative stress (OS)[6]. OS is a state when there is an imbalance between ROS and the scavenging properties of antioxidants. Under these circumstances ROS outnumber antioxidants due to either increased ROS levels, decreased antioxidant properties, or a combination of both.

Physiologically, free radicals control sperm maturation, capacitation and hyperactivation, the

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acrosome reaction (AR), and sperm-oocyte fusion. Pathologically, free radicals induce lipid peroxidation (LPO), DNA damage and apoptosis. The pathological effects of free radicals on sperm function have been described extensively in the literature, but a discussion physiological roles of free radicals in sperm function is required for better understanding of the phenomena. It is, therefore, the aim of the present review article is to explore both the beneficial and detrimental role of free radicals on sperm function.

Sources of ROS in seminal plasma

ROS that ultimately end up in the seminal plasma and exert their effects on spermatozoa can be generated from or originate from various sources that can either be endogenous or exogenous.

Endogenous sources of ROS

Semen consists of different types of cells such as mature and immature spermatozoa, round cells from different stages of spermatogenesis, leukocytes and epithelial cells. Of these, leukocytes (neutrophils and macrophages) and immature spermatozoa are the two main sources of ROS[7,8].

Peroxidase positive leukocytes — Peroxidase positive leukocytes include polymorphonuclear leukocytes (PMN) (50-60% of all leukocytes) and macrophages (20-30% of all leukocytes)[9,10]. These peroxidase positive leukocytes originate from the prostate and seminal vesicles[11]. As one of the main mechanisms to combat pathogens, leukocytes produce high levels of ROS[12]. More specifically, when activated during disease states, peroxidase positive leukocytes produce ROS through the nicotinamide

adenine dinucleotide phosphate (NADPH) system[13,14]. In addition, an increase in proinflammatory cytokines such as interleukin-8 and a decrease in the antioxidant superoxide dismutase (SOD) lead to increased levels of OS[13]. During infection, there are increased levels of leukocytes subsequently leading to more ROS production with detrimental effects on sperm function[15]. Sperm are particularly susceptible to infections in the testis and epididymis because of their lack of adequate antioxidant defense mechanisms[16]. More specifically, patients with genito-urinary tract infection have decreased sperm motility and decreased fertilization capacity[17,18].

Leukocytes, rather than immature spermatozoa, are the predominant source of ROS during sperm preparation[19]. In a study by Plante *et al.*[20] increased levels of PMN, not abnormal spermatozoa, led to decreased sperm motility. It has also been shown that leukocytes produce 1000 times more ROS than spermatozoa in human semen[20,21]. In addition, levels of ROS were correlated to the degree of leukocyte contamination[22]. *In vivo*, seminal plasma contains powerful antioxidants to scavenge the potentially dangerous ROS in the external environment[23]. However, during the *in vitro* preparation of sperm for assisted reproductive techniques, these antioxidant seminal plasma protective capacities are removed and it leaves sperm particularly susceptible to OS damage[24].

Immature spermatozoa — Normally, during spermatogenesis, developing sperm extrude their cytoplasm in preparation for fertilization. However, impaired spermatogenesis does not allow for the proper extrusion of cytoplasm. If excess cytoplasm exists, these sperm are deemed immature and functionally defective[25,26]. Excess cytoplasm (cytoplasmic droplets) activates the NADPH system, which provides electrons for free radicals through a cascade of events that ultimately results in the activation of the enzyme NADPH oxidase[26]. This production of NADPH is mediated through the enzyme glucose-6-phosphate dehydrogenase[27]. This cascade of events ultimately increases ROS production. Overall, there are two mechanisms by which spermatozoa produce ROS—(1) NADPH-oxidase system at the plasma membrane level; and (2) NADH-dependent oxidoreductase system at the mitochondrial level[28]. The mitochondrial system has been shown to be the primary source of ROS in infertile men[20].

Exogenous sources of ROS

Along with the endogenous sources of ROS, there are numerous exogenous sources of ROS, which produce ROS in pathological amounts. These sources include industrial compounds, smoking, alcohol, spinal cord injury and varicocele.

Industrial compounds — Industrial compounds have been well documented to cause adverse health effects. In male reproduction, many studies have described the negative effects of various chemicals and compounds. Environmental pollutants have shown to increase ROS such as O_2^- and H_2O_2 in male testes. More specifically, phthalate, a compound commonly found in plastics and beauty products, induces sperm DNA damage and impairs spermatogenesis[29,30]. Heavy metals like lead, pesticides, and sulfur dioxide, a widespread food preservative, all increase OS in the male testes[31-33].

Smoking — Like industrial compounds, cigarette smoking has been shown to cause widespread harmful effects on health. Specific components of cigarettes such as nicotine are associated with free radical production[34]. In sperm, cigarette smoke increases ROS production while decreasing antioxidant capacity. Cigarette smoke decreases sperm parameters such as sperm motility, morphology and concentration[35,36]. In a study by Saleh *et al.*[35], in smokers, seminal ROS production increased as measured by ROS-total antioxidant capacity (TAC) score. Smoking has also been shown to increase seminal leukocyte concentration, a potential source of ROS.

Alcohol — Ethanol metabolism increases ROS, while it simultaneously decreases antioxidants. These effects result in biomolecule damage including damage to proteins, lipids and DNA[37]. In addition, consumption of alcohol has been shown to increase ROS, if it is consumed by a person eating low nutrition diet[38].

Spinal cord injury — Over 90% of men with spinal cord injury are infertile[39]. It is thought that these men have elevated ROS levels in sperm. These high ROS levels correlate with poor sperm motility and morphology that may lead to infertility[40,41]. In addition, men with spinal cord injury have increased polymorphonuclear leukocytes (producers of ROS) in their semen[5].

Varicocele — Varicocele is the uncharacteristic dilation and tortuosity of veins in pampiniform plexus around the human male spermatic cord[42]. Varicocele appears to be associated with higher levels of ROS[8].

Increased levels of xanthine oxidase (a source of the O_2^-) and NO appear to increase ROS, thereby impairing sperm function[43]. High levels of NO have been reported in infertile men with varicocele compared to fertile men as well as infertile men without varicocele[44]. In addition, increased ROS levels in semen have been correlated with a higher varicocele grade. Those patients with varicocele grade 2 or 3 have also been shown to have higher levels of ROS in their semen[45]. In varicocele patients there are more markers of OS such as ROS induced LPO and presence of DNA damage[46,47]. Finally, those patients receiving a varicocelectomy have shown a decrease in ROS levels and increase in levels of TAC[48].

Physiological role of ROS

Besides the well-known pathological effects that are a consequence of excess ROS production, physiological levels are actually essential for proper sperm function. ROS plays an important role in transducing signals in the complex biochemical cascades of the spermatozoa. Positive effects on maturation, hyperactivation, capacitation, AR and sperm-oocyte fusion have all been well documented. The specific ROS involved in the activation of these processes is still somewhat controversial and may be species specific[3] with NO being identified as the RNS central to mediating sperm function[5] (Fig. 1).

Maturation — Spermatozoa have a characteristically tight packaging of chromatin, a process largely the result of ROS. During spermiogenesis, histone beads are replaced by smaller proteins called protamines. The majority of cysteine residues in protamines, under the slight oxidative conditions of the caudal epididymis, form disulfide bonds that result in condensed, stabilized nuclear DNA[49-51]. Because spermatozoa lack the preventative and repair systems of somatic cells or the oocyte, the enhanced stability of sperm chromatin is required for genetic integrity[52]. In addition, ROS induce changes in the mitochondrial membrane that confer protection to the major energy producer of the cell. Mature spermatozoa contain mitochondria with a keratinous-like protective coat called the mitochondrial capsule[53]. During spermatogenesis, peroxides such as H_2O_2 may induce the formation of this coat through the oxidation of phospholipid hydroperoxide glutathione peroxidase (PHPGx). Once oxidized, PHPGx, a selenoenzyme, forms an intermediate that can react with reduced protein thiol groups of the capsule to form a selenadisulfide bond. Inactivation of PHGPx and oxidation of thiol groups results in the complex, yet stable protein network of the mitochondrial capsule[54].

Capacitation and hyperactivation — Capacitation is the poorly understood maturation process that spermatozoa undergo in the female genital tract. It includes the attainment of hyperactive motility and potential to undergo acrosome reaction (AR), both of which are necessary for successful fertilization to occur. The process includes an array of molecular changes, most notably are— an efflux of cholesterol; fluxes of bicarbonate, calcium (Ca^{2+}) and other small ions that cause membrane hyperpolarization; and increase in pH, cAMP, and protein phosphorylation[55].

Hyperactivation, an essential process for successful fertilization, is considered a sub-category of capacitation. In non-hyperactive spermatozoa, low-amplitude flagellar movements are associated with slow, linear movements of the cell. Alternately, hyperactive spermatozoa exhibit high amplitude, asymmetric flagellar movement, significant side-to-side displacement of the sperm head, and an overall non-linear motility. In the female genital tract, spermatozoa with hyperactivated motility exhibit less stagnation in the oviductal epithelium and the surrounding viscous mucus. In addition, hyperactive motility provides spermatozoa with the necessary propulsion to penetrate the cumulus cells—which via secretions of progesterone may actually initiate hyperactivation at the zona pellucida (ZP) of the oocyte[56].

Results supporting the role of ROS in the initiation of hyperactivation are well accepted. Generation of ROS can be performed by a variety of methods, including the addition of xanthine + xanthine-oxidase (X + XO), glucose oxidase, potassium superoxide, H_2O_2, NADPH, fetal cord serum (FCS) and fetal cord serum ultrafiltrate (FCSU). The results from *in vitro* experiments using such ROS generating techniques has led to the suggestion that O_2^- is crucial to hyperactive motility. In hamster sperm epididymis, NO was also shown to regulate hyperactivation[57]. Hyperactivation initiated by the biological fluids FCS and FCSU was inhibited in the presence of SOD, indicating the potential activity of O_2^- *in vivo*[58]. H_2O_2 has been observed to induce a positive effect, but the delicate balance between motility activation and LPO induced by H_2O_2 may be a reason for the variable reports[3].

Whereas O_2^- is speculated to regulate hyperactivation, H_2O_2 is proposed to be central to the remaining processes of capacitation[59]. However, at low concentrations, NO is also thought to be involved in this process. Catalase prevents NO-induced capacitation, an indication that NO effects are a result of a complex mechanism involving H_2O_2[60]. In fact, the presence of ROS scavengers and nitric oxide synthase (NOS) inhibitors decreases cAMP, a well known molecule necessary for capacitation[61]. In many

ways, the molecular events accompanying capacitation are indistinguishable from those of the AR, except that the process is reversible and the time frame is significantly longer. Because of the similarities, few techniques are available that definitively distinguish between the two processes. As such, the two most common methods to assess the status of capacitation include the measurement of tyrosine phosphorylation and the ability to undergo the AR in response to external stimuli. Capacitation,
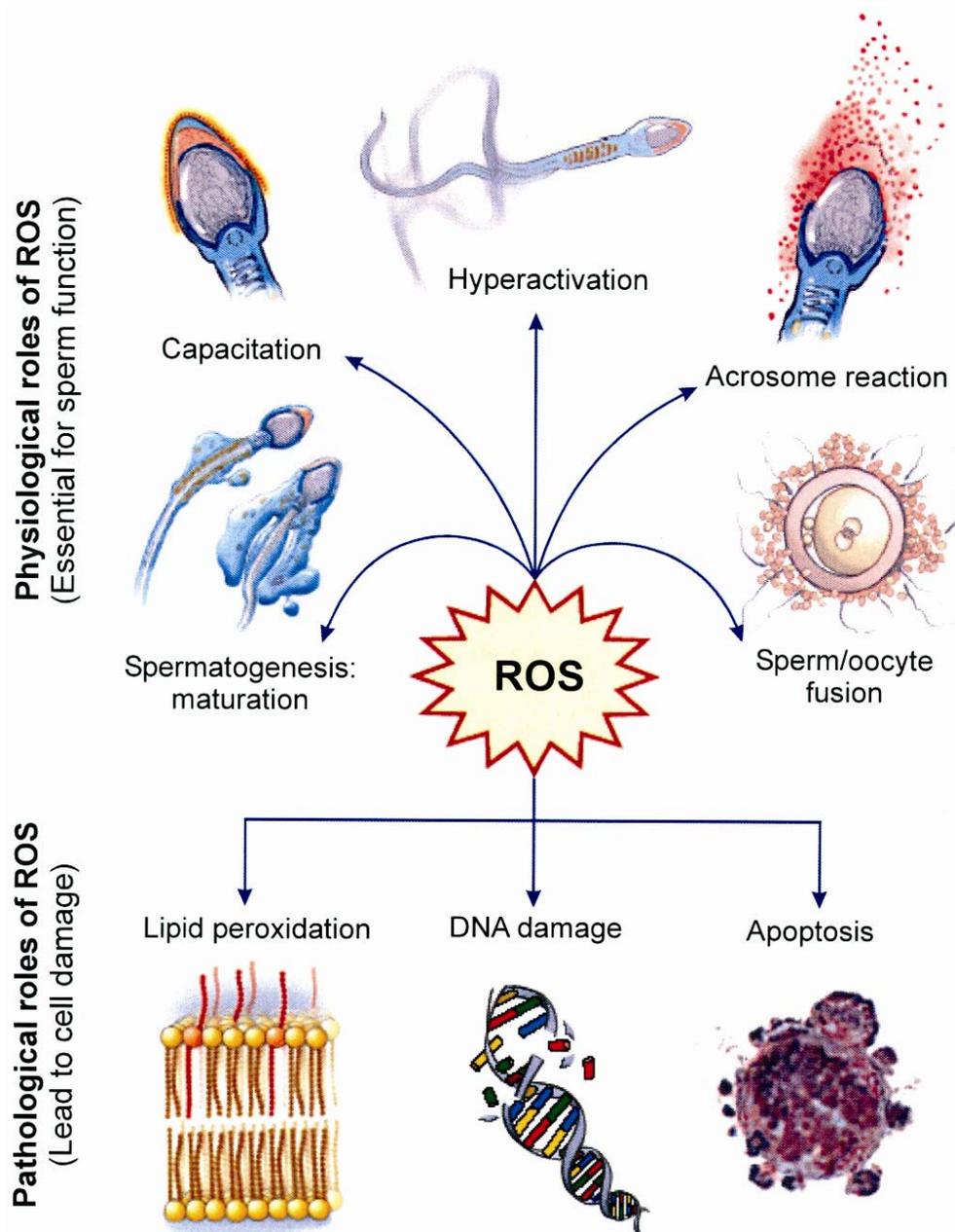

Fig. 1 — Physiological roles of ROS include maturation, capacitation, hyperactivation, the acrosome reaction and sperm-oocyte fusion. Pathological roles of ROS include lipid peroxidation, DNA damage and apoptosis.

the final maturation process, ensures that only fertile spermatozoa are able to reach, bind to, and penetrate the oocyte[55].

The general biochemical scheme involves a protein kinase A (PKA)-dependent mechanism that may be regulated by ROS. Current evidence suggests that the process is initiated by an influx of Ca^{2+} and HCO_3^- that activates the amplifier enzyme adenyl cyclase (AC). AC converts ATP to cAMP, which then activates PKA that eventually leads to tyrosine phosphorylation associated with capacitation. ROS promote capacitation by stimulating AC, inhibiting phosphotyrosine phosphatases (PTPases) and activating tyrosine kinases (TKs). By doing so, ROS thereby increase the amount of tyrosine phosphorylation, one of the final steps in the biochemical scheme. Specifically, O_2^- may activate AC via oxidation of an essential thiol group. H_2O_2, on the other hand, is hypothesized to promote tyrosine phosphorylation more indirectly through activation of TKs and inhibition of PTPases. The intermediate steps between PKA activation and tyrosine phosphorylation are unknown, although several different, interconnected biochemical pathways may ultimately regulate capacitation[61].

Acrosome reaction (AR) — Once hyperactivated motility has propelled the spermatozoa past the cumulus oophorus, a capacitated sperm may bind to ZP and initiate an exocytotic release of proteolytic enzymes. AR, as the event is termed, creates a pore in the extracellular matrix of ZP and allows spermatozoa to penetrate this physical barrier and fuse with the oocyte. As noted above, the molecular events of AR share considerable overlap with those of capacitation. Included in these similarities is phosphorylation of similar tyrosine proteins, an influx of Ca^{2+}, and activity of AC, cAMP, and PKA[62]. *In vivo*, the process is initiated upon binding to ZP. ROS have been shown to increase sperm affinity for ZP, possibly through the phosphorylation of three plasma membrane proteins at the apex of the sperm head, fertilin beta (ADAM2), spermadhesin family (includes AZN-3 and others), and P47 (the porcine homologue of SED1)[63]. *In vitro*, activation of AR has been observed upon addition of ROS[59,64]. At low concentrations, O_2^-, H_2O_2, and NO have all been shown to have positive effects on the AR[65-68]. NOS inhibitors have inhibited AR in human sperm, further evidence that NO is integral in AR. Similar to capacitation, ROS stimulates AR by activating AC,

which triggers downstream molecules (cAMP, PKA, etc) to initiate the exocytotic event[61].

Sperm-oocyte fusion — After bypassing the barrier of ZP, a spermatozoon may fuse with the oocyte and result in successful fertilization[69]. A high degree of sperm membrane fluidity is necessary for proper fusion to occur. Some degree of membrane fluidity is assured by the high poly-unsaturated fatty acid (PUFA) content of the sperm plasma membrane[70]. In studies of human spermatozoa, ROS have been shown to increase the rates of sperm-oocyte fusion[71]. The mechanism through which ROS increase membrane fluidity actually occurs during the biochemical cascade of capacitation and AR. During capacitation, by inhibiting PTPase activity, ROS prevent the dephosphorylation and deactivation of phospholipase A_2 (PLA_2) which cleaves the secondary fatty acid from the triglycerol backbone of the membrane phospholipid and increases membrane fluidity[72]. In addition, by activating AC and downstream molecules during the AR, ROS may activate PLA_2. Protein kinase C is also known to phosphorylate and therefore activate PLA_2[72].

Pathological roles of ROS

Physiologically, ROS are integral as signaling molecules for many processes involved in fertilization. However, when ROS levels overwhelm the antioxidant defense systems, pathological effects result. Because ROS are highly reactive, when they reach pathological levels they exert significant damage on biomolecules, such as proteins, lipids and nucleic acids[73]. The extent of this damage depends on many factors including duration of exposure, type of ROS and concentration as well as external factors like temperature, oxygen tension and antioxidant levels[74] (Fig. 1).

Lipid peroxidation (LPO) — The sperm plasma membrane is largely composed of PUFAs which are susceptible to oxidative damage due to the existence of double bonds[75]. The sperm membrane contains almost 50 per cent decosahexanoic acid, which contains six unsaturated double bonds in every molecule. As the LPO cascade proceeds in sperm, almost 60% of the fatty acid is lost from the membrane. LPO affects membrane structure and function such as fluidity, ion gradients, receptor transduction, transport processes and membrane enzymes[9]. As a result, functions that are necessary for normal fertilization are impaired[73]. Because LPO has

been implicated in infertility, it is important to understand the processes underlying LPO[76].

LPO can be divided into three phases, the initiation phase, the propagation phase and the termination phase. Before any of these phases commence, ROS generation is necessary. O_2^- is generated either through NADPH system, an intracellular source, or through leukocytes, an extracellular source. O_2^- can be directly protonated to form hydroperoxyl radical (HO_2) or it can be converted into H_2O_2 via SOD. H_2O_2 can then be converted into $OH^\bullet$ via Fenton reaction involving ferrous ion. These two ROS molecules, $OH^\bullet$ and HO_2, mark the beginning of initiation stage[77].

During the initiation phase, $OH^\bullet$ or HO_2 initiates LPO[77]. O_2^- and H_2O_2 are not energetic enough to initiate LPO directly[13]. As the initiation stage proceeds, unsaturated lipids undergo hydrogen abstraction to form lipid radicals. Presence of a double bond next to a methylene group makes the methylene carbon-hydrogen bonds weaker and more vulnerable to abstraction. Lipid radical then reacts with oxygen to form lipid HO_2^-, which eventually converts into lipid peroxide through antioxidant defense mechanisms. This lipid peroxide actually stabilizes the sperm plasma membrane. However, during the propagation phase, in the presence of a transition metal ion, the lipid peroxide gets broken down into alkoxyl radical and HO_2^-. These radicals subsequently act on additional lipids until lipid damage is widespread and irreversible[73,77]. In the termination phase two radicals react with each other to form a stable product and LPO ceases[76,78].

LPO damage can be quantified by measuring malondialdehyde (MDA), a stable by-product of LPO[79,80]. MDA is measured using the spectrophotometric thiobarbituric acid test. Another biological by-product of LPO is 4-hydroxynoneal, which is formed from low density lipoproteins. This compound can act on protein and DNA levels, causing significant damage. Moreover, hydroxynoneal can act as a chemical signal for neutrophils, preventing cell growth and exerting pathological effects[76].

There are many pathological effects of LPO on sperm function. Overall, LPO damages DNA and proteins through oxidation from lipid peroxyl or alkoxyl radicals. DNA damage by LPO can occur via base modifications, strand breaks or cross-linking[4]. In addition, LPO can result in a loss in membrane fluidity and as a result decreased sperm motility and

sperm-oocyte fusion[81-83]. NO has been shown as a potential ROS involved in decreasing sperm motility in humans[84,85]. The degree to which sperm motility and sperm-oocyte fusion are impaired is related to the amount of MDA[81,86]. In LPO, ROS initiate a cascade of events involving X + XO system and depleted ATP production that ultimately results in sperm immobilization[87]. In a study by de Lamirande et al[88]., increased levels of H_2O_2 induced LPO that eventually led to death of spermatozoa.

Recent studies of LPO have shown two trends. The first is that those patients with abnormal semen parameters have increased LPO. In a study by Khosrowbeygi et al[89]., patients with asthenozoospermia, asthenoteratozoospermia, and oligoasthenoteratozoospermia sperm all had higher levels of PUFA and therefore, were more vulnerable to LPO caused by ROS. Another study has shown similar results, as patients with asthenozoospermia and oligoasthenoteratozoospermia also show higher MDA levels[90]. Furthermore, LPO as measured via MDA levels correlated inversely to in vitro fertilization rates in infertile men[91]. Secondly increases in inflammation and infection have also been reported in LPO. In a study by Fraczek et al[92]., proinflammatory cytokines propagate an increase in ROS levels, causing OS leading to increased LPO and as such, altering sperm function. Another study has demonstrated that the presence of various bacteria strains in semen, for instance Escherichia coli, increases white blood cells, thereby causing OS and increased LPO[93].

DNA damage — Tight packing of DNA combined with the antioxidant defense mechanisms of semen protect DNA from damage[94]. It is perhaps for this reason that sperm are particularly resistant to DNA damage compared to many cell lines including germ cells and myoblastoid cells[95]. However, the fact still remains that sperm undergoes DNA damage and it can be a possible etiology of infertility. ROS as well as apoptosis are proposed mechanisms that can induce DNA damage[96]. In a recent study by Amiri et al[97]., NO has been correlated with increased DNA damage. In a study by Irvine et al.[98], infertile men have shown increased DNA fragmentation, decreased semen parameters and increased levels of ROS. In sperm, the mitochondrial genome is more susceptible to DNA damage than nuclear genome via H_2O_2[95]. In addition, due to Y chromosome's structure and the fact that it cannot repair double strand breaks, it is especially

susceptible to DNA damage[16]. High levels of ROS have also been correlated with DNA single and double strand breaks[99]. In a state of OS, DNA damage occurs due to oxidation of bases, strand breaks, cross-linking, deletions, frame shifts and rearrangement of chromosomes[9,100].

During intrauterine insemination and *in vitro* fertilization sperm with increased levels of DNA damage is not a cause for alarm as the concurrent LPO damage by the ROS eliminate the possibility for fertilization[74]. However, during intracytoplasmic sperm injection natural selection is bypassed and sperm with significant amounts of DNA damage have the opportunity to fertilize the oocyte[52]. Despite the fact that the sperm can have significant DNA damage, it still retains the ability to fertilize the egg, which poses significant risk to the embryo[14]. Increased DNA damage has been associated with decreased fertilization rates *in vitro* as well as increased early embryo death[101,102]. Unfortunately, there is no established clinical treatment for DNA damage[16].

Apoptosis — Often, when cellular components undergo damage, apoptosis or programmed cell death is initiated[103]. In male reproduction, abnormal spermatozoa may be eliminated via apoptosis[102]. The mechanisms of action of apoptosis in male reproduction are not well studied. However, preliminary studies have discussed that ROS serves as a stimulus that activates the mitochondria to release the signaling molecule cytochrome c[104,105]. Cytochrome c initiates a cascade of events that involve several caspases including caspase 3 and 9 that eventually leads to sperm apoptosis. In fact, ROS is associated with apoptotic caspases, including caspase 3 and 9[105]. Fas-protein may also be integral in the apoptotic pathway. When Fas-ligand or anti-Fas antibody binds to Fas, apoptosis is initiated[106]. An additional mechanism for apoptosis involves the inflammatory production of ROS, more specifically, hypochlorous acid (HOCl). HOCl is formed from H_2O_2 and chloride ion. HOCl oxidizes many cellular components, thus causing apoptosis. In a study by Said *et al.*[107], have reported that HOCl is associated with elevated levels of apoptosis in spermatozoa.

Many studies have outlined apoptosis in spermatozoa. In a study, increased ROS levels and markers for apoptosis, as measured by annexin-V staining, have been correlated in mature spermatozoa. ROS concentrations have been correlated with increased levels of DNA damage and apoptosis[105,108-110]. In deer spermatozoa it has been shown that ROS-specifically H_2O_2-induces apoptosis, while O_2^- and $OH^\bullet$ do not induce apoptosis[111]. Meanwhile studies in primate, murine and boar spermatozoa indicate that NO is correlated with apoptosis perhaps through caspase activation[112-114].

Recent literature has explored the potential for antioxidants to treat the pathological effects of ROS, including apoptotic cell death. In a study, *in vivo* antioxidant therapy has been shown to decrease sperm DNA damage, seminal levels of OS and apoptosis[115]. Association between these elements can underscore a possible etiology of male infertility. However, further studies need to be conducted to understand the mechanisms of action behind this association. In another study, taurine, a known antioxidant, reduces levels of arsenic-induced apoptosis in sperm both through mitochondrial dependent and independent pathway[116]. Melatonin, a known endogenous antioxidant, counteracts the 2-bromopropane (2-BP)-induced apoptotic damage as measured by caspase 3 activation in the testis. In addition, microcystin-LR, a bacterial toxin increases apoptotic DNA damage and a proposed mechanism is through an increase in ROS.

New research has also explored the role of external sources of ROS production to mediate apoptosis. In a study by Kumar *et al.*[109], a cell extract with known pathologic properties induces apoptosis through ROS cell damage. Furthermore, the herbicide, alachlor, potentially induces apoptotic cell death via ROS[117]. Finally, increased temperature in the male reproductive system is associated with a release of ROS that eventually causes sperm death. In this pathway, ROS has been proposed to directly signal cell death[118].

Physiological and pathological levels of ROS

Much literature, both current and past, fails to really elaborate on the exact numerical values of "physiological" levels of ROS when compared to "pathological" levels of ROS. It is hard to understand the subject completely without some numbers quantifying physiological versus pathological levels of ROS. There have been several recent attempts to elucidate this matter. Three different studies have reported three relatively similar cutoff values of ROS between low (physiological) and high (pathological) levels of ROS. Desai *et al.*[119] have reported the cutoff value to be 0.0185×10^6 counted photons per minute

(cpm)/20 × 10⁶ sperm, Agarwal *et al.*[120] have reported the cutoff value to be 0.0125×10^6 cmp/20 × 10⁶ sperm, and Allameni *et al.*[121] have reported the cutoff value to be 0.0145×10^6 cpm/20 × 10⁶ sperm. In general, values below the cutoff are low levels of ROS, while values above the cutoff are high levels of ROS. However, these experiments were not consistent with respect to samples used and subjects tested, among other things. For example, Agarwal *et al.*[120], included only infertile patients, while Desai *et al.*[119] included infertile and fertile patients in their study. Therefore it would be hard to accurately compare results. It is at least a start in the right direction to having a tangible number when referring to physiological and pathological levels of ROS.

Conclusion

This paper has discussed both the physiological and pathological roles that free radicals serve in the male reproductive system. An important perspective is that free radicals are not exclusively beneficial or exclusively detrimental. Rather, they need to be maintained at appropriate levels to ensure physiological function, while preventing pathological damage. Antioxidants represent a potential treatment for pathological levels of free radicals. However, despite the lack of clinical consensus on the efficacy of antioxidants as a potential therapy, if used, antioxidants need to keep free radicals at levels that are physiologically appropriate. Even though this paper elucidates many mechanisms dealing with free radicals and sperm, further research needs to be undertaken in order to understand the mechanisms of action behind free radicals.

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