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Role of Reactive Oxygen Species in Male Reproduction

Sabiha Fatima

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Abstract

The production of reactive oxygen species (ROS) is a normal physiological event in the male germ line. ROS are a double-edged sword, despite its role as key signaling molecules in physiological processes such as capacitation and hyperactivation, its overproduction which overwhelms the body's antioxidant defenses is thought to affect male fertility and normal embryonic development. The excess generation of ROS in semen by exogenous and endogenous factors has been recognized as detrimental etiologies for male infertilities. Spermatozoa are vulnerable to ROS attack because they are rich in mitochondria, have abundance of substrates for free radical attack and their capacity to protect themselves from oxidative stress is limited. The cytotoxic aldehydes generated as a result of lipid peroxidation are known to form adduct with the mitochondrial protein involved in electron transport chain and stimulate generation of ROS in mitochondria. ROS and their metabolites can lead to oxidative DNA damage in mitochondria and nucleus that eventually culminates in DNA fragmentation. The presence for large amount of damaged DNA is a major characteristic of defective human spermatozoa, which affect the fertility and pregnancy outcome. Thus, as a comprehensive approach, treatment of oxidative stress should involve strategies to reduce stress-provoking conditions to help reverse sperm dysfunction.

Keywords: ROS, oxidative stress, male infertility, DNA damage

1. Introduction

Infertility is a disorder affecting 10–15% couples of reproductive age worldwide [1, 2]. It is defined as the inability of a couple to achieve spontaneous pregnancy after 1 year of regular, unprotected sexual intercourse [3]. The inability to have children affects the infertile couples psychologically and it may lead to depression, suicidal tendencies and other pathological and psychological conditions [4, 5]. Although, fertility may decrease with increase in age, but often occurs as a result of anatomic defects, endocrinopathies, immunologic problems, gene

mutation, ejaculatory failures or radiation, chemotherapy and environmental exposures [6–9]. In approximately half of all the cases of infertility, male factor is the sole or major contributing factor with no identifiable cause found in over 25% of infertile males [10, 11]. In approximately 40–50% of the male infertility cases, oxidative stress-related mechanisms are found to be responsible for the impairment of the sperm function and fertilization [12]. Oxidative stress is a disturbance in the balance between the systemic manifestation of reactive oxygen species (ROS) and the ability of the body to counteract their harmful effects through neutralization by antioxidant defense mechanism [13]. ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^*) are highly reactive oxidizing agents produced continuously during metabolic processes [14]. Oxidative processes related to spermatozoa are particularly of interest as they exhibit a double-edged sword role in these cells (**Figure 1**). The physiological level of ROS is necessary to regulate a critical redox-sensitive processes such as capacitation and hyperactivation without which fertilization is impossible [15]. While its supra-physiological level affects normal spermatogenesis and sperm functions such as motility, capacitation, acrosome reaction, egg penetration and decondensation of sperm head, which is essential to achieve fertilization. Spermatogenesis is a metabolically active biological process during which haploid spermatozoa are produced in the seminiferous tubules. During this process O_2^- are generated as a natural by-product of cellular respiration. The germ cells undergoing differentiation to spermatids in testes are protected from oxidative stress by its nurse cells called sertoli cells which possess high level of antioxidant enzymes such as superoxide dismutase (SOD) as well as the reductase, transferase, and peroxidase activities of the glutathione cycle [16]. Once the spermatozoa are released from the germinal epithelium, they become vulnerable to oxidative attack as they are no longer protected by defense mechanism of sertoli cells [13, 17]. Excess ROS can lead to cellular injury by damaging DNA, lipids, and

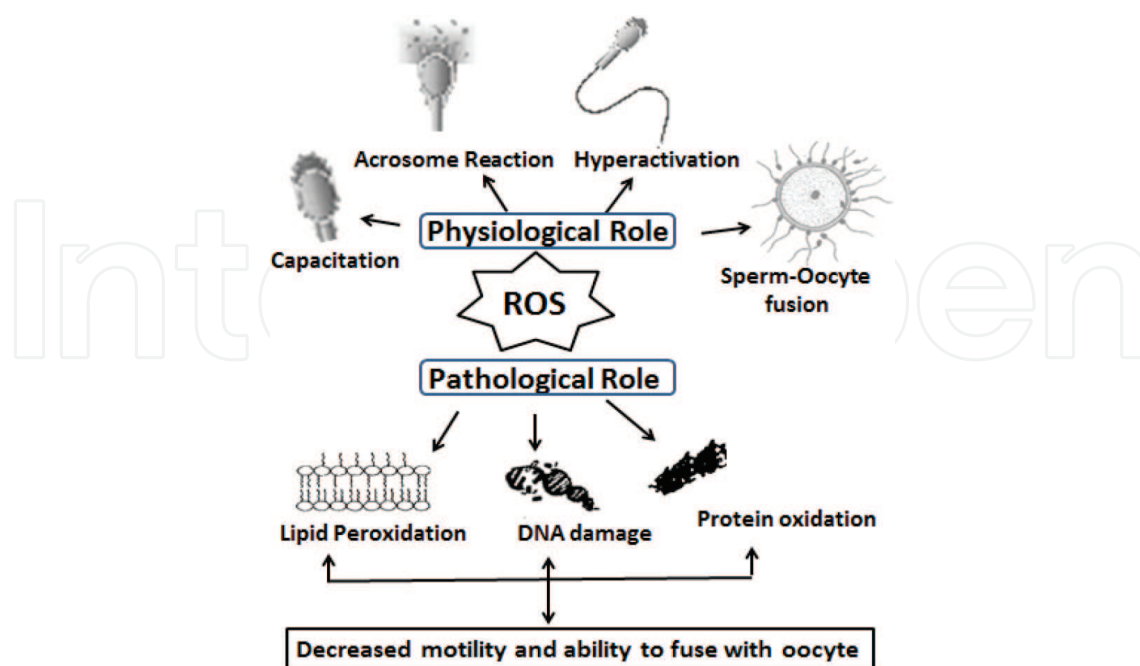


Figure 1. Physiological and pathological role of ROS in male reproduction.

proteins in the cells [18]. Thus, the ROS must be maintained at physiological levels for optimal sperm function, the maintenance of cellular homeostasis, and redox-sensitive signal transduction mechanisms affecting fertility.

2. ROS and sperm physiology

During their transit through the epididymis, spermatozoa progressively acquire the ability to move but lack fertilizing capacity [19]. They acquire the ability to fertilize in the female tract through a series of physiological changes called 'capacitation' which involves hyperactivation, acrosomal reaction, and sperm-oocyte fusion. Mammalian sperm capacitation is a redox regulated process which requires the production of different types of ROS to promote the fertilization of spermatozoa to the mature oocytes [20, 21]. The primary ROS generated in human spermatozoa is the O_2^- which appears to play a role in this process [22]. This one-electron reduction product of oxygen generated reacts with itself via dismutation reaction, which is greatly accelerated by SOD, to generate H_2O_2 . It has been reported that the capacitating populations of mammalian spermatozoa generate ROS mainly by two mechanisms: the membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme complex that is contained in the plasma membrane; and the mitochondrial nicotinamide adenine dinucleotide (NADH)-dependent oxido-reductase [23, 24]. The NADPH required by NADPH oxidase can be supplied by dehydrogenases located both in the plasma membrane and the cytosol. Studies have suggested the activation of sperm plasma membrane oxidase during capacitation and acrosome reaction [25, 26]. In mammalian spermatozoa, NADPH oxidase 5 (NOX5) are actively involved in generating O_2^- [27]. The mitochondria located in the mid-piece region of the sperm generates a low level of ROS during steady-state respiration but have the potential to accelerate this activity when these gametes enter the intrinsic apoptotic pathway [28, 29]. In addition to H_2O_2 and O_2^- , a variety of secondary cytotoxic radicals which are reported to stimulate sperm capacitation includes nitric oxide ($\cdot NO$) and peroxynitrite ($ONOO^-$) [30, 31]. The O_2^- generated from these two sources is thought to combine with $\cdot NO$ produced by nitric oxide synthase (NOS) and result in the formation of powerful oxidant $ONOO^-$, which mediates the oxidation of cholesterol to oxysterols. The oxysterols then exit the plasma membrane dramatically to enhance membrane fluidity [31, 32]. Further, the combined action of $ONOO^-$ and H_2O_2 concomitantly lead to the inhibition of tyrosine phosphatase activity while the combination of O_2^- , bicarbonate (HCO_3^-), and calcium ions (Ca^{2+}) activates soluble adenylyl cyclase, thereby stimulating cAMP production and the activation of protein kinase A (PKA) [33–35]. Activated PKA phosphorylates and inhibits protein phosphatase and activates tyrosine kinase that leads to an increase in actin polymerization, an essential process required for the development of hyperactivated motility [36, 37]. Only hyperactivated spermatozoa have increased motility to undergo acrosome reaction and acquire the characteristics required for successful fertilization. The role of low concentrations of OH^- in the initiation of hyperactivation in vitro has been well documented [38]. The hyperactivated spermatozoon traverse the cumulus oophorus surrounding ovulated eggs, it then binds and penetrate to the zonapellucida (ZP) of the oocyte and initiates an exocytotic release of proteolytic enzymes, creating a

pore in ZP's extracellular matrix. For successful fertilization, the spermatozoa then penetrate this physical zona barrier and fuse with the oocyte [39, 40]. Thus, ROS during the capacitation and acrosome reaction has been shown to increase the membrane fluidity and rates of sperm-oocyte fusion (**Figure 1**).

3. Human spermatozoa are vulnerable to oxidative stress

During spermatogenesis, germ cells produce high levels of reactive oxygen species, but fortunately a complex of antioxidant defense system and DNA repair system exists in the testis that protects genome integrity in differentiating sperm [16]. In normal spermatogenesis, the developing spermatozoa extrude most of the cytoplasm by the action of sertoli cells to change to a condensed, elongated form [41]. The lack of cytoplasm results in decreased intrinsic antioxidant defense due to the loss of most of antioxidant enzymes, rendering the cells less protected against ROS by the time they are discharged into the epididymis [42, 43]. Further, they also lack the necessary cytoplasmic-enzyme repair systems, thus they have very limited capacity for detection and repair of DNA damage [44]. Therefore, during their transit and storage into the epididymis or post-ejaculation they have no DNA repair mechanism, and thus cannot synthesize DNA, RNA, or translate proteins (such as repair enzymes) [45, 46]. The mammalian spermatozoa are vulnerable to oxidative stress not only because of their inherent free radical generating activity and lack of endogenous antioxidant protection, but also due to the abundant substrates that these cells possess for free radical attack. In mature spermatozoa, the small cytoplasm with limited defense remains confined to the mid-piece region in the vicinity of the mitochondria. As a result, the plasma membrane richly endowed with high concentrations of polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA) (22:6) and arachidonic acids (20:4) containing six and four carbon-carbon double bonds per molecule, surrounding the acrosome and the tail are not protected by the intracellular antioxidants [47–49]. In human spermatozoa, approximately 50% of the fatty acids are composed of DHA which is thought to play a major role in regulating spermatogenesis and membrane fluidity [18]. The presence of double bond in PUFAs adjacent to a methylene group weakens the methyl carbon-hydrogen bonds and makes hydrogen more susceptible to abstraction and thus vulnerable to oxidation [44, 50].

Sperm mitochondrial DNA has long been postulated as a source and often likely target of ROS oxidation as they are not protected by histones and has a very limited capacity for DNA repair with complete lack of nucleotide-excision repair pathways [51]. It is estimated that the mitochondrial DNA exhibits the mutation rate two orders of magnitude higher than that of nuclear DNA. Thus any quantitative or qualitative aberrations in mitochondrial DNA will result in the increased ROS generation which will affect the cellular functioning of the cell [52].

Despite its vulnerability to oxidative stress, maturing sperms spontaneously generate ROS during their progress through the epididymis, as its normal metabolite that aids it to acquire full fertility competence [53]. The lack of intrinsic antioxidant protection forces these cells to dependent on defense provided by seminal and epididymal enzymatic and non-enzymatic

antioxidant mechanisms. These mechanisms compensate for the deficiency in cytoplasmic enzymes in sperm [54, 55]. Thus the sperms which spend long period as an isolated cells both in male and female genital tracts (approximately 3 weeks), these limited defenses can be easily overwhelmed with an increased generation of ROS [56].

4. ROS scavenging capacity of semen

Spermatozoa like other aerobic cells are dependent on cellular respiration process which supports its life. But excessive generation of its metabolites, such as ROS, can modify cell functions. Hence, under normal condition male reproductive system must continuously inactivate ROS to maintain a balance between ROS production and its scavenging mechanism in order to keep only the small amount necessary to maintain normal cell function. Thus, in order to maintain the redox homeostasis, the mature spermatozoa with limited antioxidant defense capacity are mainly dependent on seminal plasma which is well endowed with an array of effective enzymatic and non-enzymatic antioxidant defense mechanisms [57, 58].

The main enzymatic antioxidants in the semen include superoxide dismutase (SOD), catalase, and glutathione peroxidase/glutathione reductase (GPX/GRD) system [59]. SOD is metallo-enzymes which is present in both intracellular and extracellular forms [60]. SOD spontaneously dismutase O_2^- to form H_2O_2 and catalase catalyzes the decomposition of H_2O_2 to O_2 and water (H_2O) thus preventing the lipid peroxidation of the sperm plasma membrane. Another enzyme of the antioxidant system in the semen is glutathione peroxidase (GPX), which catalyzes the reduction of hydrogen peroxide and organic peroxides, including the peroxides of phospholipids [61]. Spermatozoa have limited supply of catalase and GPX, while SOD is the main enzymatic antioxidant which protects it from oxidative stress [62]. Beside the enzymes antioxidant protective mechanism, seminal plasma is also employed by the low molecular weight, non-enzymatic antioxidants that assist enzyme activity. These include ascorbic acid (vitamin C), tocopherol (vitamin E), vitamin A, pantothenic acid, coenzyme Q10, carnitine, amino acids (taurine, hypotaurine) zinc, selenium albumin, and urate. These agents principally act by directly neutralizing free radical activity chemically and some of these antioxidants are reported to enhance sperm viability/motility as well as normal sperm morphology and required for spermatogenesis, development of spermatozoa [63, 64]. The seminal plasma antioxidants concentrations have been shown to be significantly higher in fertile men than those in infertile men [65, 66].

5. Sources of ROS in seminal plasma

Oxidants in seminal plasma originate from numerous extrinsic and intrinsic sources. The human ejaculate is composed of various types of cells, which include mature and immature cells, round cells from extraordinary degrees of spermatogenesis, leukocytes, and epithelial cells [67, 68]. Of those, leukocytes, specially neutrophils and macrophages and immature

spermatozoa are taken into consideration as the primary endogenous assets of ROS [69], while numerous life style elements including immoderate smoking and alcohol intake, and environmental elements inclusive of radiation and pollution can contribute as exogenous sources of ROS (**Figure 2**) [70, 71]. Exposure to radiation and toxins induces ROS production which impairs spermatogenesis and leads to DNA damage in human spermatozoa, which further decreases the motility and vitality of sperm cells as well as their concentration depending on the duration of exposure [72]. Cigarette smoking is found to be correlated with leukocytospermia. It has been reported that smoking can elevate the leukocyte concentration by 48% and ROS by 107% in seminal plasma [73].

5.1. Immature/abnormal spermatozoa

One of the major cellular sources of ROS in the semen is sperm cells [74]. When spermatogenesis is impaired, the cytoplasmic extrusion mechanisms are defective, and spermatozoa are released from the germinal epithelium carrying surplus cytoplasmic residues in the mid-piece [75]. These residues are rich in the cytoplasmic enzymes such as superoxide dismutase, lactic acid dehydrogenase, glucose-6-phosphate dehydrogenase (G6PDH), and creatine kinase [69, 76]. However among these enzymes, the key enzyme was thought to be G6PDH, which would be expected to enhance the intracellular availability of NADPH via the hexose monophosphate shunt. NADPH is used to fuel the generation of ROS via NADPH oxidase activity [27, 77]

5.2. Leukocytes

The main source of ROS inside semen is leukocytes. Infection or chronic inflammation may activate the leukocytes to release 1000-times more ROS than spermatozoa [78]. This high production of ROS by leukocytes plays an important role in the cellular defense system

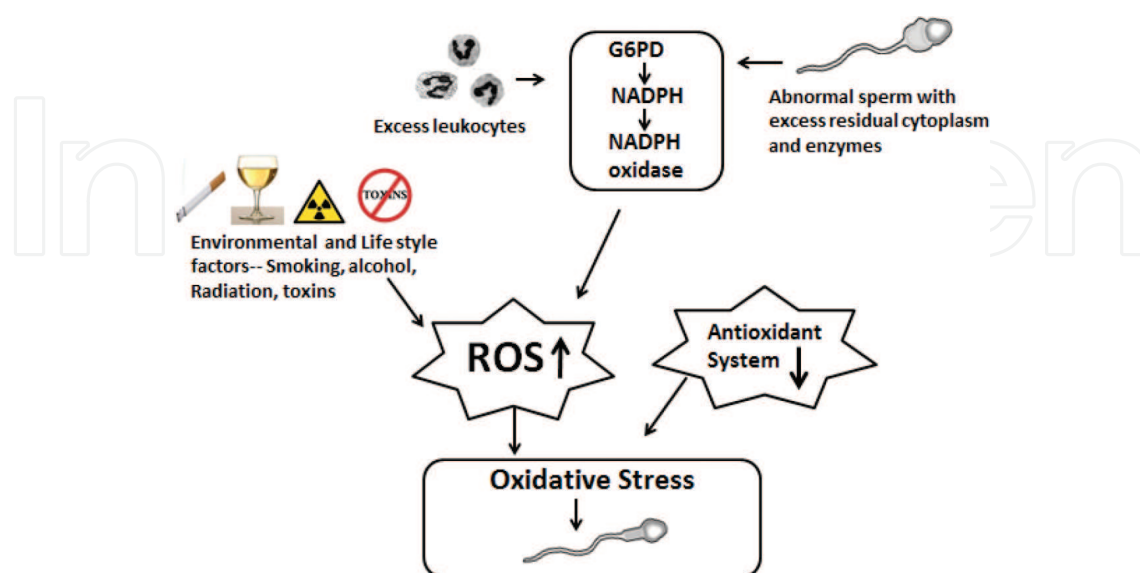


Figure 2. Extrinsic and intrinsic factors of ROS generation in seminal plasma.

against infections as well as inflammation [78]. However, the high concentrations of ROS may overwhelm seminal antioxidant defenses and damage the sperm cell [79]. Essentially, the cellular mechanisms for the generation of ROS within leukocytes and spermatozoa are same, in leukocytes, the release of the large amounts of superoxide into phagocytic vesicles for killing the pathogens [80, 81].

6. Impact of oxidative stress on spermatozoa

The exact mechanism of oxidative stress-induced decline in sperm function remains unknown but is mainly attributed to peroxidative damage to axoneme and depletion of intracellular ATP levels, followed by generation of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) owing to the oxidation of lipid membrane components and oxidation of DNA followed by fragmentation of both nuclear and mitochondrial DNA [82].

6.1. Lipid peroxidation

In PUFAs, the hydroxyl radicals attack lipids containing carbon-carbon double bond and promote the hydrogen abstraction from carbon to generate a carbon-centered lipid radical (2CH^\bullet) that then combines with oxygen to generate lipid peroxy radicals (ROO^\bullet) [83]. The ROO^\bullet radicals subsequently attacks another lipid molecule, abstract a hydrogen atom in order to stabilize itself as the lipid hydroperoxide but in the process generates another carbon-centered lipid radical that perpetuates the cascade of chemical reactions called lipid peroxidation. The process results in the generation of small molecular mass electrophilic lipid aldehydes such as 4-hydroxynonenal (4HNE), acrolein, and malondialdehyde [84]. Lipid peroxidation (LPO) is extremely harmful to spermatozoa, having a dramatic effect on both sperm movement and the competence of these cells for fertilization (**Figure 3**). Immature human sperm cells contain high levels of DHA in the cytoplasmic droplet and showed more susceptibility to LPO than normal matured sperm with lower DHA levels [85].

Added to this vulnerability, it has been shown that cytotoxic aldehydes generated as the result of oxidative stress has the ability to of triggering ROS generation by the sperm mitochondria in a self-perpetuating cycle; the greater the level of unsaturation, the greater the level of the stimulatory effect. The defective human spermatozoa contain abnormally high cellular contents of free polyunsaturated fatty acids, the levels of which are positively correlated with mitochondrial superoxide generation. The lipid aldehydes, 4HNE or acrolein bind covalently to the nucleophilic centers of vulnerable proteins, such as succinic acid dehydrogenase and form a protein adducts in the mitochondrial electron transport chain (ETC) that results in the leakage of electrons which disturbs the normal flow of electrons and reduction of oxygen to water [86, 87]. The leakage of electrons from the ETC results in the reduction of oxygen to generate O_2^- , which then by mitochondrial superoxide dismutase rapidly dismutates to H_2O_2 [88]. The excess of cytoplasm in the immature or defective spermatozoa contain superabundance of cytoplasmic enzymes. The retention of excess of SOD can only be an asset for any cell seeking to protect itself from oxidative stress if it is accompanied by a corresponding increase in the

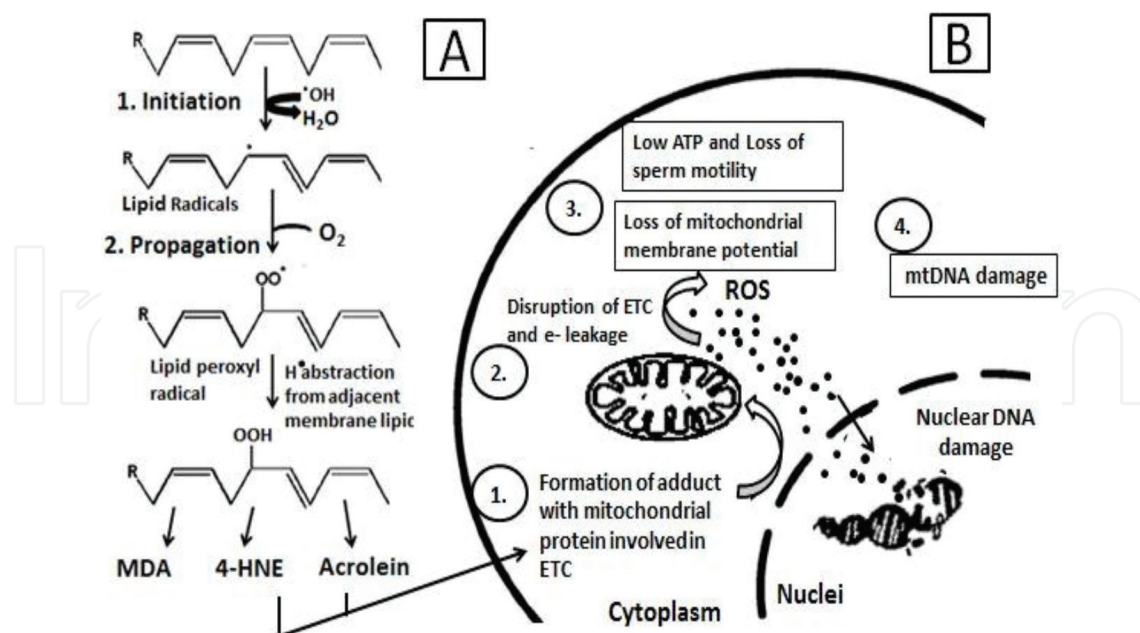


Figure 3. (A) ROS-induced initiation and propagation of lipid peroxidation (LPO) generates lipid hydroperoxides plus a new carbon-centered radical that continues the chain reaction. (B) Mitochondrial ROS generation. The electrophilic aldehydes generated as a by-product of LPO process bind to protein of electron transport chain and further promote mitochondrial ROS generation. This process results in loss of mitochondrial membrane potential, low ATP generation, loss of sperm motility, oxidative DNA damage followed by DNA fragmentation.

presence of enzymes such as glutathione peroxidase or catalase that can scavenge H_2O_2 . But excess of SOD and limited supply of glutathione peroxidase or catalase in human spermatozoa simply turns a short-lived, membrane-impermeant, relatively inert free radical $\text{O}_2^{\cdot -}$ into a long-lived, membrane-permeate reactive oxidant, H_2O_2 [89, 90]. The damage of protein and membrane lipids due to elevated levels of ROS in mitochondria might affect the process of oxidative phosphorylation causing depletion of intracellular ATP levels leading to axonemal damage, decreased sperm viability, and increased mid-piece sperm morphological defects with deleterious effects on sperm capacitation and acrosome reaction and decline of motility and fertility [91]. The mitochondrial function as a measure of inner mitochondrial membrane potential is found to be decreased in the spermatozoa of infertile men with elevated levels of ROS production and is positively correlated with the sperm concentration [92].

6.2. DNA damage in spermatozoa

Mitochondrial DNA is particularly vulnerable to free radical attack because it is essentially unprotected and has a very limited capacity for DNA repair [93]. Sperm nuclear DNA, on the other hand, is much resistant to damage because it is tightly compacted by replacing histones with small, positively charged molecules known as protamine [94, 95]. Sperm DNA maturation and appropriate packaging are vital steps in the proper development of spermatozoa.

During the late spermatogenesis in the mammalian germinal epithelium, the differentiating spermatids are highly susceptible to DNA damage due to important changes in the cytoarchitecture

and dramatic remodeling of the chromatin during which most of the histones are removed from the DNA and are first replaced by transition proteins TP1 and TP2, and then by protamines P1 and P2 which are approximately half the size of histones. P1 and P2 are normally expressed in a 1:1 ratio in human sperm, and provide a tight packaging of the sperm DNA. The chromatin remodeling is facilitated by the coordinated loosening of the chromatin by histone hyperacetylation and by the DNA topoisomerase II (topo II), which produce temporary strand breaks in the sperm DNA to relieve torsional stress that results from supercoiling [96, 97]. This forms the basic packaging unit of sperm chromatin, a toroid, which is further compacted by the intramolecular and intermolecular disulfide cross-links between cysteine residues present in protamines. The tight packaging of the sperm DNA enables the entire haploid genome to be condensed and packed in a sperm head measuring $5 \times 2.5 \mu\text{m}$. This level of protection ensures that the paternal genome is delivered in a form that allows developing embryo to accurately express genetic information. Normally, these temporary strand breaks are repaired by nuclear poly (ADP-ribose) polymerases (PARP) and topoisomerase II prior to completion of spermiogenesis and ejaculation [98]. However in pathological cases, the error in chromatin remodeling and repair mechanism leads to the generation of high level of nicked and poorly protaminated nuclear DNA with relatively high nucleohistone content or abnormally high and low P1/P2 ratios [99–101]. Thus, defect in the chromatin remodeling process causes DNA damage in spermatids during spermiogenesis, this creates a state of vulnerability whereby spermatozoa become increasingly susceptible to oxidative damage.

7. Causes of DNA damage in spermatozoa

When the protection of DNA in spermatozoa, which is dependent on its close association with cysteine rich protamines is lost, the cells become very susceptible to oxidative DNA damage induced by several extrinsic and intrinsic factors. Deoxygenated guanine (dG) is more susceptible to oxidation than other nucleosides in DNA due to its low oxidation potential [102]. The enzyme 8-oxoguanine glycosylase 1 (OGG1) immediately clips the 8OHdG residues out of the DNA generating an abasic site, But due to the absence of base excision repair enzyme, the spermatozoa are ejaculated carrying a abasic sites in their DNA [103]. Studies have reported that the spermatozoa of subfertile patients contain particularly high levels of 8-hydroxy-2'-deoxyguanosine (8OHdG), the major oxidized base adduct formed when DNA is subjected to attack by ROS [104].

DNA repair does occur during spermiogenesis but stops post-spermiogenesis because spermatozoa are transcriptionally and translationally silent. They cannot undergo programmed cell death called apoptosis, due to their inherent physical architecture, the endonucleases released from the mitochondria have no access to the DNA. Thus, abortive apoptosis initiated post-meiotically, when the ability to drive the spermiogenesis process to completion is declined and the strand breaks are not repaired due to impairment in the repair process results in high levels of DNA fragmented sperm in the ejaculate [105]. Sperm with DNA fragmentation still has the potential to fertilize and some types of strand DNA breaks in sperm can be repaired by oocytes, before the initiation of the first cleavage division, and generate normal offspring, but that

depends on the type and level of chromatin damage and the capacity of the oocyte to repair it [106]. DNA-strand breaks are extremely harmful lesions if not repaired and can lead to genomic instability and cell death. In natural conception, percentage of DNA damage has been negatively correlated to the rate of fertilization. If post fertilization oocyte make mistake in the repair process, deletions or sequence errors may be introduced, then it fabricates the possibility for *de novo* mutations, which could have a profound impact on the health and well-being of the offspring [107]. Sperm DNA damage in context to assisted reproductive technique (ART) has important clinical implications. Sperm selected for ART mostly originates from environment experiencing oxidative stress and high percentage of these sperms may have damaged DNA. If such sperms are used clinically in the form of therapy then can lead to substantial risk in pregnancy outcome. In case of intrauterine insemination (IUI) and in vitro fertilization (IVF), the use of these spermatozoa may not be cause of concern. But in case of intracellular sperm injection (ICSI), this natural selection barrier is bypassed and the spermatozoa with damaged DNA are directly injected into oocytes. Studies have reported that DNA damaged spermatozoa used in ICSI have some capacity for fertilization, but percentage of DNA damage has been negatively correlated to the rate of fertilization [108]. ROS-mediated DNA damage may be linked to an increase in early embryo death, infertility in the offspring, and high incidence of childhood cancer [109, 110]. We propose that extrinsic and intrinsic sources of ROS could make a significant contribution to the induction of OS and DNA damage in spermatozoa which can decrease pregnancy rate and affect the fertility outcome, further additional studies are clearly needed to validate this concept.

8. Management of infertility caused by oxidative stress

Oxidative stress plays an important role in the pathophysiology of male infertility, which is caused due to pathological level of ROS and the loss of antioxidant protection for the spermatozoa. There are many factors which can induce oxidative stress and can alter seminal parameters and rate of fertilization. Thorough examination and management of some of these factors may protect the ROS-induced DNA damage and improve a couple's chances of conception either naturally or via assisted reproduction.

8.1. Behavior and life style modification

Various behaviors and lifestyles factors such as alcohol consumption, cigarette smoking, obesity, excess exposure to environmental toxicants, and psychological stress are negatively correlated with spermatogenesis and may cause oxidative stress and reduction in sperm quality [111]. The increased consumption of simple sugars and high-fat food and physical inactivity are leading causes of the growing obesity. It is suggested that abnormal hormonal regulation, dysregulation of adipocytokine, and ROS generation lead to suboptimal semen quality these patients [112]. Several systemic diseases, such as diabetes mellitus, infection, and cancer are known to cause oxidative stress-induced male infertility [113, 114]. There are studies which have shown positive correlation of exercise with improvements in semen parameters, sperm DNA integrity, and pregnancy rate [115]. Nevertheless, modification in behavior and unhealthy living, regular exercise, stress free jobs, and treatment of a patient's underlying pathology should be the first steps to reduce or eliminate stress-provoking conditions to reverse sperm dysfunction.

8.2. Dietary antioxidants

As many studies suggested that oxidative stress is a major cause of unexplained male infertility, antioxidant therapy would be expected to have a therapeutic effect in such cases. There are evidences which have suggested that oral antioxidants and herbal products can also boost male reproductive functions [116, 117]. But, despite of known effect of antioxidant on oxidative stress, very few studies conducted have any validity due to small sample size, difference in dosage and duration of therapy, and lack controls [118]. In order to make the study valid, patient's selection criteria for the trial should be based on the evidence indication oxidative stress as a key element in their pathology, a thorough diagnosis is required to determine patients that need to be supplemented. However, if this strategy is pursued, great care must be taken in selecting the most appropriate antioxidants for clinical use. Since ROS plays an important role in regulating the signal transduction cascades that drive sperm capacitation, we should ensure that any antioxidants employed *in vitro* do not compromise the fertilizing potential of these cells [119].

The study of *in vitro* antioxidants is highly relevant in the era of assisted reproduction because sperm preparation techniques in ART are potential generators of exogenous stresses that make human spermatozoa vulnerable to oxidative stress and DNA damage.

9. Conclusion

Oxidative stress has been recognized as a major contributory factor to male infertility. Spermatozoa are professional generator of ROS as physiological level of ROS is necessary to regulate critical redox-sensitive processes such as capacitation, hyperactivation, acrosome reactions, and signaling processes to ensure appropriate fertilization. On the other hand, many endogenous and exogenous factors can elevate ROS production which can overwhelm their antioxidant mechanism. This results in male infertility via mechanisms involving the induction of peroxidative damage to the sperm plasma membrane, DNA damage, which significantly impairs sperm function. Lack of repair mechanism and abortive apoptosis in mature spermatozoa results in high levels of DNA fragmented sperm in the ejaculate. In natural conception, oocytes can repair some of stand DNA breaks, but that depends on the type and level of chromatin damage and the capacity of the oocyte to repair it. If post fertilization oocyte make mistake in the repair process it may lead to failure in fertilization. But if fertilization occurs, then it creates the possibility for *de novo* mutations, which could have a profound impact on the health and well-being of the offspring. When the natural balance between ROS and antioxidants is disturbed, the first restorative measure to be taken should be changes in lifestyle, maintaining a healthy and balanced diet, and antioxidant supplementation may then be taken together to improve the patient's health outcomes.

9.1. Suggestion

The conventional seminological parameter in infertile cases reflects the functional competence of the spermatozoa and the fertilizing potential of the ejaculate, but the underlying mechanisms of male fertility is not known. Thus in order to enrich the diagnostic value of this

fundamental form of investigation, the detailed examination of sperm DNA damage may be incorporated as a potentially valuable tool to investigate the functional integrity of the spermatozoa at the molecular level.

Author details

Sabiha Fatima

Address all correspondence to: sabmehdi@ksu.edu.sa

Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

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