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OXIDATIVE STRESS AND ROLE OF ANTIOXIDANTS IN NORMAL AND ABNORMAL SPERM FUNCTION

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1. ABSTRACT

Defective sperm function is the most common cause of infertility, and until recently, was difficult to evaluate and treat. Part of this difficulty was due to our incomplete understanding of the factors contributing to normal and abnormal sperm function leading to male infertility. Mammalian spermatozoa membranes are rich in high unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation. Limited endogenous mechanisms exist to reverse these damages. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and by contaminating leukocytes (leukocytospermia) has been identified as one of the few defined etiologies for male infertility. In a normal situation, the seminal plasma contains antioxidant mechanisms which are likely to quench these ROS and protect against any likely damage to spermatozoa. However, during genitourinary infection/inflammation these antioxidant mechanisms may downplay and create a situation called oxidative stress. In addition, aging and environmental toxicants are also likely to further induce this oxidative stress. Assessment of such oxidative stress status (OSS) may help in the medical treatment of this male factor infertility by suitable antioxidants.

2. INTRODUCTION

Infertility has been a major medical and social preoccupation since the dawn of humanity. Unlike other civilizations, infertility in the early Egyptian society was not a divine punishment but was considered an illness which merited proper diagnosis and treatment. However, not much was known about the anatomy or physiology of the reproductive tract until von Leeuwenhoek invented the microscope and discovered the "spermatozoid" in 1677. Despite the enormous progress in research and reasoning, most of the blame for infertility, until recently, was placed on the female. Only during the last 15-20 years, advances in understanding of gonadal/sperm function and dysfunction led to a dramatic increase in our knowledge of male infertility. Defective sperm function is the most prevalent cause of male infertility and a difficult condition to treat (1).

Many environmental, physiological, and genetic factors have been implicated in the poor sperm function and infertility. 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. Thus, it is very

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Table 1. Laboratory Assays for Evaluation of Human Semen

	Refs	
Routine Evaluation	**2**	Seminal fluid volume, Sperm count, motility, Morphology, Viability, Leukocytes in semen, Sperm antibodies
Specialized Sperm Function	**3**	Membrane integrity, Sperm-cervical mucus interaction, CASA, Capacitation, Acrosome reaction, Zona pellucida binding, Zona pellucida penetration, Oocyte-sperm fusion
Sperm Function Assays	**4**	HOST, Postcoital test, Tru-Trax, Penetrak, SPA, Acrosome reaction tests, Mannose receptor level, HZA, IVF

IVF: *in vitro* fertilization; CASA: computer-aided sperm analysis; SPA: sperm penetration assay; HZA: hemizona assay; HOST: hypo-osmotic swelling test.

important to identify the factors/conditions which affect normal sperm function. Free radical-induced oxidative damage to spermatozoa is one such condition which is recently gaining a considerable attention for its role in inducing poor sperm function and infertility. Understanding of how such conditions affect sperm function will help designing new and effective treatment strategies.

3. NORMAL AND ABNORMAL SPERM FUNCTION

The ultimate goal of a spermatozoan is the successful fertilization of ovum resulting in normal conception. In order to achieve this, the spermatozoa after spermiation must mature within the male genital tract, travel through the female reproductive system, undergo capacitation and acrosome reaction, bind to and penetrate the zona pellucidae of the ova as well as the oolemma, and finally fuse with the female pronucleus. Normal spermatozoa should properly undergo through all of these steps in order to fertilize the ova. However, many men who demonstrate normal parameters on standard semen analysis remain infertile (2). This suggests that the routine semen analysis (measurement of seminal volume, spermatozoal motility, density, viability and morphology) does not necessarily provide complete diagnostic information (3).

As a result of active research in the area of evaluation of human semen, a series of sperm function assays have been developed (Table I). However, no single test is capable of evaluating all of the steps involved in fertilization. At present only a combination of assays complementing each other can provide a comprehensive evaluation of sperm function (4). Although, ideal tests of sperm function will markedly improve the clinician's ability to diagnose male factor infertility and help in its management, evaluation of the potential causes of sperm damage leading to abnormal sperm function and infertility is an important area of investigation.

4. OXIDATIVE STRESS

"Oxidative stress" is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (5). Reactive oxygen species (ROS) have been implicated in over a hundred of disease states which range from arthritis and connective tissue disorders to carcinogenesis, aging, toxin exposure, physical injury, infection, and acquired immunodeficiency syndrome (6). The role of oxidative stress in infertility and methods for counteracting its impact on reproductive tissues with antioxidants is still in its infancy.

4.1. Reactive oxygen species and oxidative stress

ROS are highly reactive oxidizing agents belonging to the class of free-radicals. A free radical is any compound (not necessarily derived from oxygen) which contains one or more unpaired electrons. The most common ROS that have potential implications in reproductive biology include superoxide $(O_2^-)^\cdot$ anion, hydrogen peroxide (H_2O_2), peroxyl $(ROO^-)^\cdot$ radicals, and the very reactive hydroxyl $(OH^-)^\cdot$ radicals. The nitrogen-derived free radical nitric oxide $(NO^\cdot)$ and peroxynitrite anion $(ONOO^-)$ also appear to play a significant role in the reproduction and fertilization. The ultimate effects of $(NO^\cdot)$depend upon its concentration and interactions with hydrogen peroxide. Peroxynitrite (oxoperoxonitrate) anion may be formed *in vivo* from superoxide and nitric oxide and actively reacts with glutathione, cysteine, deoxyribose, and other thiols/thioethers (7). This can form a strongly nitrating species in the presence of metal ions or complexes.

The assumption that free radicals can influence male fertility has received substantial scientific support (8). The proposed mechanism for loss of sperm function upon oxidative stress has been shown to involve excessive generation of ROS (9). The H_2O_2 has both beneficial and damaging effects on sperm and thus can influence the fertilization process. Hence, free radicals and ROS are associated with oxidative stress and are likely to play a number of significant and diverse roles in reproduction. Basic and clinical research on the

involvement of ROS and antioxidants in maintaining normal sperm function is very much warranted.

4.2. Spermatozoa and leukocytes as sites of ROS production

Presence of leukocytes (predominantly granulocytes) in semen has been associated with severe male factor infertility cases (10,11). There has been much speculation as to whether the origin of ROS in semen is from spermatozoa or from infiltrating leukocytes (12,13). Iwasaki and Gagnon reported that the leukocyte free percoll fractions of semen samples obtained from non-azoospermic infertile men generate detectable levels of ROS when compared to the semen of normal and azoospermic men suggesting that damaged spermatozoa are likely to be the source of ROS (14). Also, higher levels of ROS were correlated with a decreased number of motile sperm; conversely, greater sperm motility was observed in samples with lesser amounts of detectable ROS (14). It is important for the clinician to recognize that assisted reproductive techniques (percoll gradients/sperm washing/centrifugation) may induce damage to spermatozoa by either inadvertently removing the scavenging capability of seminal plasma or by increasing ROS generation by spermatozoa (9).

4.3. Leukocytospermia and oxidative stress

The exact site of origin of these leukocytes in semen, their mode of action, and the role that bacteria, viruses and subsequent genitourinary-inflammation might have on sperm function are not clear. Experimentally, ROS production by human spermatozoa and contaminating leukocytes can be stimulated by phorbol esters and certain formyl peptides with deleterious effects on sperm motility and fertilization (13). Although the presence of leukocytes in semen did not diminish the *in vitro* fertilizing capacity of spermatozoa, the introduction of leukocytes into washed sperm preparations did reduce sperm function by the production of ROS (15). This finding seems paradoxic but does indicate that seminal plasma has significant antioxidant or ROS scavenging capacity which may prevent sperm damage by leukocytes.

An association between leukocytospermia and ROS has been recently found to correlate with increased chemokine (IL-8), and decreased SOD activity of the semen (16). This demonstrates that increased oxidative stress during leukocytospermia is caused by a defective ROS scavenging system which, in turn, can be modulated by certain proinflammatory cytokines. A significant shift towards increased production of proinflammatory chemokine (GRO-α) compared to anti-inflammatory cytokine (IL-10) during leukocytospermia suggests an active chemotactic pro-inflammatory response (17). This shift may be responsible for a significant oxidative stress to spermatozoa due to leukocytes in the semen of the infertile patient (5). Based upon these observations, it may be useful to assess the oxidative stress status (OSS) of semen in infertile or subfertile patients, particularly those with chronic genitourinary inflammation.

4.4 Oxidative stress and sperm function

Theoretically, cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. The scavenging potential in gonads and seminal fluid is normally maintained by adequate levels of antioxidants superoxide dismutase (SOD), catalase, and probably glutathione (GSH) peroxidase and reductase (5). This balance can be referred to as oxidative stress status (OSS) and its assessment may play a critical role in monitoring sperm damage and infertility (Fig 1).

A situation in which there is a shift in this ROS balance towards pro-oxidants, because of either excess ROS or diminished anti-oxidants, can be classified in terms of positive oxidative stress status (OSS). At present, there is no true ROS detection method available which will evaluate this balance. However, assessment of OSS, or a similar paradigm when monitored more objectively, would be a good indicator of sperm damage caused by oxidative stress (5). Chronic asymptomatic genitourinary inflammation can be regarded as a condition with positive OSS, which may be the real cause of idiopathic infertility in such patients. Superoxide dismutase (SOD) may directly act as antioxidant enzymes involved in the inhibition of sperm LPO (18). A high GSH/GSSG ratio will help spermatozoa to combat oxidative insult (19). It seems that the role of these biological antioxidants and their associated mechanisms is an important area for further investigation in the treatment of infertility. Though the therapeutic use of antioxidants appears attractive, until proper multicenter clinical trials have been completed, clinicians need to be aware of exaggerated antifertility claims in various commercial antioxidants.

Nitric oxide radical ($NO^{\cdot}$) and reactive nitrogen species (RNS) have recently been found to have biological roles in inflammation and in mediating many cytotoxic and pathological events (20). Synthesis of $NO^{\cdot}$ in response to infection and inflammation could contribute to poor sperm motility and function and may lead to infertility (21). RNS (*e.g.*, $NO^{\cdot}$) like ROS, may normally be useful for maintaining sperm motility but can be toxic in excess (22). Other RNS such as nitrogen dioxide ($NO_2^{\cdot}$) radical and peroxynitrite ($ONOO^-$) anion are considered to be damaging. The primary mechanism of nitric oxide -induced sperm damage is likely to be inhibition of mitochondrial respiration and DNA synthesis (23). Nitric oxide-induced toxicity is also mediated indirectly through its interaction with superoxide anions and formation of peroxynitrite anion, which when protonated, decomposes to form OH^- and NO_2, both of which are cytotoxic agents (24).

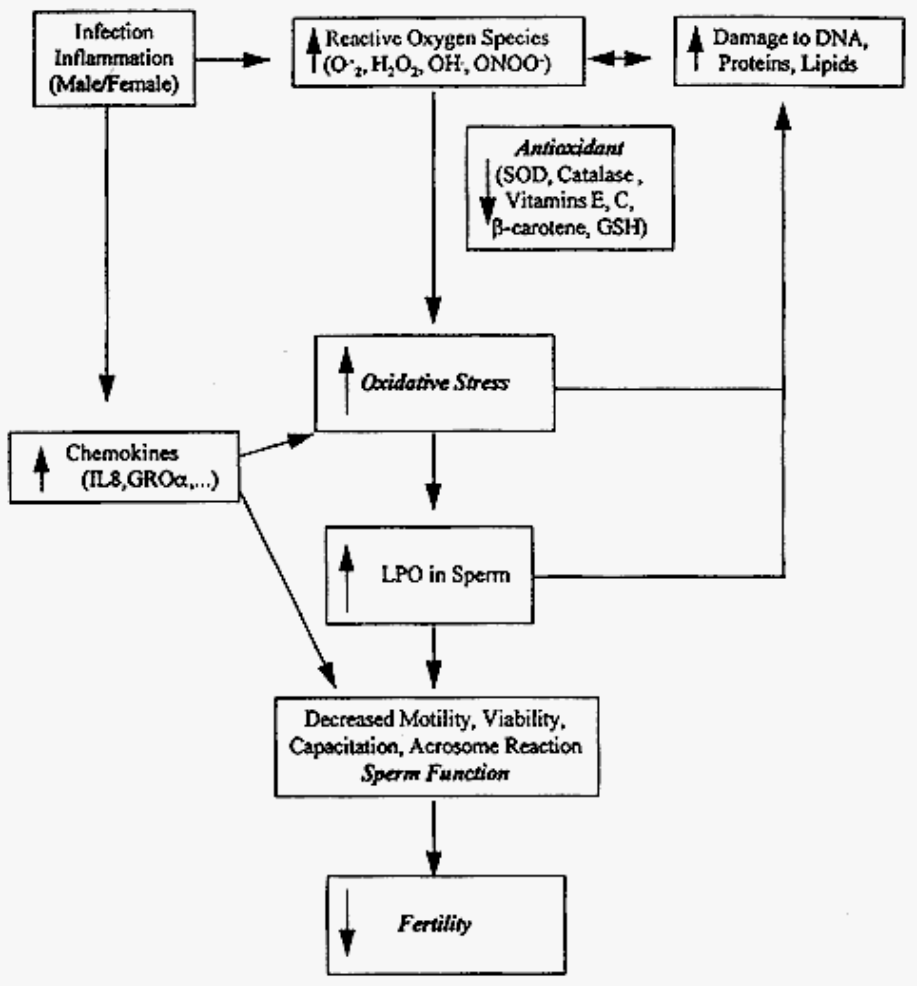

Figure 1: Scheme suggesting interacting mechanisms in the role of oxidative stress and antioxidants affecting sperm function and fertility. (The key words are in bold and are italicised. See text for further details).

5. MODE OF ACTION OF ROS

Mammalian spermatozoa are rich in polyunsaturated fatty acids and, thus, are very susceptible to ROS attack which results in a decreased sperm motility, presumably by a rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability, and increased midpiece morphology defects with deleterious effects on sperm capacitation and acrosome reaction (25). Lipid peroxidation of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to infertility (Fig 1) (18).

5.1. Lipid peroxidation of spermatozoa:

Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen activation in biology. The most common types of LPO are: (a) non-enzymatic membrane LPO, and (b) 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 (26). In spermatozoa, production of malondialdehyde (MDA), an end product of LPO induced by ferrous ion promoters, has been reported (20). 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 (27).

5.2. Biological implications of LPO and oxidative stress to spermatozoa

Spermatozoa, unlike other cells, are unique in structure, function, and susceptibility to damage by LPO (18). In order to understand the biological mechanisms of LPO in infertility, three important questions need to be addressed: (a) What are the mechanisms of LPO of sperm *in vivo*? (b) What are the consequences of damage to sperm membrane, proteins, and nucleic acids? (c) What regulates the antioxidant defense mechanisms in seminal plasma?

In general, the most significant effect of LPO in all cells is the perturbation of membrane (cellular and organellar) structure and function (transport processes, maintenance of ion and metabolite gradients, receptor-mediated signal transduction, etc.). Low levels of NADH

and glutathione, as a result of the increased activity of glutathione peroxidase to remove metabolites of LPO, will further affect cellular Ca^{2+} homeostasis. Minor alterations in sperm membranes in selected cases of dyspermia can be reversed by GSH therapy (28). Studies on how these cellular changes caused by LPO affect seminal parameters and sperm function and reversal of these effects are open to further investigations.

Besides membrane effects, LPO can damage DNA and proteins, either through oxidation of DNA bases (primarily guanine via lipid peroxyl or alkoxyl radicals) or through covalent binding to MDA resulting in strand breaks and cross-linking (26). ROS can also induce oxidation of critical -SH groups in proteins and DNA, which will alter structure and function of spermatozoa with an increased susceptibility to attack by macrophages (15). 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. In addition, the redox status of human spermatozoa is likely to affect phosphorylation and ATP generation with a profound influence on its fertilizing potential (29). Aitken *et al*. recently showed that stimulation of endogenous NADPH-dependent ROS generation in human sperm appears to regulate acrosome reaction via tyrosine phosphorylation (30). In general, the oxidizing conditions increase tyrosine phosphorylation with enhanced sperm function while reducing conditions have the opposite effect. However, this has been debated for a long time, and it is still not clear whether sperm have a NADPH-dependent oxygenase system. Nonetheless, how these mitochondrial DNA or membrane changes regulate specific sperm functions in association with altered tyrosine phosphorylation is an interesting area for further investigation. These studies may open a new series of diagnostic tool in clinical infertility to assess sperm function and damage.

6. ANTIOXIDANTS (POTENTIAL SCAVENGERS OF ROS) AND OXIDATIVE STRESS

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, 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 O_2 and H_20.

$$2\,(O_2^-)^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

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

Many studies have been reported in the literature on the role of SOD as an antioxidant in reproductive biology. SOD protects spermatozoa against spontaneous O_2 toxicity and LPO [18]. SOD and catalase also remove $(O_2^-)^-$ generated by NADPH-oxidase in neutrophils and may play an important role in decreasing LPO and protecting spermatozoa during genitourinary inflammation (30).

Glutathione peroxidase, a selenium-containing antioxidant enzyme with glutathione as the electron donor removes peroxyl (ROO^-) radicals from various peroxides including H_2O_2 (31). Glutathione reductase then regenerates reduced GSH from GSSG as shown in the following equation:

$$2GSH + H_2O_2 \xrightarrow{\text{peroxidase-(Se)}} GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{reductase}} 2GSH + NADP^+$$

A selenium-associated polypeptide, presumably glutathione peroxidase, has been demonstrated in rat sperm mitochondria which plays a significant role in this peroxyl scavenging mechanism and in maintaining sperm motility (31). It would be interesting to explore the mechanism of action of this antioxidant in human spermatozoa. In addition, GSH-peroxidase and GSH-reductase may directly act as antioxidant enzymes involved in the inhibition of sperm LPO (28). GSH has a likely role in sperm nucleus decondensation and may alter spindle microtubule formation in the ovum, thus affecting the outcome of pregnancy. In this context, the γ-glutamyl transpeptidase (γGGT), considered to be present in the midpiece and acrosomal regions of spermatozoa of certain mammalian species (*e.g.*, the boar) may further affect GSH content of oocyte at the time of sperm penetration (19, 28). Thus, in view of the great number of mitochondria in spermatozoa, these antioxidant mechanisms are important in the maintenance of sperm motility, the rate of hyperactivation, and the ability of sperm to undergo acrosome reaction during sperm preparation techniques especially in the absence of seminal plasma. Albumin, used in sperm-washing procedures, is likely to serve as an antioxidant by providing thiol groups required for "chain breaking" antioxidant activity (26). A high GSH/GSSG ratio will help spermatozoa to combat oxidative insult (5). It seems that the role of these GSH enzymes and their associated mechanisms as related to biological antioxidants in infertility is an important area for further investigation.

Within the category of chemical antioxidants, both natural and synthetic products have garnered attention by the cosmetic, nutritional, and pharmaceutical industries. Their usefulness in reproduction and management of infertility has not yet been demonstrated. Although vitamins E and C may protect spermatozoa against endogenous oxidative DNA and membrane damage they have minimal effects in improving the post-thaw sperm parameters. In this regard, carotenoids (beta-carotene), and ubiquinols may also play a role in quenching singlet oxygen and reducing lipid derived free-radicals with detrimental effects on sperm LPO (26).

Hence, the application of ROS scavengers (*e.g.*, SOD, catalase, vitamin E, GSH-enzymes) is likely to improve sperm motility and function. Pentoxifylline, a sperm motility stimulator, can also act as a suppressor or scavenger of ROS (32). The effect of vitamin E supplementation in combination with IVF techniques is a worthy notion. Further controlled clinical studies will determine if many of these putative antioxidants can improve infertility in selected groups of patients.

7. ROLE OF GENITOURINARY (GU) INFLAMMATION AND OXIDATIVE STRESS

Infection/inflammation of the genitourinary tract in infertile men is suspected when the semen analysis shows an increased number of leukocytes (>1-2 million white blood cells/ml semen) (2). Acute and sub-acute infection and inflammation of the male gonads and accessory sex glands can be associated with disturbances in both sex gland function and sperm quality (33). Some of these conditions (chlamydia infection, mumps orchitis, tuberculosis, syphilis, leprosy) can cause irreversible sterility, are invariably symptomatic, and are associated with leukocytospermia/bacteriospermia (34). Chronic infection and inflammation of the reproductive tract which can be asymptomatic also contribute to the infertile state. The impact of such asymptomatic or "silent infections" on male accessory genital organs is sometimes more severe and may involve damage to the seminiferous tubules or obstruction to the passage of sperm at the level of the epididymis or ejaculatory duct (35).

The precise role that genital tract infection plays, the exact site of origin of leukocytes, their migratory pattern, their mode of action, and the adverse effects that bacteria/virus and subsequent genitourinary-inflammation might exert on sperm function are not clear. Elevated leukocytes and granulocytes are believed to release various proinflammatory/bioactive cytokines, hydrogen peroxide, and other reactive oxygen species (15,16). These can cause oxidative stress and peroxidative damage to spermatozoa.

8. AGING AND OXIDATIVE STRESS

Some forms of infertility are caused by age-related degenerative disorders of the testis. As apparent from the declining sperm population over the last two generations, this problem is increasing in industrialized societies (36). Although the decline in sperm numbers is considered to be associated with male fetal and/or neonatal exposure to increased level of the environmental estrogen , the idiopathic male infertility may also be explained as a form of premature or differential aging of the testis induced by ischemia and oxidative stress associated with defective mitochondrial genome that controls the oxidative phosphorylation (29). Presence of retained cytoplasmic droplets on spermatozoa due to imperfect spermiation in aging testis may be a sign of' reduced fertility. It is known that lipid peroxidation (LPO) and midpiece anomalies are linked (37), and that the increased rate of LPO and creatine kinase (CK) activity in immature sperm is due to incomplete cytoplasmic extrusion during terminal spermatogenesis (38). In addition, Sertoli cells abnormality in infertile men may well be central to the development of spermatogenic failure due to faulty spermiation and maybe related to genetic defects, oxidative stress, or even aging of the gonads.

Decreased vascularity, increased spermato-genic failure, and reduced sperm output occur with senescence in a variety of animal species and in humans (39). Degenerated germ cells are presumably phagocytosed by Sertoli cells, resulting in lipid accumulations that increase progressively with age. Increased levels of lipofuscin and lipid are seen intracellularly, suggesting presence of mitochondrial dysfunction possibly compounded by oxidative stress in the older population (40). These degenerative changes in gonads associated with accumulation of lipofuscin pigment and multiple nuclei are considered to be due to ROS-induced lipid peroxidation with age (40). Most of these changes are strikingly similar to that seen in men with idiopathic testicular failure, probably due to induced gonadotoxicity. However, functional abnormalities in mature spermatozoa leading to infertility has not been demonstrated in normal aging men.

9. ASSESSMENT OF OXIDATIVE STRESS

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 luminol 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 (5). The methods commonly used for measuring ROS can be categorized into: (a) reactions involving nitroblue tetrazolium (NBT) or cytochrome c-Fe^{3+} complexes which measure ROS on the cell membrane surface, (b) reactions that measure ROS (generated inside or outside the cell) utilizing luminol-dependent chemiluminescence, and (c) the electron spin resonance methods which are more sensitive and can identify the type of ROS generated inside the cell but require skillful operation, accurate interpretations, and expensive instrumentation.

To further study their mode of action on human spermatozoa, ROS can be artificially generated under defined experimental conditions. The reaction between xanthine and xanthine oxidase results in the univalent and divalent reduction of dioxygen to generate superoxide $(O_2^-)^{\cdot}$ anion and hydrogen peroxide (H_2O_2), respectively. In the presence of ferric ions, these radicals

further generate the highly reactive hydroxyl radical (OH^-) which is especially deleterious to spermatozoa.

$$\text{Xanthine-hypoxanthine} \xrightarrow{\text{xanthine oxidase}} \text{uric acid} + O_2^{\cdot-} + H_2O_2$$

$$O_2^{\cdot-} + H_2O_2 \xrightarrow{Fe^{3+}} OH^{\cdot} + OH^- + O_2$$

Electrolysis of physiological buffer under defined conditions also generates ROS which can damage sperm motion (41). Selective modifications of these defined conditions can identify: (a) the free radicals involved, (b) their mode of action on spermatozoa, and (c) the evaluation of selective protective mechanisms.

9.1. Oxidative stress status (OSS) evaluation

The balance of ROS can be termed as the "balance of creation and destruction". Under normal circumstances, there is an appropriate balance between pro-oxidants and anti-oxidants. A shift in the levels of ROS towards pro-oxidants in semen and vaginal secretions can induce an oxidative stress on spermatozoa. Concomitantly, a decrease in antioxidant activities (*e.g.*, SOD, catalase, glutathione peroxidase and reductase, GSH) in semen correlates with idiopathic infertility (8). It is possible that an increased rate of ROS production (suggesting high oxidative stress) may inhibit the action of these antioxidant enzymes, or alternatively the inherent decreased expression of these antioxidant enzymes may cause increased oxidative stress (5). This will result in increased LPO, decreased sperm motility, viability and function, and ultimately leads to infertility (Fig 1).

Assessment of the rate of ROS production/generation using luminol as a probe can be a dynamic measure of oxidative stress (9). However, clinically the evaluation of this ROS generation is limited by a very short half life of these free radicals (12). The potential methods that can be developed for evaluation of OSS may utilize measurement of an oxidized component that remains in the body fluids (*e.g.*, TBA reactive substances; GSH/GSSG balance; the levels of unaltered tocopherol or ascorbate). Although there have been concerns about the specificity, interference, and reliability of measuring TBA-MDA activity as an indicator of LPO, this test remains one of the most efficacious methods for assessing the oxidative damage to sperm (18). Eventually, this TBA-MDA measurement will need to be combined with other assays which would be able to measure the rate of ROS production and antioxidant protection for the overall assessment of OSS in infertility. Measurement of IL-8, for example, when combined with assessment of SOD or other antioxidants in infertile patients with leukocytospermia will indicate a positive OSS in this population and can be treated accordingly (16). Thus, it would be important to assess OSS either in the semen in the male or the vaginal fluids in the female before, during, and after any clinical studies. This would be indicative that an individual with low OSS does not contribute to the infertility. If a positive correlation is observed between OSS and the outcome of the trial, a predictive value could be determined..

10. CONCLUSIONS

Oxygen toxicity is an inherent challenge to aerobic life forms, including the spermatozoa. How this toxicity affects interaction of sperm with the ovum is still unknown. Increased oxidative damage to sperm membranes (indicated by increased LPO), proteins, and DNA is associated with alterations in signal transduction mechanisms that affect fertility. Spermatozoa and oocytes possess an inherent but limited capacity to generate ROS which may help the fertilization process. A variety of defense mechanisms encompassing antioxidant enzymes (SOD, catalase, glutathione peroxidase and reductase), vitamins (E, C, and carotenoids), and biomolecules (glutathione and ubiquinol) are involved in biological systems. A balance between the benefits and risks from ROS and antioxidants appears to be necessary for the survival and normal functioning of spermatozoa. An assay system for the evaluation of oxidative stress status (OSS) may aid the clinician in the assessment of fertility status of both male and female partners. Determination of this OSS value will also theoretically identify the subgroups of responders and non-responders to any putative antioxidant therapy.

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