Review Article

Role of Oxidative Stress in Male Infertility: An Updated Review

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ABSTRACT

Current evidence links oxidative stress (OS) to male infertility, reduced sperm motility, sperm DNA damage and increased risk of recurrent abortions and genetic diseases. A review of PubMed, Medline, Google Scholar, and Cochrane review databases of published articles from years 2000–2018 was performed focusing on physiological and pathological consequences of reactive oxygen species (ROS), sperm DNA damage, OS tests, and the association between OS and male infertility, pregnancy and assisted reproductive techniques outcomes. Generation of ROS is essential for reproductive function, but OS is detrimental to fertility, pregnancy, and genetic status of the newborns. Further, there is a lack of consensus on selecting OS test, type, and duration of antioxidants treatment as well as on the target patients group. Developing advanced diagnostic and therapeutic options for OS is essential to improve fertility potential and limit genetic diseases transmitted to offspring.

KEYWORDS: *Antioxidants, male infertility, oxidative stress, sperm DNA damage*

INTRODUCTION

Infertility is defined as the inability to achieve pregnancy after 1 year of regular unprotected sexual intercourse.[1] The global prevalence of infertility varies between 2.5%–15%, correlating to at least 30 million infertile men worldwide.[2] Infertility has been linked to several emotional, physical, and sociocultural problems.[3] One of the mechanisms proposed for idiopathic male infertility is oxidative stress (OS). Male infertility accounts for about 40% of all cases and it is known that some conditions such as varicocele, cryptorchidism, hypogonadism, and genetic factors can cause infertility. However, no underlying cause can be identified for primary or secondary infertility in approximately 25% of couples which is termed idiopathic infertility.[4] One of the proposed mechanisms for idiopathic infertility is OS and reactive oxygen species (ROS).

Increased ROS along with decreased antioxidant defense result in redox imbalance, reduced sperm motility and sperm DNA damage. Spermatozoa are highly susceptible to the deleterious effects of ROS due to the large amounts of unsaturated fatty acids found in their cell membranes. Reactive oxygen species promote peroxidation of lipids, resulting in intracellular oxidative burden. The sequence of events involves lipid peroxidation, loss of membrane integrity with increased permeability, reduced sperm motility, structural DNA damage, and apoptosis.[5-7] Several intrinsic and extrinsic factors have been associated with increased OS in the male reproductive system.

The World Health Organization (WHO) has published references values for seminal fluid analysis parameters.[8] Decreased sperm concentration – defined as $< 15 \times 10^6$ sperm/ml – is termed oligozoospermia; whereas asthenozoospermia corresponds to progressive sperm motility of <32% or total sperm mobility under 40%. Teratospermia is defined as normal sperm motility of <4% using Kruger's strict criteria.[9] The combination of all these abnormalities is termed oligoasthenoteratozoospermia.

Currently, there is a lack of agreement on which patients should be tested for OS, as well as which test to perform. There are also controversies on types, dose, and duration of antioxidants treatment of in patients with excessive ROS levels.[10] Therefore, the aim of this review is

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to provide an update on current evidence regarding ROS production, tests and the association between OS and male infertility as well as pregnancy and assisted reproductive techniques (ART) outcomes.

Methods

This review of literature included a systematic search strategy performed in electronic scientific databases PUBMED, Medline, Google Scholar, and Cochrane review to include published articles from years 2000 to 2018. The search involved keywords including combinations of search terms "oxidative stress," "reactive oxygen species," "semen parameters," "male infertility," "sperm function," "antioxidants," "semen analysis," "oxidative stress tests," "pregnancy outcomes," and "assisted reproductive techniques." Articles were perused, and their reference lists were checked for relevant publications. We included articles published in English only.

Oxidative Stress Definition

OS is defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of available antioxidants resulting in redox paradox.[11] Sperm cells are vulnerable to ROS because of the abundance of polyunsaturated fatty acids in their plasma membrane and cytoplasm[12] and limited antioxidant capacity and DNA repair system.[13] Certain levels of ROS are required for maturation of spermatozoa, acrosome reaction, capacitation, hyperactivation, and sperm-oocyte fusion.[14] Excessive ROS production, however, overwhelms the neutralizing capability of antioxidants (enzymatic and nonenzymatic) in the seminal plasma. ROS are formed as natural byproducts of oxygen during metabolism and have important roles in cell signaling and homeostasis.[15] Sources of ROS can be endogenous or exogenous [Figure 1] and body antioxidant defense mechanism aims to neutralize the harmful effects of these pro-oxidants molecules.

Sources of Reactive Oxygen Species

Intrinsic

Redox reactions in aerobic metabolism yield ROS as byproducts. In mitochondria, these reactions require nicotinamide adenine dinucleotide (NADH) as an electron donor and acceptor in the electron transport chain, which allows synthesis of adenosine triphosphate (ATP)[16] [Figure 1]. Seminal fluid ROS can also originate from cytoplasmic glucose-6-phosphate dehydrogenase.[17] Varicocele, the most common etiology of male infertility, has been linked with the increased oxidative burden and ROS-induced sperm DNA damage;[14] as well as with increased scrotal temperature.[18] These findings have been corroborated with evidence describing reduced levels of seminal lipid peroxidation and sperm DNA damage after varicocelectomy.[18] Moreover, increased temperature has also been related to increased ROS

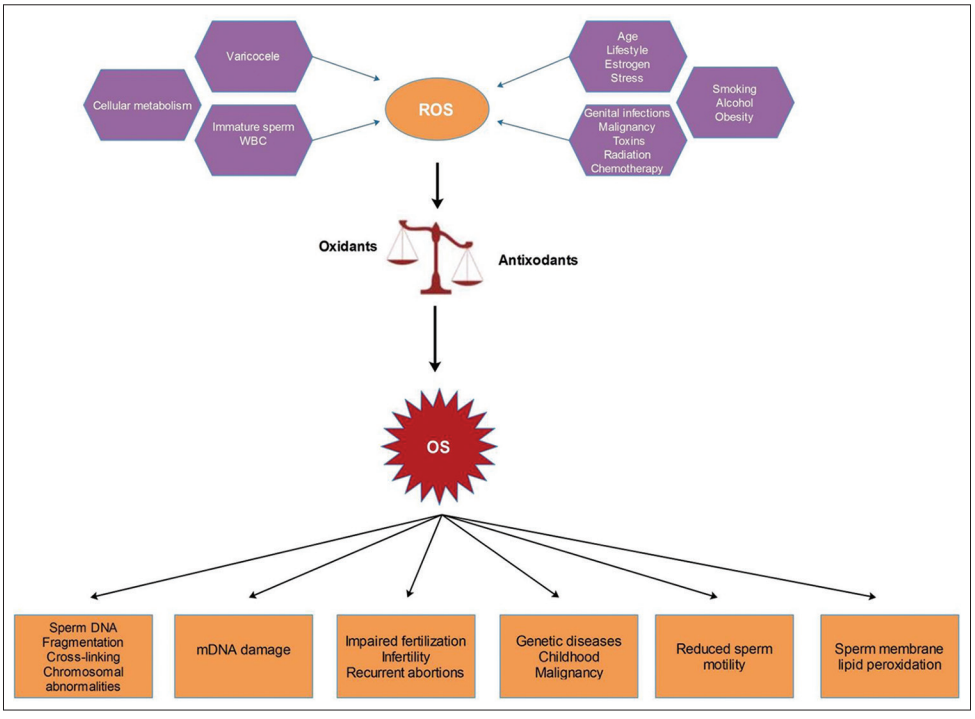

Figure 1: Sources of reactive oxygen species in the body and their pathological consequences on semen, fertility and health. ROS = Reactive oxygen species, OS = Oxidative stress

production and negative effects on other seminal fluid parameters.[19,20] In particular, elevated ROS have been associated with male accessory gland infection, including the urethra, prostate, deferent ducts, seminal vesicles, epididymis, or testes.[21] This has been attributed to the capacity of seminal leukocytes to produce 1000-fold more ROS and free radicals than any other cell with aerobic metabolism.[22] Infections have also been independently associated with increased ROS.[23,24] Furthermore, hyperglycemia is another important factor for male infertility, as strong correlations have been identified between prediabetes and diabetes mellitus with increased OS and altered sperm parameters.[25]

Extrinsic

Extrinsic factors such as smoking, alcohol intake, and exposure to radiation and industrial heavy metals have been associated with increased ROS and male infertility [Figure 1]. Smoking has been associated with reduced sperm concentration, motility, and altered morphology.[26] Smoking also elicits a chronic inflammatory response which recruits leukocytes to the genital tract and causes a substantial increase in seminal ROS levels,[27] as well as increased sperm DNA damage.[13]

Seminal fluid abnormalities have been associated with excessive alcohol intake, including decreased spermatogenesis, abnormal sperm morphology, decreased seminal fluid volume, low levels of testosterone, and increased OS.[28] Indeed, alcohol abuse results in increased production of acetaldehyde which promotes the generation of ROS due to its interactions with proteins and lipids.[11]

Altered sperm function and increased DNA damage have been associated with industrial exposure to heavy metals such as lead, cadmium, iron, and copper, as well as exposure to phthalates, pesticides, and pollution.[20,29] Malignancies are another important extrinsic source of ROS, along with the accompanying exposure to radiation and chemotherapy. Men treated with chemotherapy medications such as cisplatin, doxorubicin, or cyclophosphamide have been linked to increased OS.[30] Radiotherapy has also been associated with increased OS, while low-level radiation therapy appears to modulate NADH oxidase activity promoting sperm death.[31,32]

Antioxidants in Semen

Both enzymatic and nonenzymatic antioxidant protective systems have been identified in semen, which positively interact with each other to counteract the adverse effects of ROS.

Enzymatic antioxidants

Superoxide dismutase, catalase, and glutathione (GSH) peroxidase form the main antioxidant system in semen.[33] These metalloenzymes are present in both the intracellular and extracellular space. Superoxide dismutase catalyzes the dismutation of the superoxide anion, utilizing the copper and zinc molecules in its active center.[34] There are two main isoforms of superoxide dismutase (SOD) enzyme: SOD-1, with about 75% of the antioxidant, and SOD-3, with the remaining 25%.[35] Seminal SOD activity has been positively correlated with sperm concentration and motility.[36]

Catalase acts on hydrogen peroxide, resulting in its decomposition to water and molecular oxygen. The heme system with an iron atom in the center is the prominent characteristic of this enzyme, which can be found in the cytoplasm, endoplasmic reticulum, and various other organelles.[37] This enzyme is synthesized in the prostrate and catalase activity involves capacitation of nitric oxide and involvement of hydrogen peroxide.[33,38] Catalase level has been positively correlated with progressive sperm motility in normospermic individuals.[39] GSH peroxidase is responsible for the catalytic reduction of hydrogen peroxide and organic peroxides, including the peroxides of phospholipids.[34] This enzyme contains selenium in its active site and is primarily located in the mitochondrial matrix of spermatozoa. A specific isoform protects sperm DNA from oxidative damage and chromatin condensation.[40] GSH peroxidase activity was reduced in individuals diagnosed with severe asthenozoospermia, oligozoospermia, and teratozoospermia.[41]

Nonenzymatic antioxidants

Numerous nonenzymatic antioxidants are present in semen, including Vitamins A, E, C, and B complex, GSH, coenzyme Q10, carnitine, and minerals such as zinc, copper, selenium, and chromium.[42] GSH is a tripeptide thiol with a wide array of biological functions, including the preservation of the intracellular redox status and detoxification of exogenous and endogenous compounds. Structurally, it stems from a combination of three amino acids; cysteine, glycine, and glutamine. GSH possesses an innate reducing power that protects cells against OS, in particular, due to its sulfhydryl group (SH). GSH is found in both its oxidized and reduced forms. The antioxidant mechanisms of GSH are mediated by associated enzymes such as GSH peroxidase and GSH reductase.[43]

Vitamin E or alpha-tocopherol is a fat-soluble molecule found in almonds, avocados, spinach, and sweet potatoes. It has potent antioxidant properties, by neutralizing free radicals and inhibiting ROS damage to cell membranes;

resulting in prevention of lipid peroxidation and enhancement of other antioxidants. According to the European Commission Directive 2008/100/EC, the recommended daily intake of Vitamin E is 12 mg.[44] In an interventional placebo-controlled trial on infertile men conducted by Greco *et al.*,[45] minimal sperm DNA damage was observed after 2-month supplementation of Vitamin E (1 g/day) and Vitamin C (1 g/day).

Vitamin C is a water-soluble vitamin with antioxidant properties found in citric fruits and fresh berries. Numerous studies have evaluated the effect of Vitamin C supplementation on sperm function. A study performed on male rats showed ascorbic acid could revert testicular OS induced by cyclophosphamide.[46] In another study, reduced levels of Vitamin C and increased ROS levels were detected in the seminal fluid of men with asthenozoospermia.[47] Carotenoids are a group of organic compounds found in orange, red, yellow, and pink vegetable dyes, which act as precursors for Vitamin A, whose integral component is retinol. These are naturally occurring antioxidants, necessary for maintaining the integrity of cell membranes. They are also involved in the regulation of spermatogenesis. Carotenoid deficiency can lead to decreased sperm motility and male infertility.[48]

Zinc is the second-most abundant metal in the human body and a cofactor for various enzymes involved in DNA transcription and protein synthesis, playing a pivotal role in reproduction. Zinc participates in various reproductive processes such as steroidogenesis, testicular development, gonadal differentiation, production of luteinizing hormone and follicle stimulating hormone, formation and maturation of spermatozoa, acrosome reaction, and fertilization.[49,50] The WHO currently estimates zinc deficiency to affect one-third of the population worldwide. Zinc, along with various antioxidant enzymes, may improve sperm parameters and increase the likelihood of pregnancy for men with oligoasthenoteratozoospermia.[51] Selenium is another essential trace element, which intervenes in sperm formation and testosterone synthesis.[52] At least 25 selenoproteins have been identified in humans and animals, involved in the maintenance of sperm structural integrity. Several randomized clinical trials have tried selenium in combination with other antioxidants with promising results.[53,54]

Physiological Role of Reactive Oxygen Species in Male Fertility

The development of male germ cells yields significant amounts of ROS which constitutes a principal source of OS in spermatozoa.[55] Reactive oxygen species modulate sperm chromatin condensation by adjusting the number of germ cells and inducing apoptosis or proliferation of spermatozoa.[56] ROS are also involved in the processes of capacitation, acrosome reaction, mitochondrial stability, and sperm motility in mature sperm. ROS can function as messengers, by modulating the NADPH oxidase enzyme complex in the cell membrane, and intervening in the respiratory chain within mitochondria. In spermatozoa, superoxide anion metabolism is regulated by the NADH oxidoreductase enzyme, which works in close conjunction with the mitochondrial respiratory chain and xanthine oxidase found in sperm and seminal plasma.[33] Immature spermatozoa with cytoplasmic residues show increased production of ROS when compared to sperm with normal morphology.[17,57]

Seminal leukocytes are another source of ROS, producing 1000 times more of these molecules than sperm cells under physiological conditions. This is because seminal leukocytes represent the first line of defense against offending infectious agents, using primarily oxidative and inflammatory mechanisms.[22] However, this can become a double-edged sword, as an imbalance between oxidants and antioxidants could result in cellular injury. Indeed, ROS generated to counteract infectious agents can also damage host cells, which can result in the disintegration of the cell membrane or sperm DNA damage.

Pathological Effects of Reactive Oxygen Species on Male Fertility

The rationale behind the use of antioxidants for the treatment of male infertility relies on excessive levels of ROS and free radicals cause altered sperm function and sperm DNA damage. A study Desai *et al.* found that sperm characteristics were significantly lower in infertile men with high levels of ROS in semen as assessed through chemiluminescence.[32] Reactive oxygen species alter DNA integrity in the sperm nucleus by inducing breakage of DNA strands, base modifications, and chromatin cross-linking [Figure 1].[58] Moreover, spermatozoa have limited defense mechanisms against ROS-induced DNA damage.

Human ejaculate contains sperm cells with various degrees of maturity, along with leukocytes, epithelial cells and round cells from different stages spermatogenesis. Among these cells, peroxidase-positive leukocytes and immature spermatozoa produce significant amount of free radicals.[59] Spermatozoa are especially susceptible to oxidative damage due to the presence of abundant polyunsaturated fatty acids in their plasma membrane.

These fatty acids are important as they provide membrane fluidity, a key feature for several membrane fusion events such as acrosome reaction and sperm-egg interactions. However, these unsaturated fatty acids render them vulnerable to free radical attacks and ongoing lipid peroxidation.[60]

Nevertheless, in around 85% of cases, the sperm genome is protected from free radical damage as it is bound to central nucleoprotamines.[13] Deficient protamination has been observed in infertile men, representing yet another source of ROS-induced DNA damage[61] which is compounded by the limited capacity for sperm DNA repair seen during spermatogenesis.[62] ROS-mediate disruption of mitochondrial membranes leads to caspase activation, resulting in apoptosis. The apoptotic pathways involve cytochrome c release, which augments the levels of ROS, DNA damage, and apoptosis.[63]

DNA bases are also prone to OS-induced damage with base modifications, strand-breaks, and chromatin cross-linking. Indeed, OS and apoptosis are key events involved in causing DNA damage in the germ line.[64] The major role of ROS in the etiology of sperm DNA damage in infertile men has been corroborated in multiple studies.[65-68]

Spermatozoa carry a complete haploid genome to the ovum to form a new individual. Condensation of the nuclear material in the sperm nucleus is essential for this process to be successful. This condensation is promoted by the unique process of protamination, which involves the replacement of histones by positively charged protamines, which in turn form tight toroidal complexes. This is essential, as chromatin organization is necessary for fertilization and early embryonic development.[69] However, normal sperm appears to possess varying degrees of fragmented DNA; although, infertile men appear to have larger proportions of fragmented DNA.[70]

Both extrinsic and intrinsic factors are involved in the pathogenesis of fragmented DNA. The latter include poor chromatin structure and limited repair capacity. Intrinsic factors include abortive apoptosis and defective maturation.[71,72] Accumulating evidence suggests extrinsic factors are responsible for the increased DNA fragmentation found in the epididymis and ejaculated sperm in comparison to testicular sperm.[73] Recent research posits OS as another extrinsic cause of sperm DNA fragmentation (SDF),[74] as ROS can surpass the limited antioxidant mechanisms of sperm and damage polyunsaturated fatty acids in membranes, resulting in SDF.[75,76]

Sperm DNA Damage Induced by Reactive Oxygen Species

Although ROS seem to play a physiological role in the acrosome reaction, normal sperm function, activation, motility, and capacitation;[77] their potentially deleterious effects cannot be overlooked. Spermatozoa are especially vulnerable to ROS as they contain large amounts of polyunsaturated fatty acids in their plasma membrane and cytoplasm. OS could induce a rapid loss of intracellular ATP, resulting in axonemal damage with decreased sperm viability and mobility and increased mid-piece structural defects, with deleterious effects on sperm capacitation and the acrosome reaction. Lipid peroxidation of the sperm membrane is a key mediator of ROS-induced sperm damage, leading to infertility [Figure 1].[78,79]

Hydrogen peroxide is the principal ROS in human spermatozoa, while excessive production of ROS by abnormal spermatozoa or leukocytes appears to be associated with male infertility.[75] Moderately elevated concentrations of hydrogen peroxide cause sperm immobilization, mostly through depletion of intracellular ATP and reduced phosphorylation of axonemal proteins, with no impact on viability. In contrast, higher concentrations of hydrogen peroxide promote lipid peroxidation and cell death.[80,81]

In a study by Pasqualotto *et al.*,[82] the levels of antioxidants in seminal plasma from infertile men were significantly lower than in fertile controls, and the levels of ROS produced by spermatozoa were negatively correlated with sperm quality. In semen of infertile men, pathological levels of ROS are likely to be the result of increased ROS production and impaired antioxidant capacity.[83]

Exogenous or endogenous sources of ROS can induce sperm DNA damage that in turn may cause childhood diseases such as autosomal dominant disorders, neuropsychiatric disorders, and childhood cancers like retinoblastoma.[84,85] OS tends to target on telomeres, which are key genome protectors. Telomeres erode faster when exposed to OS, resulting in telomere dysfunction, chromosome instability, and apoptosis; all of which have been related to aging and carcinogenesis.[86]

This form of DNA damage could be particularly important in recurrent spontaneous abortion (RSA). Various paternal factors have been linked to RSA, including ROS-induced sperm DNA damage. In a study on 25 couples with idiopathic RSA and 25 proven fertile controls, ROS levels and DNA damage were significantly higher among the men in the RSA group.[87] Mitochondrial dysfunction and OS have been associated

with cancer, cellular senescence, apoptosis and aging; as well as with isolated cases of asthenozoospermia.[88] Antioxidants may prevent telomere loss and promote genomic stability in cells with mitochondrial dysfunction, corroborating the association with OS. Furthermore, nuclear transfer protected the genomes from telomere dysfunction and reconstitution of the mitochondria, thereby promoting cell survival.[89]

Lipid peroxidation cascade contributes to the production of free radicals and induces the production of lipid aldehydes such as acrolein, 4-hydorxynonenal (4-HNE), and malondialdehyde (MDA).[12] These have been linked with OS and damage to nuclear and mitochondrial DNA, with shorter telomeres, formation of the base product 8-hydroxy-deoxyguanine (8-OHdG), and fragmentation of mitochondrial DNA. They can also affect sperm plasma membranes, thus affecting their motility and ability to fuse with the oocyte. Production of 8-OHdG facilitates DNA damage by limiting the repairing capacity of spermatozoa.[90] Because fragmented DNA carries a high mutagenic potential, the oocyte may skip the base-excision repair and correction of 8-OHdG-associated changes, resulting in genomic hypermutability and instability, as well as infertility.[91] A high incidence of genetic aberrations in embryos have been attributed to ROS-induced OS in the male germ line; in association with conditions such as childhood cancers, neuropsychiatric disorders such as autism and schizophrenia, and dominant gene mutations such as Apert syndrome and achondroplasia.[13]

Antioxidant Therapy in Male Infertility

The rationale for oral antioxidant therapy is because seminal OS is due to increased ROS production and/or decreased levels of seminal antioxidants.[60] The different oral antioxidants available belong to the exogenous antioxidant category and they include Vitamin C, Vitamin E, coenzyme Q10, N-acetyl cysteine, carnitines, trace elements such as zinc, selenium, pentoxifylline, and a combination of these oral antioxidants. Numerous studies have been conducted to assess the effectiveness of oral antioxidant supplementation for the treatment of male infertility. Most of the studies showed an improvement in one or more of seminal fluid parameters,[92,93] whereas some studies reported no positive effect [Table 1].[94-96]

Tests to Measure Reactive Oxygen Species in Semen

Assessment of sperm ROS levels among infertile men can aid in determining which individuals who may benefit from antioxidant therapy. Various tests have been developed to detect seminal ROS levels which can be classified into direct and indirect assays [Tables 2 and 3]. Currently, there are no infertility guidelines that recommend routine ROS measurement and there is still an ongoing debate on which type of patients have to be tested for the oxidative burden. Asthenozoospermia in a semen sample is probably a marker of ROS.[11] Hyperviscosity has also been suggestive of increased OS because it is attributed to increased malondialdehyde levels. Increased leukocytes or round cells which is one of the principal sources of ROS may suggest further testing for OS. Abnormal sperm morphology due to cytoplasmic residues also correlates with high levels of ROS.[10] The hypo-osmotic swelling test suggests membrane damage in the sperm due to lipid peroxidation and this might imply higher levels of ROS in semen. Besides, some studies recommend ROS testing in individuals with idiopathic infertility.[110]

Reactive Oxygen Species Effects on Pregnancy Loss and Outcomes

The effects of SDF on natural pregnancy and pregnancy outcome have been recognized.[111] The Danish First Pregnancy Planner study illustrated a correlation between infertility and an SDF index of >30%. There was a significant association between a high sperm SDF and increased time to conceive naturally and lower fertility potential.[112] Similarly, in 500 couples with no infertility history who discontinued contraception for the purpose of pregnancy and they were enrolled in the Longitudinal Investigation of Fertility and the Environment study, SDF was associated with low fecundity.[113] A meta-analysis included 616 couples demonstrated that three studies showed an odds ratio of 7.01 suggestive of an association between high SDF and failure to achieve natural pregnancy.[114]

OS has become a growing concern for researchers and clinicians because of association with decreased fertilization, poor embryonic development, pregnancy loss, potential birth defects such as autism and cancers.[11] The chromatin in the sperm DNA is vulnerable to OS and there are base-pair modifications along with DNA fragmentation. Sperm and oocyte DNA damage may interfere with implantation and ultimately result in abortion. Evidence suggests that about 80% of the chromosomal aberrations are of paternal origin in humans.[115] Further, sperm DNA damage has been implicated in apoptosis, poor fertilization rate, higher frequency of miscarriage, and morbidity in offspring.[11]

Emerging evidence suggests that in ART, there is a correlation between high SDF and an increased risk of miscarriage. In a systematic review reported by Rilcheva

Table 1: Antioxidant therapy in male infertility

Author, years	Groups/number of participants	Controlled	Type of antioxidant and dose	Intervention period	Results
Safarinejad, 2011[97]	Idiopathic oligoasthenoteratozoospermia/211	Yes	EPA and DHA acids, 1.84 g/day versus placebo	32 weeks	Increase in total sperm count and concentration. Both EPA and DHA positively correlated with plasma superoxide dismutase and catalase activity
Wirleitner *et al*., 2012[98]	Oligoasthenoteratozoospermia and nonoligoasthenoteratozoospermia/147	Yes	Fertilovit M-Plus Vitamin C-100 mg Vitamin E-100 mg Folic acid-500 µg Zinc-25 mg Selenium-100 µg N-acetyl L-cysteine 50 mg L-carnitine 300 mg Citrulline 300 mg GSH reductase 50 mg Lycopene 4 mg Co-enzyme Q10 15 mg twice daily	2 months	Increased sperm concentration and motility. No significant improvement in morphology
Safarinejad *et al*., 2012[99]	Idiopathic oligoasthenoteratozoospermia/228	Yes	Coenzyme Q10-200 mg per day	26 weeks	Increased sperm density, motility and morphology. Decreased follicle stimulating hormone activity
Safarinejad, 2012[100]	Idiopathic oligoasthenoteratozoospermia/287	No	Coenzyme Q10-300 mg twice daily	12 months	Increased mean sperm concentration, progressive motility and normal morphology
Busetto *et al*., 2012[101]	Idiopathic asthenoteratozoospermia/114	No	L-carnitine-145 mg Acetyl L carnitine-64 mg, fructose 250 mg, citric acid 50 mg Selenium 50 µg CoQ10 20 mg Zinc 10 mg Ascorbic acid 90 mg Cyanocobalamine 1.5 µg Folic acid 200 mcg/once a day	4 months	Increased progressive sperm motility, and no significant improvement in sperm concentration and morphology
Chen *et al*., 2012[102]	Oligozoospermia and asthenozoospermia/64 and 42	Yes	Oligospermia Tamoxifen 10 mg bid Tamoxifen 10 mg + Vitamin E 100 mg tid Asthenospermia Levocarnitine 1 bottle bid Levocarnitine 1 bottle bid, Vitamin E 100 mg tid	3 months	Progressive increase in sperm motility in oligospermic men. There was nonsignificant improvement in sperm motility in asthenozoospermic individuals

Contd...

Table 1: Contd...

Author, years	Groups/number of participants	Controlled	Type of antioxidant and dose	Intervention period	Results
Cavallini, 2006[8]	Idiopathic oligoasthenoteratozoospermia/55	Yes	L-carnitine 1 g bid L-carnitine 500 mg bid +30 mg cinnoxicam every 4 days	3 months	Improvement in morphology and number of spermatozoa. Increased percentage of pregnancy following ICSI. Nonsignificant improvement in the number of fertilised oocytes and embryos transferred
Abad *et al*., 2013[103]	Asthenoteratozoospermia/20	Yes	L-carnitine-1500 mg Vitamin C-60 mg Coenzyme Q10-20 mg Vitamin E-10 mg Zinc-10 mg Vitamin B9-200 µg Vitamin B12-1 µg Selenium-50 µg	0 h, 2 h, 6 h, 8 h and 24 h	Increase in sperm concentration, motility, vitality and morhological parameters
Nadjarzadeh *et al*., 2014[104]	Idiopathic oligoasthenoteratozoospermia/60	Yes	200 mg/day CoQ10 or placebo	3 months	Improved semen parameters
Raigani *et al*., 2014[105]	Oligoasthenoteratozoospermia/83	Yes	Folic acid (5 mg/day) Zinc sulfate (220 mg/day) or placebo	16 weeks	Increased sperm concentration with combined treatment
Hadwan *et al*., 2014[106]	Asthenozoospermia/60	Yes	Zinc sulfate (220 mg/day) bid	3 months	Increased in semen volume, sperm count and forward motility
Cyrus *et al*., 2015[107]	Clinical varicocele/115	Yes	Vitamin C (250 mg) Bid or placebo	3 months	No effect on sperm count but improved sperm motility and morphology
Haghighian *et al*., 2015[108]	Idiopathic asthenozoospermia/44	Yes	Alpha lipoic acid (600 mg) or placebo	12 weeks	Sperm count, concentration, and motility were significantly improved
ElSheikh *et al*., 2015[94]	Idiopathic oligoasthenozoospermia/90	Yes	I: Vitamin E (400 mg/day) II. Clomiphene citrate (25 mg/day) III: Combination of drugs with same dosage	6 months	There was no significant increase in sperm concentration but only in the Vitamin E group. Combination therapy showed increased sperm concentration and motility
Bozhedomov *et al*., 2017[109]	Oligo or astheno or teratozoospermia/173	Yes	L-carnitine fumarate (1 g), acetyl L-carnitine (0.5 g) twice daily, combination of Vitamins A, E, C, selenium, zinc, clomiphene (25 mg) bid	3-4 months	Improve in the concentration of spermatozoa but no effect on sperm morphology, motility and pregnancy rates

EPA=Eicosapentaenoic acid, DHA=Docosahexaenoic acid, ICSI=Intracytoplasmic sperm injection, GSH=Glutathione

et al., it was found that after *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), there were increased chances of pregnancy loss due to high SDF with a combined odds ratio of 2.48.[116] Another systematic review with 16 individual cohorts and 2969 couples corroborated the above results and it observed a

Table 2: Direct assays of oxidative stress

Test	Method of measurement	Function	Advantages	Disadvantages
Chemilum-inescence assay	Charged or uncharged probes undergo oxidation/reduction with generation of light as by-product	Deduce oxidation or reduction through the generation of light	High sensitivity and specificity. Robust test	Large and expensive equipment Time consuming Requires higher sample volume Interfering variables like semen age, volume, and temperature control
Flow cytometry	When excited by light of differing wavelengths, the incubation with the dye emits fluorescence	Measurement of ROS	It requires a small number of spermatozoa - patients with low sperm count- and can measure multiple markers simultaneously	Expensive tool that is not practical for widespread clinical use
Electron spin resonance	Obtains the absorption spectra of spin energy among unpaired electrons in an applied magnetic field	Detection of free radicals	Broad usage covering various parameters like: Observation of free radicals Analysis of free radicals characteristics Quantitative analysis of free radicals Kinetic analysis Good for high levels of ROS production	Free radicals can react with another molecule other than spin-trapping agent Interference factors like neutralization
Cytochrome c reduction	Superoxide radicals and reduced ferricytochrome c are identified	Evaluation of ROS on the cell membrane	Good in detecting high levels of ROS Quantifies superoxide released during respiratory burst of neutrophils or enzymes	If enzyme activity is less, relative insensitivity to the detection of NADPH oxidase activity
Nitroblue tetrazolium test	Nitroblue tetrazolium turns yellow to purple/blue when exposed to ROS	Localization of reaction between leukocytes or sperm cells and superoxide ions	Detects neutrophils at a concentration of 0.5×10^6/mL or higher Easy and cost-effective	Subjective interpretation of a positive or negative neutrophil
Thiobarbituric acid assay	Based on the reaction of a chromogenic agent, 2-thiobarbituric acid with MDA	Used to evaluate the resistance of sperm to oxidative stress	Can assess sperm MDA levels Needs expensive microplate readers	Expensive equipment is required
Xylenol orange-based assay	Oxidants in semen samples oxidise the ferrous ion-o-dianisidine complex to ferric ion. Ferric ion forms a colored compound with xylenol that can be detected using a spectrophotometer	Colorimetric automated assay	Measures the net oxidative imbalance between ROS production and antioxidant concentration	Limited widespread utility due to cost
FITC-labelled lectins	Used to detect the sperm acrosome status	Used to detect sperm peroxidase using plant lectins labeled with a fluorescent agent (FITC) to detect a group of sperm peroxidases	Detects sperm acrosome status	Difficult to detect true and false acrosome reaction Cannot detect sperm viability and acrosomal reaction status in one picture Fluorescent signal can fade sometimes

ROS=Reactive oxygen species, MDA=Malondialdehyde, NADPH=Nicotinamide adenine dinucleotide phosphate, FITC=Fluorescein isothiocyanate

Table 3: Indirect assays of oxidative stress

Test	Method of measurement	Function	Advantages	Disadvantages
Myeloperoxidase or Endtz test	Peroxidase positivity is assessed through staining using benzidine as a buffer	Detection of granulocytes in semen	Specifically distinguishes WBC's especially producing granulocytes from immature germ cells in semen	Cannot be used to detect ROS production in spermatozoa
Lipid peroxidation levels	Detection of MDA and toxic 4-HNE through colorimetric and thiobarbituric acid assays	Identification of by-products of lipid peroxidation	MDA is a colored substance that can be measured by fluorometry or spectrophotometry. Low sperm concentration of MDA can be measured through sensitive HPLC equipment or spectrofluorometric measurement of iron-based promoters	Not a widely-used test in clinic practice
MiOXSYs	Assessment of electron transfer in millivolts from a reducing agent to the oxidant using a galvanostat-based system	Measurement of oxidation-reduction potential	Easy to employ in a clinical setting Can be used in patients with low semen volume	Larger cohort studies to establish the reference value are needed
Total antioxidant capacity	Evaluates the reductive ability of the antioxidants within the semen against an oxidative agent such as hydrogen peroxide and measures the effect on the substrate	Assesses the cumulative effect of antioxidants within the semen	Rapid colorimetric method Total antioxidants in seminal plasma can be measured	Does not measure individual or enzymatic antioxidants. Requires expensive assay kit and microplate reader
Gpx activity	The activity of Gpx is measured by the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3	Based on the principle that Gpx catalyzes the reaction between hydrogen peroxide and reduced GSH	Gpx protects the sperm from lipid peroxidation and the DNA damage can be significant if the Gpx levels are lower	Some studies show that Gpx activity does not correlate with sperm motility or concentration
Comet assay	Allows DNA migration in an agarose gel under an electric field. The loose DNA forms a pattern of migration that resembles a comet	Single-cell gel electrophoresis assay to assess DNA damage	Can detect extent of DNA damage equivalent to 50-single strand breaks per cell Can be employed in men with low sperm concentrations (requires only 100 cells for analysis)	No consensus reached on the standardization protocol

GSH=Glutathione, GPx=GSH peroxidase, 4-HNE=4-hydorxynonenal, MDA=Malondialdehyde, WBC's=White blood cells, ROS=Reactive oxygen species, HPLC=High-performance liquid chromatography

2.16-fold increase in the risk of pregnancy loss after IVF and ICSI with semen specimens with high SDF.[117] Both systematic reviews stated that the significant correlations between miscarriage rates and high SDF were independent of the method of fertilization used. A study involving 25 fertile sperm donors and 20 recurrent pregnancy loss (RPL) couples showed double-stranded DNA breaks analyzed by Comet assay among RPL sperm donors without any female factor.[118] A more recent study showed that there was a positive association between RSA and high SDF.[119]

Reactive Oxygen Species, Fertility Capacity and Assisted Reproduction Techniques Outcomes

Reactive oxygen species and oxidation-reduction potential (ORP) play significant roles in the fertility process and in the outcome of ART. In a prospective case-control study carried out in 1168 infertile and 100 fertile men, semen analysis parameters, the ORP and SDF were compared. The study concluded that infertile men had significant lower semen parameters and higher ORP and SDF levels, and that a significant positive correlation exists between ORP, SDF, and sperm head defects.[120] In a meta-analysis by Van Waart *et al.*, pregnancy rates achieved with intrauterine insemination (IUI) were correlated with normal sperm morphology and it was found a significant improvement in the pregnancy rate associated with >4% sperm morphology, and hence, they concluded that sperm morphology assessment by strict criteria is a good predictor of IUI outcome.[121] However, more recent studies failed to show an association of ART outcomes in groups with or without isolated teratozoospermia is semen.[122] Hotaling *et al.*, reported that in four retrospective studies, isolated teratozoospermia was not associated with lower pregnancy rates following IVF or

ICSI. Hence, the predictive power of sperm morphology on ART outcomes is still debatable.[123,124]

The existing literature about the consequences of sperm DNA damage on ART outcomes is still controversial. Li *et al.* performed a meta-analysis and found that sperm DNA damage had a negative impact on IVF clinical pregnancy rates but did not affect the IVF and ICSI fertilization and the ICSI clinical pregnancy.[125] Another meta-analysis by Zini *et al.*, evaluating the influence of sperm DNA damage on spontaneous pregnancy loss after IVF and ICSI, showed the detrimental effect of sperm DNA damage on ART outcomes and suggested a clinical test to evaluate the DNA damage before IVF or ICSI procedures.[126] However, the Practice Committee of the American Society for Reproductive Medicine concluded that the data in the existing literature does not support the adverse consequences of sperm DNA damage on ART and natural pregnancy outcomes, but recommends future research to validate the clinical utility of sperm integrity tests.[127] A more recent meta-analysis by Simon *et al.* including 41 studies showed that DNA damage in the sperm significantly correlated with adverse ART outcomes with IVF or ICSI techniques. The sperm DNA damage was evaluated using various tests such as Comet assay, sperm chromatin structure assay, terminal deoxyuridine nick end labeling assay, and sperm chromatin dispersion assay.[128] Therefore, despite that studies demonstrated correlation between sperm DNA damage and adverse ART events, the evidence is inconclusive as there is no standardization of the tests that has been used to detect sperm DNA damage.

Conclusion

Although reactive oxygen species are essential for some reproductive processes such as capacitation and acrosome reaction, increased ROS along with decreased antioxidant defense result in OS status which ultimately leads to sperm membrane lipid peroxidation, reduced motility, sperm DNA damage, poor pregnancy, and ART outcomes and increased risk of genetic diseases in offspring. Various diagnostic and therapeutic options have been developed for OS. However, there is lack of agreement on selecting OS test, type, and duration of antioxidants treatment as well as on defining the target patients group. Further studies are warranted to overcome these limitations, improve fertility potential and reduce the risk of genetic diseases and malignant tumors in newborns.

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Conflicts of interest

There are no conflicts of interest.

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