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Review article

Role of oxidants in male infertility: rationale, significance, and treatment

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Recently, the generation of oxidants, also described as reactive oxygen species (ROS), in the male reproductive tract has become a real concern because of their potential toxic effects, at high levels, on sperm quality and function [1] [2] . ROS are highly reactive oxidizing agents belonging to the class of free radicals. As is true for all cells living under aerobic conditions, spermatozoa constantly face the oxygen (O_2) paradox. O_2 is required to support life, but its metabolites, such as ROS, can modify cell functions, endanger cell survival, or both [3] ; hence, ROS must be inactivated continuously to maintain only the small amount necessary to maintain normal cell function. A battery of different antioxidants normally protects against oxidants [4] . Oxidative stress develops when oxidants outnumber antioxidants, peroxidation products develop, and these phenomena cause pathologic effects [5] [6].

In the context of human reproduction, a balance normally exists between ROS production and antioxidant scavenging activities in the male reproductive tract. As a result of such balance, only minimal amounts of ROS remain, and they are needed for the regulation of normal sperm functions, such as sperm capacitation, the acrosome reaction, and sperm–oocyte fusion [7] [8] . The production of excessive amounts of ROS in semen can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma and causes oxidative stress [1] [2] [5] .

Recent reports indicate that high levels of ROS are detected in the semen of 25% to 40% of infertile men [3] [9] . Spermatozoa are particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) [10] , and their cytoplasm contains low concentrations of scavenging enzymes [3] . In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic antioxidant defenses by depending on the protection afforded by the seminal plasma [11] [12] .

Production of high levels of ROS in the reproductive tract is detrimental not only to the fluidity of the sperm plasma membrane but also to the integrity of DNA in the sperm nucleus [7] . Strong evidence suggests that DNA fragmentation commonly observed in the spermatozoa of infertile men is mediated by high levels of ROS [13] [14] .

This review discusses the mechanisms by which oxidants develop in semen and their role in physiology and pathology of sperm. Topics include the clinical implications of the presence of high levels of seminal ROS to the fertility potential of infertile men. In addition, research is summarized on treatment strategies for infertile men in whom oxidative stress has a significant role.

Mechanisms of oxidant generation in human semen

A variety of semen components, including morphologically abnormal spermatozoa, precursor germ cells, and leukocytes, are capable of generating ROS. Seminal leukocytes and morphologically abnormal spermatozoa are the main sources of ROS in human ejaculates [15] .

Reactive oxygen species production by spermatozoa

Clear evidence suggests that human spermatozoa can produce ROS [16] [17] [18] . Levels of ROS production by sperm correlate negatively with quality of sperm in the original semen [19] . The link between poor sperm quality and increased ROS generation lies in the retention of excess residual cytoplasm (cytoplasmic droplets) in abnormal spermatozoa. When spermatogenesis is impaired, the cytoplasmic extrusion mechanisms are defective. Spermatozoa released from the germinal epithelium carrying surplus residual cytoplasm are thought to be immature and functionally defective [20] . Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose-6-phosphate-dehydrogenase (G_6PD). This enzyme controls the rate of glucose flux through the hexose monophosphate shunt, which, in turn, controls the intracellular availability of nicotinamide adenine dinucleotide phosphate (NADPH) (Fig. 1). The latter is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH-oxidase [21] .

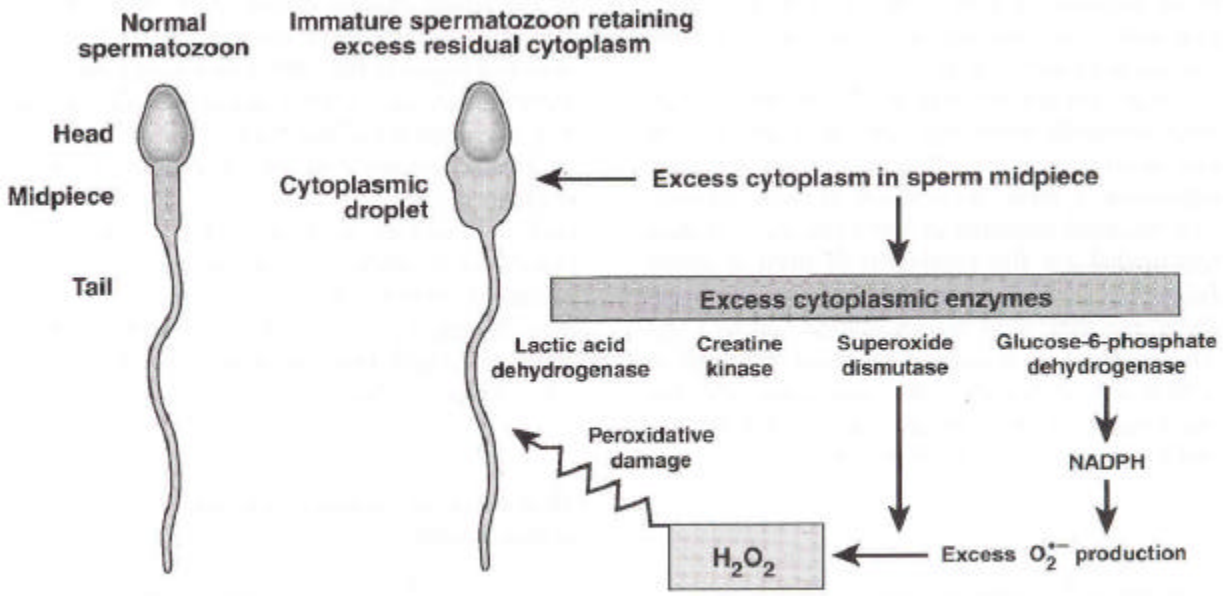

Fig. 1. Mechanism of ROS production by immature spermatozoa (spermatozoa with excess residual cytoplasm). H_2O_2, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; $O_2^{\cdot-}$, superoxide anion.

Spermatozoa may generate ROS in two ways: (1) as a result of the NADPH-oxidase system at the level of the sperm plasma membrane, and (2) as a result of the NADH-dependent oxidoreductase (diphorase) at the level of mitochondria [16] . The mitochondrial system is the major source of ROS in spermatozoa from infertile men [22] . The primary ROS generated in human spermatozoa is the superoxide anion ($O_2^{\cdot}$). This one-electron reduction product of O_2 secondarily reacts with itself in a dismutation reaction, which is greatly accelerated by superoxide dismutase, to generate hydrogen peroxide (H_2O_2). In the presence of transition metals such as iron and copper, H_2O_2 and $O_2^{\cdot}$ can interact to generate the extremely pernicious hydroxyl radical ($OH^{\cdot}$) (Haber-Weiss reaction) as shown in the following equation: $O_2^{\cdot} + H_2O_2 \rightarrow OH^{\cdot} + OH + O_2$.

Alternatively, the hydroxyl radical can be produced from hydrogen peroxide (Fenton reaction), which requires a reducing agent such as ascorbate or ferrous ions, as shown in the equation: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^{\cdot} + OH$. The hydroxyl radical is thought to be an extremely powerful initiator of the lipid peroxidation cascade and can precipitate loss of sperm functions.

A study performed at the authors' center indicated that sperm production of ROS is significantly increased by the repeated cycles of centrifugation involved in the sperm preparation techniques used for assisted conception [23] . The duration of centrifugation was found to be more important than the force of centrifugation in inducing sperm production of ROS [24] . Sperm production of ROS increases with the increase of sperm concentration and decreases with time [25] . Results from recent studies indicate a significant variation in ROS production in subsets of human spermatozoa at different stages of maturation [17] [26] . Following isolate gradient fractionation of ejaculated sperm, ROS production was found to be highest in immature sperm with abnormal head morphology and cytoplasmic retention and lowest in mature sperm and immature germ cells. The levels of ROS production by immature sperm were directly correlated with sperm nuclear DNA damage values in mature sperm and inversely correlated with the recovery of motile mature sperm. These findings led to the hypothesis that oxidative damage of mature sperm by ROS-producing immature sperm during their comigration from seminiferous tubules to the epididymis may be an important cause of male infertility.

Reactive oxygen species production by leukocytes

Peroxidase-positive leukocytes are the major source of ROS in semen [27] [28] [29] . Peroxidase-positive leukocytes include polymorphonuclear leukocytes, which represent 50% to 60% of all seminal leukocytes, and macrophages, which represent 20% to 30% of all seminal leukocytes [30] [31] . Peroxidase-positive leukocytes in semen are contributed largely by the prostate and seminal vesicles [32] . The capacity of leukocytes to generate ROS depends on their activation, which may occur in response to a variety of stimuli, including inflammation and infection [33] . During activation, NADPH production is increased, and the myeloperoxidase system of leukocytes is activated, leading to a respiratory burst with subsequent release of high levels of ROS [34] . Such an oxidative burst is thought to be an effective early defense that kills the microbes in cases of infection [35] .

Sperm damage from ROS produced by leukocytes may occur when seminal plasma is removed during sperm preparation for assisted reproduction [28] , or when seminal leukocyte concentrations are abnormally high, such as in leukocytospermia [29] . Sperm damage from leukocyte-derived ROS may happen even at leukocyte concentrations below the World Health Organization's cutoff value for leukocytospermia, that is, greater than 1×10^6 peroxidase-positive leukocytes/mL of semen [36] . In addition, results from the most recent studies performed at the authors' center indicate that seminal leukocytes may have a role in stimulating ROS production by human spermatozoa [37] . The exact mechanisms underlying such stimulation are not clear and may involve direct sperm–leukocyte contact or may be mediated by soluble products released by the leukocytes.

Reactive oxygen species and sperm physiology

Limited amounts of ROS can intervene physiologically in the regulation of some sperm functions [38] . It has been observed that the addition of low amounts of ROS to human spermatozoa enhances their ability to bind zona pellucida, an effect that is reversed by the addition of vitamin E. More recently, the incubation of spermatozoa with low concentrations of H_2O_2 was found to stimulate sperm capacitation, hyperactivation, and the ability of spermatozoa to undergo the acrosome reaction and oocyte fusion [3] [39] . ROS other than H_2O_2, such as nitric oxide and $O_2^{\cdot}$, were also found to promote sperm capacitation and the acrosome reaction [40] . Sperm capacitation is a priming process that prepares spermatozoa in vivo in such a way that they reach the site of fertilization at the appropriate time and rapidly undergo the acrosome reaction upon contacting the zona pellucida. During the initial stages of capacitation, the intracellular concentration of calcium starts to rise, ROS generation is initiated, cyclic adenosine monophosphate (cAMP) concentrations increase, and spermatozoa develop a highly vigorous form of motility known as hyperactivation. When spermatozoa reach the oocyte, they fall under the influence of progesterone, which induces a sudden influx of extracellular calcium into the acrosomal region of the sperm head. As a result, spermatozoa become highly sensitized and ready to undergo the acrosome reaction [39] .

Reactive oxygen species and sperm toxicity

Virtually every human ejaculate is contaminated with potential sources of ROS, such as peroxidase-positive leukocytes and morphologically abnormal spermatozoa. It follows that some of the sperm cells in every ejaculate incur oxidative damage and a concomitant loss of function. The extent of damage caused by ROS depends not only on the nature and amount of ROS involved but also on the moment and duration of ROS exposure and the extracellular factors, such as temperature, oxygen tension, and the composition of the surrounding environment, including ions, proteins, and ROS scavengers.

Lipid peroxidation of sperm plasma membrane

Lipid peroxidation can be defined broadly as “oxidative deterioration of PUFA: ie, fatty acids that contain more than two carbon–carbon double bonds” [41] . Lipid peroxidation attacks the fluidity of sperm plasma membrane, with subsequent loss of the ability for oocyte fusion. The propagation of lipid peroxidation through sperm populations depends on the antioxidant strategies employed by spermatozoa and seminal plasma. One of the by-products of lipid peroxidation is malondialdehyde, which has been used as an end product in biochemical assays to monitor the degree of peroxidative damage to spermatozoa [38] . This assay correlates closely with the degree to which sperm motility and the capacity for oocyte fusion are impaired [42] [43] .

Impairment of sperm motility

Increased formation of ROS has been correlated with reduction of sperm motility [11] [44] . The link between ROS and reduced motility may be explained by a cascade of events that result in a decrease in axonemal protein phosphorylation and sperm immobilization [45] . Another hypothesis is that H_2O_2 can diffuse across the membranes into the cells and inhibit the activity of enzymes such as G_6PDH, leading to a decrease in the availability of NADPH and a concomitant accumulation of oxidized glutathione and reduced glutathione. These changes can cause a decrease in the antioxidant defenses of the spermatozoa, which ultimately leads to the peroxidation of membrane phospholipids [46] .

Sperm DNA damage

Two factors protect spermatozoal DNA from oxidative insult: the characteristic tight packaging of sperm DNA and the antioxidants in seminal plasma [47] . Exposing the sperm to artificially produced ROS causes DNA damage in the form of modification of all bases, production of base-free sites, deletions, frame shifts, DNA cross-links, and chromosomal rearrangements [48] . Oxidative stress is also associated with high frequencies of single and double DNA strand breaks [14] . This information may have important clinical implications, particularly in the context of assisted reproductive techniques (ART). Spermatozoa selected for ART usually originate from an environment experiencing oxidative stress, and a high percentage of these sperm may have damaged DNA [13] [49] . There is a substantial risk that spermatozoa carrying damaged DNA are used in this form of therapy [50] . When intrauterine insemination (IUI) or in vitro fertilization (IVF) is used, such damage may not be a cause of concern because the collateral peroxidative damage to the sperm plasma membrane ensures that fertilization cannot occur with a DNA-damaged sperm. When intracytoplasmic sperm injection (ICSI) is used, this natural selection barrier is bypassed, and a spermatozoon with damaged DNA is directly injected into the oocyte [7] [50] .

Whether DNA-damaged spermatozoa used in ICSI can impair the process of fertilization and embryo development is not clear. A recent study indicated that spermatozoa with significantly damaged DNA retain the capacity for fertilization [7] ; however, the percentage of sperm with DNA damage negatively correlates with fertilization rates [14] [49] . In addition, a recent study linked sperm DNA damage to increased rates of early embryo death [51] .

Antioxidant defense mechanisms

The seminal plasma contains enzymatic antioxidants, such as superoxide dismutase, the glutathione peroxidase/glutathione reductase system, and catalase [52] , as well as nonenzymatic antioxidants, such as ascorbate (vitamin C) [53] , -tocopherol (vitamin E) [54] , taurine, and hypotaurine [55] . The seminal plasma of fertile men has a higher total antioxidant capacity (TAC) than the seminal plasma of infertile men [56] . Nevertheless, the pathologic levels of ROS detected in the semen of infertile men are more likely caused by increased ROS production than by reduced antioxidant capacity of the seminal plasma [12] .

Antioxidant defense mechanisms include three levels of protection: prevention, interception, and repair. Prevention of ROS formation is the first line of defense against an oxidative insult. An example is the binding of metal ions, iron and copper ions in particular, which prevents them from initiating a chain reaction [4] . When transition metals become loosely bound to ROS, they can produce more reactive oxidants, particularly $OH^{\cdot}$ [57] . Free radicals have a tendency toward chain reaction, that is, a compound carrying an unpaired electron will react with another compound to generate an unpaired electron as "radical begets radical." The basic problem is to intercept a damaging species from further activity, which is the process of deactivation leading to the formation of nonradical end products [4] . -Tocopherol, a chain-breaking antioxidant, inhibits lipid peroxidation in membranes by scavenging peroxyl ($RO^{\cdot}$) and alkoxyl ($ROO^{\cdot}$) radicals, thereby breaking the chain reaction. The ability of -tocopherol to maintain a steady-state rate of peroxyl radical reduction in the plasma membrane depends on the recycling of -tocopherol by external reducing agents such as ascorbate or thiols. In this way, -tocopherol is able to function again as a free radical chain-breaking antioxidant, even though its concentration is low [58] . Protection from the effects of oxidants also can be brought about by repairing the damage once it has occurred. Unfortunately, spermatozoa are unable to repair the damage induced by oxidative stress, because they lack the cytoplasmic enzyme systems required to accomplish the repair [38] [52] .

Methods to assess seminal oxidative stress

Reactive oxygen species–total antioxidant capacity score

Because oxidative stress is caused by an imbalance between levels of ROS production and antioxidant protection in semen, it is conceivable that assessment of seminal oxidative stress will rely on measurement of ROS as well as TAC of semen. Levels of seminal ROS can be measured accurately by a chemiluminescence assay [59] , and TAC of the seminal plasma can be assessed by an enhanced chemiluminescence assay [60] . The fact that neither ROS nor TAC alone can adequately quantify seminal oxidative stress led to the logical conclusion that combining these two variables may be a better index for diagnosis of the overall oxidative stress affecting spermatozoa. This conclusion was behind the development of a statistical formula described as the ROS-TAC score for assessment of seminal oxidative stress in infertile men [61] . This score minimizes the variability of the individual parameters of oxidative stress (ROS alone or TAC alone). The ROS-TAC score was based on a group of normal healthy fertile men who had very low levels of ROS. The composite ROS-TAC score is representative of the fertile population, and scores significantly lower (<30) indicate infertility [61] .

Measurement of reactive oxygen species in neat semen

More recently, the authors' group introduced an additional test for accurate and reliable assessment of oxidative stress by measurement of ROS levels directly in neat (raw) semen using a chemiluminescence assay [62] . The test was subjected to quality control studies and proved to be an accurate measure for seminal oxidative stress status. The maximum ROS level observed in neat semen from normal healthy donors, with normal genital examination and normal standard semen parameters, was 1.5×10^4 cpm/20 million sperm/mL. Infertile men were reliably classified into oxidative stress–positive (1.5×10^4 cpm/20 million sperm/mL) or oxidative stress–negative ($<1.5\times10^4$ cpm/20 million sperm/mL), regardless of their clinical diagnosis or the results of standard semen analysis. Assessment of ROS directly in neat semen had diagnostic and prognostic capabilities identical to those based on the ROS-TAC score (Table 1) [63] . Levels of ROS in neat semen were strongly correlated with levels of ROS in washed semen and with ROS-TAC scores.

Table 1. **Median (25th and 75th percentile) values of reactive oxygen species (ROS) in neat semen, ROS in washed semen, total antioxidant capacity (TAC) in seminal plasma, and ROS-TAC score in donors, oxidative stress (OS)–negative, and OS-positive patients**

Variable	Donors (n = 9)	OS-negative (n = 11)	OS-positive (n = 23)	A	B	C
ROS in neat semen ($\times10^4$ cpm)	0.3 (0.2, 0.9)	0.3 (0.2, 1)	19 (6, 143)	0.9	0.0001	0.0001
ROS in washed semen ($\times10^4$ cpm)	10 (4, 17)	79 (13, 177)	1468 (514, 11496)	0.03	0.0003	0.0008
TAC (Trolox equivalent)	908 (736, 1129)	797 (575, 966)	739 (627, 1047)	0.31	0.24	0.94
ROS-TAC score	53 (51, 55)	49 (47, 52)	36 (32, 44)	0.4	0.001	0.001

Results were analyzed by Wilcoxon rank-sum test; *P*<0.05 was significant.*Abbreviations:*
A, *P* value of donors versus OS-negative patients; B, *P* value between donors versus OS-positive patients; C, OS-negative patients versus OS-positive patients.

Clinical significance of seminal oxidative stress testing

Accurate assessment of seminal levels of oxidative stress may help to diagnose infertility cases in which oxidative stress has a major role (Table 2). Levels of ROS were found to be increased significantly in men with spinal cord injury and were associated with poor sperm motility and morphology [9] . A similar increase of seminal ROS was observed in infertile men with varicoceles [18] . Patients with varicocele were also found to have low levels of TAC in their seminal plasma and might benefit from antioxidant supplementation [18] . In a recent study, varicocelectomy resulted in a significant increase in pregnancy and live birth rates for couples who underwent IUI, although standard semen parameters were not improved in all patients [64] . Such improvement in pregnancy rates following varicocelectomy may result from the correction of a functional factor not tested during routine semen analysis, such as seminal oxidative stress. The new measures of seminal oxidative stress, such as the ROS-TAC score and ROS levels in neat semen, are tools for successful discrimination between fertile and infertile men [37] [61] . Infertile men with chronic prostatitis or prostatodynia were found to have lower ROS-TAC scores than normal sperm donors [65] . Similarly, infertile men with male-factor or idiopathic diagnoses had significantly lower ROS-TAC scores than normal sperm donors. In addition, infertile men with male-factor diagnoses who eventually were able to initiate a successful pregnancy had significantly higher ROS-TAC scores than men who failed to initiate a pregnancy [66] . The ROS-TAC score may serve as an important measure in identifying men with a clinical diagnosis of infertility who are likely to initiate a pregnancy over time [33] . The average ROS-TAC score for fertile men who had undergone vasectomy was nearly identical to that of fertile donors [60] .

Table 2. Mean and standard deviation between reactive oxygen species (ROS), total antioxidant capacity (TAC), and ROS-TAC score in subgroups of infertility patients and controls

Diagnosis	ROS Log (ROS + 1)	*P* value versus controls*	TAC (Trolox equivalent)	*P* value versus controls*	ROS-TAC score	*P* value versus controls*
Control (n = 24)	1.39±0.73	—	1650.93±532.22	—	50.00±10.00	—
Varicocele (n = 55)	2.10±1.21	0.02	1100.11±410.30	0.0002	34.87±13.54	0.0001
Varicocele with prostatitis (n = 8)	3.25±0.89	0.0002	1061.42±425.11	0.03	22.39±13.48	0.0001
Vasectomy reversal (infertile, n = 23)	2.65±1.01	0.0004	1389.89±723.92	0.30	33.22±15.24	0.0002
Vasectomy reversal (fertile, n = 12)	1.76±0.86	0.80	1876.93±750.82	0.62	49.35±12.25	1.00
Idiopathic infertility(n = 28)	2.29±1.20	0.01	1051.98±380.88	0.0003	32.25±14.40	0.0001

* **Pairwise *P* values from Student's *t*-test adjusted using Dunnett's method.**

Treatment strategies

Given the major role of oxidative stress in the pathogenesis of male infertility, treatment strategies with the goal of reducing levels of seminal oxidative stress are necessary for natural and assisted conception.

Elimination of reactive oxygen species sources from semen

Once seminal oxidative stress is diagnosed, treatment plans should focus on identification and elimination of the sources of enhanced ROS production in semen. The differentiation between spermatozoal and leukocyte sources of ROS may significantly affect therapeutic strategies for infertility cases in which oxidative stress has a causative role. Seminal leukocytes should be

considered potentially detrimental and must be monitored carefully. The underlying etiologic factors for abnormal leukocyte infiltration into semen (leukocytospermia), such as inflammation, infection, and cigarette smoking, should be determined and properly addressed. The interaction between ROS-producing cells in semen (leukocytes or abnormal spermatozoa) and normal spermatozoa must be minimized. Sperm preparation techniques used for ART, such as density gradient and swim-up methods, can be used for separation of spermatozoa with good quality that may have a potential to fertilize; however, clinicians should be aware that the repeated cycles of centrifugation routinely performed in these procedures may induce ROS generation by spermatozoa. Such increases in ROS levels together with lack of antioxidant protection owing to removal of seminal plasma may expose the spermatozoa to risk of oxidative damage. The use of sperm preparation techniques with shorter centrifugation periods may reduce the risk of oxidative stress–induced injury to sperm.

Antioxidant supplementation

It has been postulated that protective agents against ROS, such as antioxidants, may be useful therapeutic agents for male infertility [67] ; however, the results of clinical trials in which antioxidants have been used for the treatment of infertile men are controversial [68] . The results of 6 months of supplementation with vitamin E indicated an improvement in sperm motility limited to the patients whose original sperm motility was greater than 15% [69] . A combination of vitamin E and C administered orally for 2 months resulted in improvement of sperm concentration [13] . Administration of glutathione via intramuscular injection for 2 months resulted in improvement of sperm concentration, motility, and morphology [70] . To the contrary, some studies have found no changes in sperm characteristics following treatment with vitamin C or E [71] [72] [73] . In a recent prospective clinical trial, the treatment of infertile men with a combination of vitamins A plus E and essential fatty acids improved the sperm concentration in oligozoospermic men but did not improve sperm motility or morphology [74] .

Levels of seminal ROS and TAC were not predetermined in these studies before antioxidant supplementation. Accurate assessment of levels of seminal ROS and TAC may help to identify subgroups of infertility patients with high ROS, low TAC, or both who may benefit from antioxidant supplementation.

The results of in vitro trials using antioxidants are certainly not better than the results of in vivo trials, and the potential advantages of antioxidants in assisted reproduction are the subject of major debate [75] [76] . During sperm preparation for ART, spermatozoa are washed free from their seminal plasma and left vulnerable to oxidative stress owing to a lack of antioxidant protection. It has been speculated that supplementing the sperm preparation media with antioxidants may provide some degree of protection to the spermatozoa against oxidative stress–induced damage [77] . On this basis, several studies have examined the potential role of antioxidant supplementation in vitro [78] . Vitamin E has been shown to inhibit sperm lipid peroxidation in vitro [79] , to enhance the ability of spermatozoa to fuse with zona-free hamster oocytes [38] , and to improve zona pellucida binding [80] . Supplementing the sperm preparation media with a combination of vitamins C and E was associated with decreased ROS production by the sperm [81] . In another study, supplementation with superoxide dismutase was associated with improved rates of acrosome reaction and preservation of sperm motility [8] . Baker et al (1996) have shown that glutathione, hypotaurine, and catalase can reduce the loss of motility caused by leukocyte-generated ROS. Lewis et al [82] recommended adding low-molecular weight antioxidants such as ascorbate to sperm during preparation for ART, especially in asthenozoospermic patients, on the basis of low levels of ascorbate in those patients. They also indicated that it would be of more clinical benefit to add these antioxidants directly to the sperm rather than using dietary supplements, because defective seminal vesicle function may be one of the causes of reduced ascorbate levels in these patients.

When vitamin E or C was added to the sperm preparation media during density gradient separation using Percoll, spermatozoa were protected from DNA damage [77] ; however, when both of these vitamins were used together, DNA damage increased. Twigg et al [47] found that albumin can be an important means of neutralizing lipid peroxide–mediated damage to the sperm plasma membrane and DNA.

Sperm motion characteristics were reduced significantly when a combination of vitamins E and C was used, with the greatest reduction observed with the highest concentrations [81] . It was concluded that supplementing the sperm preparation media with vitamins E and C was not beneficial.

No definitive conclusion has been reached, with some investigators overestimating and others underestimating the potential benefits of antioxidants in the treatment of male infertility.

Future directions

Future research efforts should be directed toward understanding why spermatozoa of some patients become overreactive in the generation of ROS. Insight into the molecular basis of these defects is vital to identify the cause of unexplained male infertility. If the error in spermatogenesis that leads to such atypical activity (excessive ROS production) could be defined, it would provide a rational basis for the design of effective therapies. The period of sperm differentiation during which this self-destructive activity first appears must be determined. Further efforts are required to identify the sperm population at risk of collateral peroxidative damage to the sperm membrane. An interesting area of future research is to investigate precisely the oxidative damage to sperm DNA and its effects on male fertility potential and the outcome of ART.

Summary

Interest in the physiologic and pathologic effects of ROS on male fertility is growing. The controlled generation of very low amounts of ROS seems to regulate the acquisition of sperm-fertilizing ability. High levels of ROS endanger sperm function and viability. Oxidative stress can arise as a consequence of excessive production of ROS or impaired antioxidant defense mechanisms in semen. Oxidative stress precipitates a range of pathologies that are thought to affect the male reproductive system. ROS-mediated peroxidative damage to the sperm plasma membrane may account for the defective sperm functions observed in a high proportion of infertility patients. High levels of ROS in semen have been correlated with reduced sperm motility and damage to sperm nuclear DNA.

Determining the levels and the sources of excessive ROS production in human semen and precise evaluation of the scavenger system may be useful tools to develop therapeutic strategies for male infertility. Therapeutic interventions directed toward the isolation of mature spermatozoa by in vitro separation techniques may benefit infertile men in whom oxidative stress has a significant role. Nevertheless, this approach may be limited by the fact that the prolonged centrifugation involved in these procedures may enhance ROS production by spermatozoa. Clinical trials using antioxidants in vivo and in vitro have resulted in a major debate, and further research is required before one can be optimistic about a role for antioxidants in the treatment of infertile men. A double-blind, placebo-controlled study underway at the authors' center is evaluating the effects of treating infertile men with a combination of vitamins E and C after adjusting for all causes of controversy.

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