Chapter

Effects of Oxidative Stress on Spermatozoa and Male Infertility

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Abstract

Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the body's own natural antioxidant defences, resulting in cellular damage. Spermatozoa oxidative stress is intimately linked to several reproductive pathologies including the failure of spermatozoa cryopreservation and spermatozoa-egg recognition and fertilization. In this light, this review focuses on (i) the effects of oxidative stress on spermatozoa and application of antioxidants; (ii) production of ROS during cryopreservation; and (iii) oxidative stress in male infertility. This literature describes both a physiological and a pathological role of ROS in fertility. A delicate balance between ROS necessary for physiological activity and antioxidants to protect from cellular oxidative injury is essential for fertility.

Keywords: spermatozoa, oxidative stress, antioxidants, cryopreservation, infertility

1. Effects of oxidative stress on spermatozoa

1.1 What is oxidative stress?

Oxidative stress occurs when a system has an imbalance between oxidation and reduction reactions, leading to generation of excess oxidants or molecules that accept an electron from another reactant [1]. A free radical is a molecule or element with an unpaired electron that is extremely reactive in an attempt to reach an electronically stable state. ROS are free radical derivatives of oxygen (O_2) containing molecules. Some of the clinically important ROS identified include peroxyl ($\cdot ROO^{-}$) and hydroxyl ($\cdot OH^{-}$) radicals, superoxide ($\cdot O_2^{-}$) anion, and H_2O_2. Nitrogen compounds such as nitric oxide (NO) and peroxynitrite anion (ONOO) also appear to play a role in oxidation and reduction reactions. Common molecules that receive the unpaired electron are lipids in membranes and carbohydrates in nucleic acids [2]. This leads to potential cellular membrane and DNA damage when ROS are greater than the antioxidant-carrying capacity (**Figure 1**).

1.2 Production of ROS

The process of mitochondrial oxidative phosphorylation uses nicotinamide adenine dinucleotide (NADH) as an electron donor and O_2 as an electron acceptor in the electron transport chain, coupling both reduction and oxidation reactions with the synthesis of adenosine triphosphate (ATP), and about 1–5% O_2 transformed into ROS [3]. Another intrinsic source of spermatic ROS production is cytoplasmic glucose-6-phosphate dehydrogenase (G-6-PDH). This cytoplasmic source of ROS

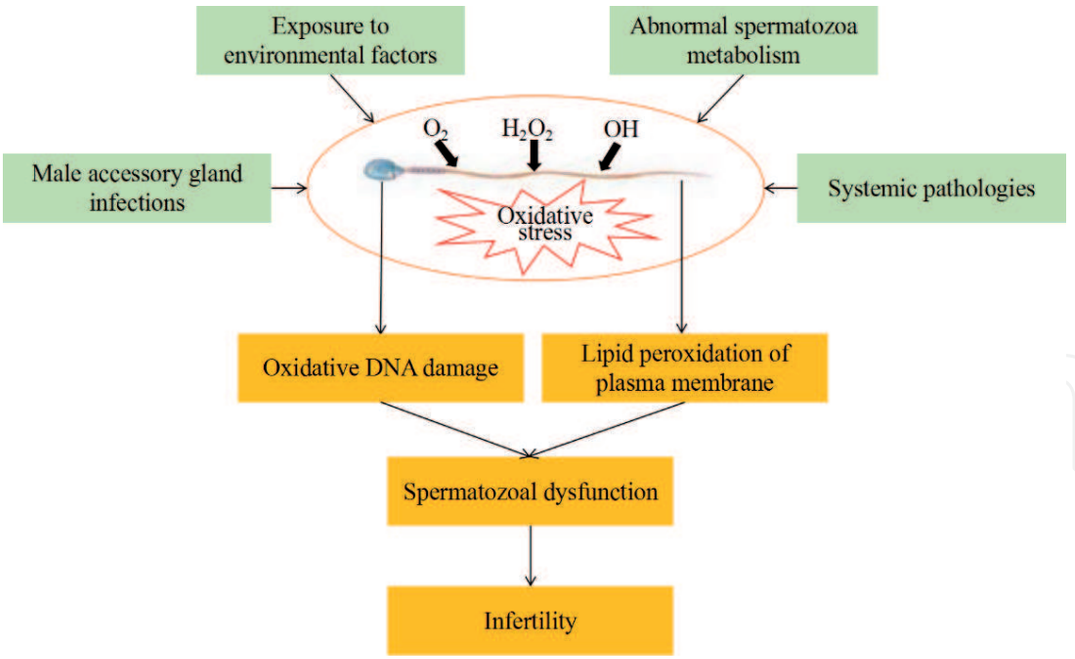

Figure 1.
Factors contributing to oxidative stress-induced male infertility.

may explain why increased spermatic cytoplasm could be linked to infertility [4]. In addition to leucocytes, infection in semen has also been implicated as a source of ROS. Exposure to heavy metals (e.g., cadmium, lead, iron and copper), pesticides, phthalate and pollution can lead to spermatozoa damage by excessive ROS [5]. Smoking has also been associated with decreased spermatic function. But industrial exposure not only induces oxidative stress but also disrupts the hypothalamic–pituitary-gonadal axis to inhibit the release of GnRH, LH and FSH in human and animal [6, 7].

1.3 Pathological effects on spermatozoa

Only the balance of ROS and antioxidants can keep the optimal spermatozoa function. Low level of ROS has been shown to be essential for fertilization, acrosome reaction, hyperactivation, motility and capacitation [8, 9]. ROS induces cyclic adenosine monophosphate (cAMP) in spermatozoa that inhibits tyrosine phosphatase, leading to tyrosine phosphorylation [10]. In particular, capacitation not only requires ROS, but also it can be inhibited by catalase (CAT) [11]. It has been described that high level of ROS can promote the acrosome reaction with the mechanism of ROS-modulated tyrosine phosphorylation [12].

1.3.1 Lipid peroxidation of plasma membrane

Lipids are present in spermatozoa plasma membrane in the form of polyunsaturated fatty acids (PUFA), most susceptible to oxidative damage [13, 14]. Once there is generation of lipid peroxide radical, it will react with the neighboring lipid molecule, triggering a chain reaction that can lead to >50% oxidation of the spermatozoa plasma membrane [15]. Byproducts of lipid oxidization include mutagenic and genotoxic molecules malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), leading indirectly to DNA damage [16]. Buffalo spermatozoa are more prone to oxidative damage than that of cattle, since it is rich in polyunsaturated fatty acids like arachidonic acids and docosahexaenoic acids [17].

1.3.2 DNA damage

Free radicals have the capability to directly damage spermatozoa DNA via single- and double-strand DNA breaks, cross-links and chromosomal rearrangements [18, 19]. ROS also can cause various types of gene mutations such as point mutations and polymorphism, resulting in decreased semen quality [20]. Other mechanisms such as denaturation and DNA base-pair oxidation also may be involved. Although most of the spermatozoa genome (85%) is bound to central nucleoprotamines that protect it from free radical attack [21], infertile men often have deficient protamination, which may make their sperm DNA more vulnerable to ROS damage [22]. A common byproduct of DNA oxidation, 8-hydroxy-2-deoxyguanosine (8-OH-2- deoxyguianosine), has been considered a key biomarker of this oxidative DNA damage [23].

1.3.3 Motility

Decreased motility has been shown to be due to ROS-induced peroxidation of lipids in the spermatozoa membrane decreasing flexibility and by inhibition of motility mechanisms [24, 25]. The axosome and associated dense fibers of the middle pieces in spermatozoa are covered by mitochondria that generate energy from intracellular stores of ATP. It is well established that ROS can induce axonemal and mitochondrial damage, resulting in the immobilization of spermatozoa [26, 27]. In addition, ROS-induced damage of mitochondrial DNA leads to decreased ATP and energy availability and leads to activation of caspases and ultimately apoptosis, impeding spermatozoa motility [28, 29]. H_2O_2 can diffuse across the membranes of spermatozoa and inhibit the activity of some vital enzymes such as glucose-6-phosphate dehydrogenase (G6PD), which is an enzyme controlling the intracellular availability of NADPH. This is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH oxidase [30]. Another hypothesis involves a series of interrelated events resulting in a ROS-reduced motility due to a decrease in axonemal protein phosphorylation and mitochondrial membrane damage and leakage of intracellular enzymes [31]. Meanwhile, cytochrome c release during the apoptotic pathway further increases levels of ROS, promoting DNA damage and fragmentation [32]. Especially after frozen–thawed cycles, spermatozoa with higher levels of oxidative stress have higher levels of caspase activation that can trigger apoptosis [33].

1.3.4 Apoptosis

High levels of ROS disrupt the mitochondrial membranes, inducing the release of the cytochrome c protein and Ca^{2+} and activating the caspase-inducing apoptosis [34]. Apoptosis in spermatozoa also may be initiated by ROS-independent pathways involving the cell surface protein Fas, which is a type I membrane protein that belongs to the tumor necrosis factor-nerve growth factor receptor family and mediates apoptosis [35]. Mitochondrial exposure to ROS also results in the release of apoptosis-inducing factor (AIF), which directly interacts with the DNA to cause DNA fragmentation in spermatozoa [36, 37].

1.3.5 Fertilization, pregnancy and miscarriage

Lipid peroxides and DNA damage are the most typical oxidative stress injury in sperm. Lipid peroxides are spontaneously generated in the sperm plasma

membrane, which induce decrease in fertility during storage of semen [38]. In addition, the importance of sperm DNA damage is brought to light when studies correlated the degree of DNA damage with various indices of fertility such as the fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate and live birth rate of the offspring. If sperm DNA is unable to decondense after entering the ooplasma, fertilization may not take place or a postfertilization failure may occur when sperm DNA is defective by ROS. Higher miscarriage rate is observed with ROS-induced sperm DNA damage [39]. High-level sperm DNA fragmentation induced was related to lower pregnancy rates in in vitro fertilization (IVF) but not in intracytoplasmic sperm injection (ICSI) cycles, whereas it was associated with higher miscarriage rates in both IVF and ICSI cycles. In addition, ROS actively participate in metabolic pathways during sperm activation, which leads to cholesterol efflux, cyclic adenosine monophosphate (cAMP) production and tyrosine phosphorylation, important events that contribute to fertilization competence [40]. However, it has been also described that appropriate ROS (hydrogen peroxide stimulation) can promote the acrosome reaction and sperm hyperactivation with the mechanism of ROS-modulated tyrosine phosphorylation [41], thereby assisting the sperm's transit through the cumulus and zona pellucida [42].

2. Production of ROS during cryopreservation

Cryopreservation of spermatozoa is an applicable technique, but it may influence the post-thaw qualities of spermatozoa, including morphology, motility, viability and DNA integrity. The imbalance between the presence of ROS and spermatozoa antioxidant activity is a main cause of cryodamage of spermatozoa [43]. The specific cell structure and plasma membrane of spermatozoa, a large number of mitochondria, low cytoplasm and incomplete antioxidant system in cytoplasm make them possibly vulnerable to damage from free radicals [43]. Susceptibility to cold temperatures is also linked to a high ratio of unsaturated to saturated fatty acid content of the spermatozoa plasma membrane. Bull, ram and boar spermatozoa are more sensitive to cooling than rabbits, dogs and human, due to a higher ratio of unsaturated to saturated fatty acids [44]. Antioxidants are the main defense factors against oxidative stress induced by free radicals [45]. Supplementation of cryopreservation extenders with antioxidants provides a cryoprotective effect on bull, ram, goat, boar, canine and human spermatozoa quality, thus minimizing the detrimental effect of ROS and improving quality of post-thaw spermatozoa (**Table 1**).

Vitamin E (α-tocopherol) is a highly potent chain-breaking lipophilic antioxidant residing on the cell membrane which can break the covalent links that ROS have formed between fatty acid side chains in membrane lipids [83]. Addition of α-tocopherol in rabbit, equine, bovine, boar and ram, aiming to improve semen quality, led to inconsistent results [46]. Combined with vitamin C, vitamin E enhanced motility and viability of cooled spermatozoa [47, 48]. Askari et al. (1994) showed that vitamin E improved hypo-osmotic swelling scores and the post-thaw motility slightly. Moreover, α-tocopherol supplementation at 200 uM concentration may protect the spermatozoa against stress oxidative by reducing lipid peroxidation and DNA fragmentation [67].

The GSH content and its antioxidant defensive capacity alter during the freezing–thawing process, possibly because of oxidative stress and cell death [84], so that addition of GSH to the freezing extender has variable outcomes. Varghese et al. reported that addition of 5 mM of GSH to human spermatozoa freezing media improved the DNA integrity, but failed in reducing the lipid peroxidation and in

Antioxidant	Effects	Species	References
Vitamin C	Improve semen quality	Rabbit, equine, bovine, boar and ram	[46]
	Enhance motility and viability	Ram and goat	[47, 48]
GSH	Improve DNA integrity	Human	[49]
	Improve progressively motile, protect plasma membrane integrity	Red deer	[50]
	Ameliorate acrosome ultrastructure	Ram	[51]
	Increase motility, viability and fertilization	Boar	[52]
Vitamin C	Improve motility, acrosome and membrane integrity	Bovine	[53]
	Reduce DNA damages	Infertile human	[54]
	Reduce DNA damage and lipid peroxidation	Boar	[55]
Ergothioneine	Protect DNA integrity	Bull	[56]
Melatonin	Improve spermatozoa characteristics	Goat, rat, boar, ram, mouse and human	[57–63]
	Improve spermatozoa function	Boar	[64]
	Enhance hyperactivation	Hamster	[65]
Selenium	Ameliorate motility, viability, membrane integrity and total antioxidant capacity	Bovine	[66]
Zinc	Improve hypo-osmotic swelling (HOS), reduce lipid peroxidation and DNA fragmentation	Mammalian	[67]
Amino acids	Membrane stabilizer and inhibit spermatozoa capacitation	Ram	[68]
	Enhances membrane integrity, viability and motility, reduce lipid peroxidation and DNA damage	Bull, ram, goat, boar and fish	[69–74]
	Reduce DNA fragmentation	Fish	[75]
Natural herbs	Improve motility and viability, reduce DNA damage	Human	[76, 77]
	Enhance motility and viability and minimize DNA damage	Human, rat	[78]
	Improve viability and motility and prevent peroxidatation	Boar, canine, bull and ovine	[79–82]

Table 1.
Proposed antioxidants in spermatozoa cryopreservation.

increasing the motility [49]. Recently, Gadea et al. showed that GSH supplementation to freezing media reduced human spermatozoa ROS levels and increased the level of sulphhydryl groups on membrane proteins in spite of increasing the percentage of motile and progressively motile spermatozoa after addition of GSH to the thawing media. Longer exposure to GSH and main damaging effect on spermatozoa membrane before the dilution in the thawing extender may elucidate this difference in viability [85]. It seems that boar spermatozoa benefited from the supplementation with this antioxidant at 1 and 5 mM [86]. GSH (at 1 mM) improved the quality of red deer post-thawing spermatozoa, especially regarding kinematic parameters and mitochondrial status [50]. In ram semen, Camara et al. (2011) found no

enhancement adding GSH (0.5–2 mM) to the freezing extender, but the concentration at 2 and 5 mM ameliorated the ultrastructure of the acrosome which resulted in obtaining even lower motility at 7 mM [51]. Also, adding 1–2 mM glutathione to the ram semen extender increased the activities of GPX and SOD, decreased free radicals and improved the survival rate of post-thawed spermatozoa. Addition of SOD or CAT to boar spermatozoa freezing extender not only increased spermatozoa motility and viability but also decreased post-thaw ROS generation which led to a rising in in vitro fertilizing potential of thawed spermatozoa [52]. These findings comply with results showing that the addition of CAT and SOD to the extender improved the survival and in vitro fertility of liquid stored ram spermatozoa [87].

The intake of vitamin C (ascorbic acid) could result in decreasing of GSH-Px in opposition to GSH increase and improved spermatozoa motility, acrosome and membrane integrity [53]. The addition of ascorbic acid before cryopreservation reduced DNA damages only in infertile men [54]. Because ascorbic acid is rapidly oxidized into inactive dehydroascorbate when exposed to highly oxidative environment [88], it is difficult to maintain its scavenging activities during exposure of spermatozoa to high oxidative environments for extended periods of time. Ascorbic acid 2-O-α-glucoside (AA-2G) is characterized by high resistance to thermal and oxidative degradation in neutral solutions and non-reducing conditions. Addition of AA-2G to the freezing extender improved the post-thaw quality of boar spermatozoa through the protection of spermatozoa against DNA damage and the lipid peroxidation caused by oxidative stress during cryopreservation [55].

Ergothioneine is an important low-molecular-weight thiol which scavenges singlet oxygen [89] and hydroxyl and peroxyl radicals [90]. It exists in millimolar concentrations in some tissues and has been linked to the metabolism of iron, copper and zinc. Increasing concentration of ergothioneine in semen extenders preserved DNA integrity of spermatozoa against cryodamage [56].

Melatonin (N-acetyl-5-methoxytryptamine, MT) is mainly synthesized and secreted by the pineal gland in reaction to changes in dark–light cycles [91]. It can stimulate the activity of antioxidant enzymes such as SOD and GSH-Px [92]. MT scavenges a variety of reactive oxygen and nitrogen species with powerful non-enzymatic antioxidant property [93]. MT can improve spermatozoa characteristics in goat [57], rat [58], boar [59], ram [60], mouse [61] and human [62, 63]. In addition, it had a dose-dependent effect on all parameters of spermatozoa motility. 1 uM MT did not succeed in improving the function of boar semen stored at 17°C [64], but 1 nM MT can enhance hyperactivation of hamster spermatozoa [65].

It has been indicated that dietary Se supplementation enhanced reproductive function in mice, sheep and cattle [94, 95] and also brought about the improvement in post-thaw spermatozoa quality [66]. Lack of Se has been related to reproductive problems and diminished spermatozoa quality in mice, pigs, sheep and cattle [96], but excessive Se intake also has been connected to an impaired spermatozoa quality [97]. In frozen–thawed buffalo spermatozoa, extenders containing 1 and 2 ug mL^{-1} Se significantly ameliorated spermatozoa motility, viability, membrane integrity and total antioxidant capacity. It also exerts its effects in a dose-dependent manner so that it had deleterious effects on spermatozoa parameters at high levels of 4 and 8 ug mL^{-1}.

Amino acids have an important biological role for prevention of cell damage during cryopreservation. L-cysteine (L-Cys) is a naturally occurring sulfur containing non-essential amino acid, which penetrates the spermatozoa membrane easily to participate in the intracellular GSH biosynthesis [98]. It protects the membrane lipids and proteins via indirect scavenging of free radicals; also it acts as a membrane stabilizer and inhibitor of spermatozoa capacitation [68]. Moreover, L-Cys is metabolized to taurine after passing into cells. Taurine transformed to acyl-taurine

after combination with a fatty acid in plasma membrane which improves surfactant properties and osmoregulation of the spermatozoa membrane [99, 100]. It has been reported that L-Cys enhances motility and morphology of spermatozoa, reducing lipid peroxidation of plasma membrane and preventing DNA damage from ROS of post-thaw bull [69], ram [70], goat [71] and fish [72, 73] spermatozoa, and improves the viability, the chromatin structure, and membrane integrity of boar spermatozoa during chilled storage [74]; in combination with docosahexaenoic acid (DHA)-enriched hen egg yolk, L-cysteine significantly improved progressive motility and acrosome integrity of boar spermatozoa. Also, the cysteine enhanced the post-thaw Merino ram spermatozoa mitochondrial activity without improving motility after the freezing–thawing cycle. 5 or 10 mM was the optimum concentration of L-cysteine for improving the quality of frozen–thawed boar spermatozoa. Methionine had a positive effect on the sperm viability and increased the post-thaw spermatozoa motility and reduced DNA damage of fish spermatozoa [101, 102]. DNA fragmentation in gilthead seabream (*S. aurata*) and European sea bass (*D. labrax*) was significantly reduced by taurine and hypotaurine [75]. The concentration of 50 mM taurine provided the most pronounced protective effect in improving post-thaw quality of red seabream sperm [103].

The addition of natural herbs also improves the cryoprotective effect of spermatozoa. Addition of genistein to the cryoprotectant has a significant antioxidant protective effect on the frozen–thawed spermatozoa. It causes a reduction in ROS production and makes an improvement in the sperm motility and viability; it also reduces DNA damage caused by the process of cryopreservation [76, 77]. The high concentrations of genistein decreased the proportion of motile mice spermatozoa which was approved in human spermatozoa, too [104]. In ram spermatozoa, addition of either resveratrol or quercetin (5–20 ug/mL for each compound) to a Tris-egg yolk-glycerol extender decreased the mitochondrial membrane potential [105]. Quercetin at 50 uM enhanced spermatozoa motility and viability and minimized post-thawed human spermatozoa DNA damage and also proved its potential role in protecting spermatozoa against H_2O_2-mediated spermatozoa damage on spermatozoa parameters and lipid peroxidation by reducing the levels of MDA and improving activities of antioxidant enzymes in rats [78]. The antioxidant properties of *Rhodiola sacra* aqueous extract (RSAE)-enriched freezing extender with or without glycerol had substantial impacts on concentrations of MAD and GSH, apart from the quality of frozen–thawed boar spermatozoa. Likewise, the optimal concentration of RSEA in extender ranged from 4 to 8 mg L^{-1} with and without glycerol, even if the influence of 6 mg L^{-1} RSEA on spermatozoa quality was more enhanced in glycerol-free extender than glycerol-containing extender [106]. The effects of adding rosemary to semen freezing extenders in several species have been reported, including boar [79], canine [80] and ovine [81]. Rosemary-enriched freezing extender efficiently improved motility and prevented peroxidatation of epididymal boar spermatozoa, showing a significant correlation between rosemary concentration and concentration of MDA [107]. Added 10 g L^{-1} rosemary extract to the freezing extender of bull semen before cryopreservation and showed its effects on increasing viability, motility and average path velocity as well as on decreasing lipid peroxidation after thawing [82].

3. Oxidative stress in male infertility

A decline in fertility rates is becoming an increasingly prevalent issue worldwide. Infertility affects up to 15% of the population globally [108], and furthermore, male infertility is responsible in about 20% of cases but may contribute to 40% of

infertile couples [109]. The leading cause of male infertility stems from a loss of spermatozoa function, ultimately resulting in a loss of fertilization potential [110]. This loss in function is causatively linked to oxidative stress within the spermatozoa driven by the presence and/or overproduction of intracellular ROS [111].

Several studies have shown conflicting results for the effect of the antioxidant therapy on male fertility, whilst a number of studies conveyed a favorable effect on basic semen parameters, advanced spermatozoa function tests and pregnancy rates. But, the ideal balance of the redox system necessary for optimal spermatozoa function is not known, and overconsumption of antioxidants may result in reductive stress that could cause detrimental effects on human health and well-being. Impairment of mitochondrial activity [112], reduction in blood–brain barrier permeability [113] and attenuation of endothelial cell proliferation [114] are consequences that have been reported to occur secondary to reductive stress. **Table 2** shows the mechanism of action of several commonly used antioxidants for the treatment of male infertility. The list of antioxidants used in treatment of male infertility is presented in **Table 3**.

Vitamin E is well accepted as the first line of defence against lipid peroxidation, protecting polyunsaturated fatty acids in cell membranes through its free radical quenching activity in biomembranes at an early state of free radical attack. MDA concentration was prevented by treatment with vitamin E; it may help in the prevention of against production of free radicals and quenches free hydroxyl radicals and superoxide anions, thereby reducing lipid peroxidation initiated by ROS at the level of plasma membranes [126]. Its antioxidant activity is similar to that of glutathione peroxidase. In infertility of male, the percentage of motile spermatozoa is significantly related to spermatozoa vitamin E content [127]. Lower levels of vitamin E were observed in the semen of infertile men [128]. Insufficient intake of vitamin E produced deleterious effects on the process of normal spermatozoa [129]. One of the earlier studies investigating vitamin E alone (300 mg daily) on infertile men reported significant improvement in spermatozoa motility [121]. Combined with clomiphene citrate treatments, vitamin E significantly improved spermatozoa concentration and motility of patients with idiopathic oligoasthenozoospermia (OAT) [130]. Another observational study investigated a daily regimen of vitamin E (400 mg) + selenium (200 lg), for a period of 100 days, on infertile men with idiopathic asthenoteratospermia. Results revealed that 52.6% of patients showed a significant improvement in spermatozoa motility, morphology or both [131]. On the other hand, a few other studies failed to reproduce any significant effect on semen

Antioxidants	Antioxidant mechanism	Typical daily dose
Vitamin E	Neutralizes free radicals	200–600 mg
Vitamin C	Neutralizes free radicals	200–1000 mg
Selenium	Enhancement of antioxidant enzyme activity	100–200 mg
Zinc	Inhibition of NADPH oxidase and scavenges hydroxyl radicals	15–40 mg
Carnitines	Neutralizes free radicals and acts as an energy source	1–3 g
CoQ10	Scavenges free radicals of mitochondrial electron transport system	60–300 mg
Lycopene	Scavenges free radicals	4–6 mg

Vitamin E, tocopherol; vitamin C, ascorbic acid; NADPH, nicotinamide adenine dinucleotide phosphate.

Table 2.
Mechanism of action of commonly used antioxidants and clinical dosage.

Clinical applications	Antioxidants daily	References
Oligozoospermia	Vitamin E (180 mg), vitamin A (30 mg) and essential fatty acids (600 mg)	[22]
	LC (2 g)	[115]
	CoQ10 (300 mg)	[116]
	Selenium (200 mg)	[117]
	Folic acid (5 mg) + zinc (66 mg)	[118]
	Lycopene (2 mg)	[119]
Asthenozoospermia	Zinc (400 mg), vitamin E (20 mg) and vitamin C (10 mg)	[120]
	CoQ10 (300 mg)	[116]
	Selenium (200 mg)	[117]
	Lycopene (2 mg)	[119]
Teratozoospermia	Selenium (200 mg)	[117]
	Zinc (400 mg), vitamin E (20 mg) and vitamin C (10 mg)	[120]
Improving DNA integrity	Vitamin E (1 g) + vitamin C (1 g)	[121]
	Vitamin C (400 mg), vitamin E (400 mg), b-carotene (18 mg), zinc (500 mmol) and selenium (1 mmol)	[29]
	LC (1500 mg), vitamin C (60 mg), CoQ10 (20 mg), vitamin E (10 mg), zinc (10 mg), folic acid (200 lg),	[32]
Improving ART	vitamin E (200 mg)	[33]
	Vitamin E (600 mg)	[122]
	Vitamin C (1 g) + vitamin E (1 g)	[123]
Improving live birth rate	CoQ10 (300 mg)	[116]
	Vitamin E (300 mg)	[124]
	Zinc (5000 mg)	[12]
	Vitamin E (1 g) + vitamin C (1 g)	[121]
	Carnitines: LC (2 g) + LAC (1 g)	[125]

Table 3.
Proposed antioxidants in various clinical treatments.

parameters using vitamin E as a single treatment [123, 125] or in combination with other antioxidants [132].

In the male reproductive system, vitamin C (ascorbic acid) is known to protect spermatogenesis and plays a key role in spermatozoa integrity and fertility both in men by increasing testosterone levels and preventing spermatozoa agglutination. It exists at a concentration 10 times higher in seminal plasma than in blood serum [133] and contributes up to 65% of the total antioxidant capacity of seminal plasma found intracellularly and extracellularly [134, 135]. Semen of infertile men with asthenozoospermia was found to contain lower vitamin C levels and higher ROS levels than those obtained from fertile controls [117]. Vitamin C as a single agent which is used to treat heavy smokers, with a daily dose of 200 or 1000 mg or placebo for 1 month, significantly improved spermatozoa quality [136]. Receiving 500 mg daily vitamin C with a combination of zinc, vitamin E and vitamin C for a total of 3 months after undergoing varicocelectomy significantly improved spermatozoa motility and morphology on varicocelectomy patients [118, 137].

Carnitines [L-carnitine (LC) and L-acetyl carnitine (LAC)] are water-soluble antioxidants involved in spermatozoa metabolism, fuelling important activities like spermatozoa motility [138]. The carnitine and acetylcarnitine can significantly improve spermatozoa motility or kinetics in patients with asthenozoospermia [120, 139]. In vitro studies of spermatozoa cultured in media containing carnitines had higher motility and viability. They exhibit their antioxidant activities through scavenging superoxide anions and hydrogen peroxide radicals, thereby inhibiting lipid peroxidation. A combined treatment of LC (2 g) and LAC (1 g) for 2 months' duration to placebo in men with OAT showed significant improvement in all semen parameters; however, the most significant increase was in spermatozoa motility. Low-grade varicocele and idiopathic infertility patients treated with LC and LAC in comparison with placebo had significant improvement in all semen parameters [140]. On the contrary, LC (1000 mg) and LAC (500 mg) daily treated asthenozoospermic men for 12 weeks and failed to show any significant improvement in spermatozoa motility [141].

CoQ10 is a vital antioxidant omnipresent in almost all body tissues. It is particularly present at high concentrations in spermatozoa mitochondria involved in cellular respiration and plays an integral role in energy production [142]. This contribution rationalizes its use as a promotility and antioxidant molecule. Furthermore, CoQ10 inhibits superoxide formation, delivering protection against OS-induced spermatozoa dysfunction. A significant negative correlation between CoQ10 levels and hydrogen peroxide has been reported, and a linear correlation between CoQ10 levels in seminal plasma spermatozoa count and motility was detected [115]. 300 mg CoQ10 for 26 weeks obtained a significant increase in sperm density and motility [143]. A systemic review of clinical trials on 332 infertile men revealed that treatment with CoQ10 (200–300 mg daily) resulted in a significant increase in spermatozoa concentration and motility [144].

Antioxidant properties of selenium are thought to stem from its ability to augment the function of glutathione. More than 25 selenoproteins exist, such as phospholipid hydroperoxide glutathione peroxidase (PHGPX) [145] and spermatozoa capsular selenoprotein glutathione peroxidase [146], to maintain spermatozoa structural integrity [147]. Selenium deficiency has been most commonly associated with morphological spermatozoa midpiece abnormalities and impairment of spermatozoa motility [148]. A significant increase (74%) in total normal spermatozoa concentration was noted amongst the subfertile group receiving combined therapy [116] with a combination of both folic acid and zinc for 26 weeks of treatment. Selenium has been less frequently investigated for the treatment of subfertile men. As previously noted, with selenium (200 mg) supplements for 26 weeks, results showed a significant improvement in all semen parameters. A strong correlation was seen between the sum of the selenium and mean spermatozoa concentration, motility and percentage normal morphology [149]. Furthermore, the combination of selenium with vitamin E resulted in an increase in spermatozoa motility [124, 150]. But in the contrary report, treatment with selenium (300 mg) daily for 48 weeks did not result in a significant influence on semen parameters of a group of normozoospermic men [122].

Zinc plays a vital role in the metabolism of RNA and DNA, signal transduction, gene expression and regulation of apoptosis. Its antioxidant properties are thought to result from its ability to decrease production of hydrogen peroxide and hydroxyl radicals through antagonizing redox-active transition metals, such as iron and copper [151]. Zinc concentrations of seminal plasma were found to be significantly lower in subfertile men [152]. Spermatozoa flagellar abnormalities, such as hypertrophy and hyperplasia of the fibrous sheath, axonemal disruption, defects of the inner microtubular dynein arms and abnormal or absent midpiece, are all associated with zinc deficiency [153]. Zinc given for 3 months in men with asthenozoospermia obtained a significant improvement in spermatozoa concentration, progressive motility and fertilizing capacity and a reduction in the incidence of anti-spermatozoa antibodies [153]. Oral zinc supplementation

successfully restored seminal catalase-like activity and improved spermatozoa concentration and progressive motility in a group of asthenozoospermic men [154].

Lycopene is a naturally synthesized carotenoid presented in fruits and vegetables. Its powerful ROS quenching abilities make it a major contributor to the human redox defense system [155]. Lycopene is detected at high concentrations in human testes and seminal plasma with levels that tend to be lower in infertile men [156]. The treatment with 2 mg lycopene twice daily for 3 months significantly improves spermatozoa concentration and motility in 66% of patients, respectively. However, the effects were only significant in patients who had baseline spermatozoa concentrations of $>5 \times 10^6$ sperm/mL [119].

4. Conclusion

Spermatozoa possess an inherent but limited capacity to generate ROS which may help the fertilization process. Antioxidants improve the motility and fertilizing ability of spermatozoa. A balance between the benefits and risks from ROS and antioxidants appears to be necessary for the survival and normal functioning of spermatozoa. Antioxidants in extenders may minimize the detrimental effect of ROS and improve the quality of frozen–thawed spermatozoa in animals and human. From the other point of view, the divergent effect of each antioxidant supplementation, improving different parameters of frozen–thawed sperm quality, is attributed to animal species, extender medium and type of molecule and concentration used for each species. Although a beneficial influence was generally observed for antioxidants in reversing ROS-induced spermatozoa dysfunction and in improving pregnancy rates, evaluation of ROS and the use of antioxidants are not routine in clinical practice. The dose and duration of these antioxidants should also be determined and standardized. There should be an effort to develop optimum combinations of antioxidants to supplement spermatozoa media. Finally, this study suggests that further research should be done to determine the appropriate antioxidant compounds as well as certain dose of antioxidants whether used clinical practices or cryopreservation. Moreover the future studies should concern the spermatozoa fertilization and pregnancy rate as a research emphasis.

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References

[1] Henkel RR. Leukocytes and oxidative stress: Dilemma for sperm function and male fertility. Asian Journal of Andrology. 2011;**13**:43-52

[2] Tremellen K. Oxidative stress and male infertility a clinical perspective. Human Reproduction Update. 2008;**14**:243-258

[3] Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. The Biochemical Journal. 1973;**134**:707-716

[4] Gomez E, Buckingham DW, Brindle J, Lanzafame F, Irvine DS, Aitken RJ. Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: Correlation with biochemical markers of the cytoplasmic space, oxidative stress, and sperm function. Journal of Andrology. 1996;**17**:276-287

[5] Gharagozloo P. Treating Male Infertility Secondary to Sperm Oxidative Stress. US Patent Application. 2015;**15**:939-953

[6] Queiroz EK, Waissmann W. Occupational exposure and effects on the male reproductive system. Cadernos de Saúde Pública. 2006;**22**:485-493

[7] Ni K, Steger K, Yang H, Wang H, Hu K, Zhang T. A comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic, and astheno/oligozoospermic clinical varicocele. Andrology. 2016;**4**:816-824

[8] Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: An overview of the literature. Reprod. Biomed. 2004;**8**:616-627

[9] Gagnon C, Iwasaki A, De Lamirande E, Kovalski N. Reactive oxygen species and human spermatozoa. Annals of the New York Academy of Sciences. 1991;**637**:436-444

[10] Tremellen K, Miari G, Froiland D, Thompson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. The Australian and New Zealand Journal of Obstetrics and Gynaecology. 2007;**47**:216-221

[11] Omu AE, Dashti H, Al-Othman S. Treatment of asthenozoospermia with zinc sulphate: Andrological, immunological and obstetric outcome. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 1998;**79**:179-184

[12] Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnoxicam and L -carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. Journal of Andrology. 2004;**25**:761-772

[13] Kodama H, Kuribayashi Y, Gagnon C. Effect of sperm lipid peroxidation on fertilization. Journal of Andrology. 1996;**17**:151-157

[14] Griveau JF, Dumont E, Renard P, Callegari JP, Lannou DL. Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. Journal of Reproduction and Fertility. 1995;**103**:17-26

[15] Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: A randomized, double-blind, placebo-controlled trial. Fertility and Sterility. 2006;**85**:1409-1414

[16] Huang XX, Zhang AH, Hong F. Expression of MDA and 4-HNE AFB 1-induced rat hepatic putative preneoplastic lesions alleviated by Li_2CO_3 and their significance. Carcinogenesis, Teratogenesis and Mutagenesis. 2005;**3**:222-228

[17] Singh P, Chand D, Georgie GC. Effect of vitamin E on lipid peroxidation in buffalo *Bubalus bubalis*. Indian Journal of Experimental Biology. 1989;**27**:14-16

[18] Kemal DN, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertility and Sterility. 2000;**74**:1200-1207

[19] Aitken RJ, Krausz C. Oxidative stress, DNA damage and the Y chromosome. Reproducción. 2001;**122**:497-506

[20] Spiropoulos J, Turnbull DM, Chinnery PF. Can mitochondrial DNA mutations cause sperm dysfunction? Molecular Human Reproduction. 2002;**8**:719-721

[21] Ammar O, Haouas Z, Hamoud B, Hamdi H, Hellara I, Jlali A, et al. Relationship between sperm DNA damage with sperm parameters, oxidative markers in teratozoospermic men. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2019;**233**:70-75

[22] Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility—A preliminary report. International Urology and Nephrology. 2002;**34**:369-372

[23] Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC. DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:93-95

[24] Lenzi A, Lombardo F, Gandini L, Alfano P, Dondero F. Computer assisted sperm motility analysis at the moment of induced pregnancy during gonadotropin treatment for hypogonadotropic hypogonadism. Journal of Endocrinological Investigation. 1993;**16**:683-686

[25] Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radical Biology and Medicine. 1999;**26**:869-880

[26] Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. Journal of Reproduction and Fertility. 1987;**81**:459-469

[27] Peris IS, Bilodeau JF, Dufour M, Bailey J. Impact of cryopreservation and reactive oxygen species on DNA integrity, lipid peroxidation, and functional parameters in ram semen. Molecular Reproduction and Development. 2007;**74**:878-892

[28] Menezo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P. Antioxidants to reduce sperm DNA fragmentation: An unexpected adverse effect. Reproductive Biomedicine Online. 2007;**14**:418-421

[29] Gual-Frau J, Abad C, Amengual MJ, Hannaoui N, Checa MA, Ribas-Maynou J. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. Human Fertility (Cambridge, England). 2015;**18**:225-229

[30] Aitken RJ. Molecular mechanisms regulating human sperm function. Molecular Human Reproduction. 1997;**3**:169-173

[31] de Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. Journal of Andrology. 1992;**13**:379-386

[32] Geva E, Bartoov B, Zabludovsky N, Lessing JB, Lerner-Geva L, Amit A. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertility and Sterility. 1996;**66**:430-434

[33] Safarinejad MR. The effect of coenzyme Q(10) supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: An open-label prospective study. International Urology and Nephrology. 2012;**44**:689-700

[34] Lee E, Ahn MY, Kim HJ, Kim IY, Han SY, Kang TS, et al. Effect of di(n-butyl) phthalate on testicular oxidative damage and antioxidant enzymes in hyperthyroid rats. Environmental Toxicology. 2007;**22**:245-255

[35] Krammer PH, Behrmann I, Daniel P, Dhein J, Debatin KM. Regulation of apoptosis in the immune system. Current Opinion in Immunology. 1994;**6**:279-289

[36] Paasch U, Sharma RK, Gupta AK, Grunewald S, Mascha EJ, Thomas JAJ. Cryopreservation and thawing is associated with varying extent of activation of apoptotic machinery in subsets of ejaculated human spermatozoa. Biology of Reproduction. 2004;**71**:1828-1837

[37] Cande C, Cecconi F, Dessen P, Kroemer G. Apoptosis-inducing factor (AIF): Key to the conserved caspase-independent pathways of cell death? The Journal of Cell Science. 2002;**115**:4727-4734

[38] Twigg J, Fulton N, Gomez E, Irvine DS, Aitken RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: Lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Human Reproduction. 1998;**13**:1429-1436

[39] Carrell D, Liu L, Peterson C, Jones K, Hatasaka H, Erickson L. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Archives of Andrology. 2003;**49**:49-55

[40] Aitken RJ, Nixon B. Sperm capacitation: A distant landscape glimpsed but unexplored. Molecular Human Reproduction. 2013;**19**:785-793

[41] Zhao J, Zhang Q, Wang YG, Li YP. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: A systematic review and meta-analysis. Fertility and Sterility. 2014;**102**:998-1005

[42] de Lamirande E, Gagnon C. A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa. International Journal of Andrology. 1993;**16**:21-25

[43] Slaweta R, Wasowicz W, Laskowska T. Selenium content, glutathione peroxidase activity, and lipid peroxide level in fresh bull semen and its relationship to motility of spermatozoa after freezing-thawing. Zentralblatt für Veterinärmedizin. Reihe A. 1988;**35**:455-460

[44] Noiles EE, Bailey J, Storey BT. Temperature dependence of the water permeability, Lp, of murine sperm shows a discontinuity between 4 and 0°C. Cryobiology. 1995;**32**:220-238

[45] Upreti GC, Jensen K, Munday R, Duganzich DM, Vishwanath R,

Smith JF. Studies on aromatic amino acid oxidase activity in ram spermatozoa: Role of pyruvate as an antioxidant. Animal Reproduction Science. 1998;**51**:275-287

[46] Hatamoto LK, Sobrinho CB, Nichi M, Barnabe V, Barnabe R, Cortada C. Effects of dexamethasone treatment (to mimic stress) and Vitamin E oral supplementation on the spermiogram and on seminal plasma spontaneous lipid peroxidation and antioxidant enzyme activities in dogs. Theriogenology. 2006;**66**:1610-1614

[47] Benhenia K, Rahab H, Smadi MA, TakfarinasIdres HB, Ouada MI. Beneficial and harmful effects of cyclodextrin-vitamin E complex on cryopreserved ram sperm. Animal Reproduction Science. 2018;**195**:266-273

[48] Amidi F, Farshad A, Khor AK. Effects of cholesterol-loaded cyclodextrin during freezing step of cryopreservation with TCGY extender containing bovine serum albumin on quality of goat spermatozoa. Cryobiology. 2010;**61**:94-99

[49] Varghese A, Das S, Bhattacharya A, Bhattacharya S, Mandal M, Agarwal A. Effect of cryoprotective additives-reduced glutathione, acetyl-L-carnitine on sperm membrane lipid peroxidation, DNA integrity and recovery of motile human sperm. Fertility and Sterility. 2005;**84**:410-411

[50] Luis AL, Manuel AR, Olga GA, Mercedes A, Maroto-Morales A, Anel L, et al. Reduced glutathione and Trolox (vitamin E) as extender supplements in cryopreservation of red deer epididymal spermatozoa. Animal Reproduction Science. 2012;**135**:37-46

[51] Silva S, Soares A, Batista A, Almeida F, Nunes J, Peixoto C, et al. In vitro and in vivo evaluation of ram sperm frozen in tris egg-yolk and supplemented with superoxide dismutase and reduced glutathione. Reproduction in Domestic Animals. 2011;**46**:874-881

[52] Roca J, Carvajal G, Lucas X, Vazquez JM, Martinez EA. Fertility of weaned sows after deep intrauterine insemination with a reduced number of frozen-thawed spermatozoa. Theriogenology. 2003;**60**:77-87

[53] Hu JH, Tian WQ, Zhao XL, Zan LS, Wang H, Li QW, et al. The cryoprotective effects of ascorbic acid supplementation on bovine semen quality. Animal Reproduction Science. 2010;**121**:72-77

[54] Branco CS, Garcez ME, Pasqualotto FF, Erdtman B, Salvador M. Resveratrol and ascorbic acid prevent DNA damage induced by cryopreservation in human semen. Cryobiology. 2010;**60**:235-237

[55] Yoshimoto T, Nakamura S, Yamauchi S, Muto N, Nakada T, Ashizawa K, et al. Improvement of the postthaw qualities of Okinawan native pig spermatozoa frozen in an extender supplemented with ascorbic acid 2-O-α-glucoside. Cryobiology. 2008;**57**:30-36

[56] Motohashi N, Mori I, Sugiura Y. Complexing of copperion by ergothioneine. Chemical and Pharmaceutical Bulletin. 1976;**24**:2364

[57] Ramadan T, Taha T, Samak M, Hassan A. Effectiveness of exposure to longday followed by melatonin treatment on semen characteristics of Damascus male goats during breeding and non-breeding seasons. Theriogenology. 2009;**71**:458-468

[58] Sonmez M, Yuce A, Turk G. The protective effects of melatonin and vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated

male rats. Reproductive Toxicology. 2007;**23**:226-231

[59] Lane RL, Whitaker BD. Melatonin and tannic acid supplementation in vitro improve fertilization and embryonic development in pigs. Animal Reproduction. 2018;**15**:118-123

[60] Ashrafi I, Kohram H, Naijian H, Bahreini M, Poorhamdollah M. Protective effect of melatonin on sperm motility parameters on liquid storage of ram semen at 5°C. African Journal of Biotechnology. 2011;**10**:66-70

[61] Sarabia L, Maurer I, Bustos-Obregon E. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on mouse sperm DNA. Ecotoxicology and Environmental Safety. 2009;**72**:663-668

[62] Espino J, Bejarano I, Ortiz A, Lozano GM, Garca JF, Pariente JA, et al. Melatonin as a potential tool against oxidative damage and apoptosis in ejaculated human spermatozoa. Fertility and Sterility. 2010;**94**:1915-1917

[63] Ortiz A, Espino J, Bejarano I, Lozano GM, Monllor F, Garcia JF, et al. Highendogenousmelatoninconcentrations enhance sperm quality and short-term in vitro exposure to melatonin improves aspects of sperm motility. Journal of Pineal Research. 2011;**50**:132-139

[64] Martin-Hidalgo D, Baron F, Bragado M, Carmona P, Robina A, Garcia-Marin L, et al. The effect of melatonin on the quality of extended boar semen after long-term storage at 17°C. Theriogenology. 2011;**75**:1550-1560

[65] Fujinoki M. Melatonin-enhanced hyperactivation of hamster sperm. Reproduction. 2008;**136**:533-541

[66] Abdel-Halim BR, Helmy NA. Effect of nano-selenium and nano-zinc particles during in vitro maturation on the developmental competence of bovine oocytes. Animal Production Science. 2018;**58**:2021-2028

[67] Karl K, Michal Z, Erma Z, Drobnis MS, Sutovsky P. Zinc ion flux during mammalian sperm capacitation. Nature Communications. 2018;**9**:206

[68] Coyan K, Baspınar N, Bucak MN, Akalin PP. Effects of cysteine and ergothioneine on post-thawed merino ram sperm and biochemical parameters. Cryobiology. 2011;**63**:1-6

[69] Bilodeau JF, Blanchette S, Gagnon C, Sirard MA. Thiols prevent H_2O_2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology. 2001;**56**:275-286

[70] Andreea A, Stela Z. Role of antioxidant additives in the protection of the cryopreserved semen against free radicals. Romanian Biotechnology Letters. 2010;**15**:33-41

[71] Bucak MN, Uysal O. The role of antioxidants in freezing of Saanen goat semen. The Indian Veterinary Journal. 2008;**85**:148-150

[72] Szczesniak-Fabianczyk B, Bochenek M, Smorag Z, Ryszka F. Effect of antioxidants added to boar semen extender on the semen survival time and sperm chromatin structure. Reproductive Biology. 2003;**3**:81-87

[73] Stejskal K, Svobodova Z, Fabrik I, Adam V, Beklova M, Rodina M, et al. Content of cysteine, reduced and oxidized glutathione in spermatozoa of representatives of Acipenseriformes (*Acipenser baerii* and *A. ruthenus*) as well as teleosts (*Perca fluviatilis* and *Sander lucioperca*). Journal of Applied Ichthyology. 2008;**24**:519-521

[74] Ogretmen F, Inanan BE, Kutluyer F, Kaym M. Effect of semen extender supplementation with cysteine on post-thaw sperm quality, DNA damage,

and fertilizing ability in the common carp (*Cyprinus carpio*). Theriogenology. 2015;**83**:1548-1552

[75] Cabrita E, Ma S, Diogo P, Martínez-Paramo S, Sarasquete C, Dinis MT. The influence of certain amino acids and vitamins on post-thaw fish sperm motility, viability and DNA fragmentation. Animal Reproduction Science. 2011;**125**:189-195

[76] Thomson L, Fleming S, Aitken R, De Iuliis G, Zieschang JA, Clark A. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Human Reproduction. 2009;**24**:2061-2070

[77] Sierens J, Hartley J, Campbell M, Leathem A, Woodside J. In vitro isoflavone supplementation reduces hydrogen peroxide-induced DNA damage in sperm. Teratogenesis, Carcinogenesis, and Mutagenesis. 2002;**22**:227-234

[78] Abdallah FB, Zribi N, Ammar-Keskes L. Antioxidative potential of Quercetin against hydrogen peroxide induced oxidative stress in spermatozoa in vitro. Andrologia. 2011;**43**:261-265

[79] Malo C, Gil L, Gonzalez N, MartInez F, Cano R, De Blas I, et al. Anti-oxidant supplementation improves boar sperm characteristics and fertility after cryopreservation: Comparison between cysteine and rosemary (*Rosmarinus officinalis*). Cryobiology. 2010;**61**:142-147

[80] Gonzalez N, Gil L, Martinez F, Malo C, Cano R, Mur P, et al. Effect of natural antioxidant rosemary in canine soya freezing extender. Reproduction in Domestic Animals. 2010;**45**:88

[81] Gil L, Mascaro F, Mur P, Gale I, Silva A, Gonzalez N. Freezing ram semen: The effect of combination of soya and rosemary essences as a freezing extender on post-thaw sperm motility. Reproduction in Domestic Animals. 2010;**45**:91

[82] Daghigh-Kia H, Olfati-Karaji R, Hoseinkhani A, Ashrafi I. Effect of rosemary (*Rosmarinus officinalis*) extracts and glutathione antioxidants on bull semen quality after cryopreservation. Spanish Journal of Agricultural Research. 2014;**12**:98-105

[83] Jeong YJ, Kim MK, Song HJ, Kang EJ, Ock SA, Kumar BM, et al. Effect of α-tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes. Cryobiology. 2009;**58**:181-189

[84] Bilodeau JF, Chatterjee S, Sirard MA, Gagnon C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Molecular Reproduction and Development. 2000;**55**:282-288

[85] Gadea J, Molla M, Selles E, Marco M, Garcia-Vazquez F, Gardon J. Reduced glutathione content in human sperm is decreased after cryopreservation: Effect of the addition of reduced glutathione to the freezing and thawing extenders. Cryobiology. 2011;**62**:40-46

[86] Gadea J, Garcıa-Vazquez F, Matas C, Gardon JC, Canovas S, Gumbao D. Cooling and freezing of boar spermatozoa: Supplementation of the freezing media with reduced glutathione preserves sperm function. Journal of Andrology. 2005;**26**:396-404

[87] Maxwell W, Stojanov T. Liquid storage of ram semen in the absence or presence of some antioxidants. Reproduction, Fertility, and Development. 1996;**8**:1013-1020

[88] Linster CL, Van Schaftingen E, Vitamin C. Biosynthesis, recycling and

degradation in mammals. The FEBS Journal. 2007;**274**:1-22

[89] Dahl TA, Midden WR, Hartman PE. Some prevalent biomolecules as defenses against singlet oxygen damage. Photochemistry and Photobiology. 1988;**47**:357-362

[90] Asmus KD, Benasson RV, Bernier JL, Houssin R. One electron oxidation of ergothioneine and analogues investigated by pulse radiolysis: Redox reaction involving ergothioneine and vitamin C. The Biochemical Journal. 1996;**315**:625-629

[91] Panke ES, Rollag MD, Reitter RJ. Pineal melatonin concentrations in the Syrian hamster. Endocrinology. 1979;**104**:194-197

[92] Jang H, Kim YH, Kim BW. Ameliorative effects of melatonin against hydrogen peroxide-induced oxidative stress on boar sperm characteristics and subsequent in vitro embryo development. Reproduction in Domestic Animals. 2010;**45**:943-950

[93] Raygan F, Ostadmohammadi V, Bahmani F, Reiter RJ, Asemi Z. Melatonin administration lowers biomarkers of oxidative stress and cardio-metabolic risk in type 2 diabetic patients with coronary heart disease: A randomized, double-blind, placebo-controlled trial. Clinical Nutrition. 2019;**38**:191-196

[94] Chiachun T, Hong C, Haifun R. The effects of selenium on gestation, fertility, and offspring in mice. Biological Trace Element Research. 1991;**30**:227-231

[95] Sanders D. Use of selenium in problem cattle herds. Modern Veterinary Practice. 1984;**65**:136-138

[96] Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: An updated review of literature. Arab Journal of Urology. 2018;**16**:35-43

[97] Jamali NU, Kaka A, Khatri P, Malhi M, Naeem M, Memon AA, et al. Effect of in vitro selenium addition to the semen extender on the spermatozoa characteristics before and after freezing in Kundhi Buffalo Bull and in vivo fertility rate. Pakistan Journal of Zoology. 2019;**51**:317-323

[98] Uysal O, Bucak M. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. Acta Veterinaria. 2007;**76**:383-390

[99] Amidi F, Pazhohan A, Nashtaei MS, Khodarahmian M, Nekoonam S. The role of antioxidants in sperm freezing: A review. Cell and Tissue Banking. 2016;**17**:745-756

[100] Esteves SC, Sharma RK, Thomas AJ, Agarwal A. Evaluation of acrosomal status and sperm viability in fresh and cryopreserved specimens by the use of fluorescent peanut agglutinin lectin in conjunction with hypo-osmotic swelling test. International Brazilian Journal of Urology. 2007;**33**:364-376

[101] Lahnsteiner F. The role of free amino acids in semen of rainbow trout *Oncorhynchus mykiss* and carp *Cyprinus carpio*. Journal of Fish Biology. 2009;**75**:816-833

[102] Kutluyer F, Ogretmen F, Inanan BE. Effects of semen extender supplemented with L-methionine and packaging methods (straws and pellets) on post-thaw goldfish (*Carassius auratus*) sperm quality and DNA damage. CryoLetters. 2015;**36**:336-343

[103] Liu Q, Wang X, Wang W, Zhang X, Xu S, Ma D. Effect of the addition of six antioxidants on sperm motility, membrane integrity and mitochondrial function in red seabream (*Pagrus major*) sperm cryopreservation.

Fish Physiology and Biochemistry. 2014;**41**:413-422

[104] Bajpai M, Doncel G. Involvement of tyrosine kinase and cAMP-dependent kinase cross-talk in the regulation of human sperm motility. Reproduction. 2003;**126**:183-195

[105] Silva E, Cajueiro J, Silva S, Soares P, Guerra M. Effect of antioxidants resveratrol and quercetin on in vitro evaluation of frozen ram sperm. Theriogenology. 2012;**77**:1722-1726

[106] Zhao HW, Li QW, Ning GZ, Han ZS, Jiang ZL, Duan YF. Rhodiola sacra aqueous extract (RSAE) improves biochemical and sperm characteristics in cryopreserved boar semen. Theriogenology. 2009;**71**:849-857

[107] Malo C, Gil L, Cano R, MartInez F, Gale I. Antioxidant effect of rosemary (*Rosmarinus officinalis*) on boar epididymal spermatozoa during cryopreservation. Theriogenology. 2011;**75**:1735-1741

[108] Trussell J. Optimal diagnosis and medical treatment of male infertility. Seminars in Reproductive Medicine. 2013;**31**:235-236

[109] Jarow JP, Sharlip ID, Belker AM, Lipshultz LI, Sigman M, Thomas AJ. Best practice policies for male infertility. The Journal of Urology. 2002;**167**:2138-2144

[110] Liu D, Baker H. Defective spermzona pellucida interaction: A major cause of failure of fertilization in clinical in-vitro fertilization. Human Reproduction. 2000;**15**:702-708

[111] Guthrie H, Welch G. Effects of reactive oxygen species on sperm function. Theriogenology. 2012;**78**:1700-1708

[112] Singh F, Charles AL, Schlagowski AI, Bouitbir J, Bonifacio A, Piquard F. Reductive stress impairs myoblasts mitochondrial function and triggers mitochondrial hormesis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2015;**1853**:1574-1585

[113] Mentor S, Fisher D. Aggressive antioxidant reductive stress impairs brain endothelial cell angiogenesis and blood brain barrier function. Current Neurovascular Research. 2017;**14**:71-81

[114] Lamosova D, Jurani M, Greksak M, Nakano M, Vanekova M. Effect of rooibos tea (*Aspalathus linearis*) on chick skeletal muscle cell growth in culture. Comparative Biochemistry and Physiology. 1997;**116**:39-45

[115] Safarinejad MR. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. The Journal of Urology. 2009;**182**:237-248

[116] Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: A double-blind, placebo controlled, randomized study. The Journal of Urology. 2009;**181**:741-751

[117] Wong WY, Merkus HM, Thomas CM, Menkveld R, Zielhuis GA, Steegers-Theunissen RP. Effects of folic acid and zinc sulfate on male factor subfertility: A double-blind, randomized, placebo-controlled trial. Fertility and Sterility. 2002;**77**:491-498

[118] Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Medical Principles and Practice. 2008;**17**:108-116

[119] Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility a preliminary report. International

Urology and Nephrology. 2002; **34**:369-372

[120] Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, et al. A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetylcarnitine treatment in men with asthenozoospermia. Fertility and Sterility. 2004;**81**:1578-1584

[121] Suleiman SA, Ali ME, Zaki ZM, El-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: Protective role of vitamin E. Journal of Andrology. 1996;**17**:530-537

[122] Hawkes WC, Alkan Z, Wong K. Selenium supplementation does not affect testicular selenium status or semen quality in north American men. Journal of Andrology. 2009;**30**:525-533

[123] Moilanen J, Hovatta O. Excretion of alpha-tocopherol into human seminal plasma after oral administration. Andrologia. 1995;**27**:133-136

[124] Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Archives of Andrology. 2003;**49**:83-94

[125] Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell ID, Cooke ID. A double-blind randomized placebo crossover controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertility and Sterility. 1995;**64**:825-831

[126] Matough FA, Budin SB, Hamid ZA, Louis SR, Alwahaibi N, Mohamed J. Palm vitamin E reduces oxidative stress, and physical and morphological alterations of erythrocyte membranes in streptozotocin-induced diabetic rats. Oxidants and Antioxidants in Medical Science. 2012;**1**:59-68

[127] Therond P, Auger J, Legrand A, Jouannet P. Alpha-tocopherol in human spermatozoa and seminal plasma: Relationships with motility, antioxidant enzymes and leukocytes. Molecular Human Reproduction. 1996;**62**:739-744

[128] Omu AE, Fatinikun T, Mannazhath N, Abraham S. Significance of simultaneous determination of serum and seminal plasma alpha-tocopherol and retinol in infertile men by high-performance liquid chromatography. Andrologia. 1999;**31**:347-354

[129] Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Molecular Aspects of Medicine. 1997;**18**:S213-S219

[130] ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad S, Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenozoospermia: A prospective, randomized trial. Andrology. 2015;**3**:864-867

[131] Moslemi MK, Tavanbakhsh S. Selenium-vitamin E supplementation in infertile men: Effects on semen parameters and pregnancy rate, Int. The Journal of Gene Medicine. 2011;**4**:99-104

[132] Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: A randomized, placebo-controlled, double-blind study. Human Reproduction. 1999;**14**:1028-1033

[133] Jacob RA, Pianalto FS, Agee RE. Cellular ascorbate depletion in healthy men. The Journal of Nutrition. 1992;**122**:1111-1118

[134] Shrilatha B. Muralidhara, early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Reproductive Toxicology. 2007;**23**:578-587

[135] Naziroglu M. Enhanced testicular antioxidant capacity in streptozotocin-induced diabetic rats. Protective role of vitamins C and E and selenium. Biological Trace Element Research. 2003;**94**:61-71

[136] Dawson EB, Harris WA, Teter MC, Powell LC. Effect of ascorbic acid supplementation on the sperm quality of smokers. Fertility and Sterility. 1992;**58**:1034-1039

[137] Cyrus A, Kabir A, Goodarzi D, Moghimi M. The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: A double blind placebo controlled clinical trial. International Brazilian Journal of Urology. 2015;**41**:230-238

[138] Kumar R, Gautam G, Gupta NP. Drug therapy for idiopathic male infertility: Rationale versus evidence. The Journal of Urology. 2006;**176**:1307-1312

[139] Balercia G, Regoli F, Armeni T, Koverech A, Mantero F, Boscaro M. Placebo-controlled, double-blind, randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. Fertility and Sterility. 2005;**84**:662-671

[140] Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. Journal of Andrology. 2004;**25**:761-772

[141] Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: A randomized, double-blind, placebo-controlled trial. Fertility and Sterility. 2006;**85**:1409-1414

[142] Minamiyama Y, Ichikawa H, Masui T, Kobayashi K, Takemura S, Oka M, et al. Oral administration of reduced coenzyme Q10 ameliorates the endocrine-disrupting chemical-induced sperm toxicity in rats. Free Radical Biology and Medicine. 2016;**100**:S105-S106

[143] Mancini A, Conte B, De Marinis L, Hallgass ME, Pozza D, Oradei A. Coenzyme Q10 levels in human seminal fluid: Diagnostic and clinical implications. Molecular Aspects of Medicine. 1994;**15**:S249-S255

[144] Lafuente R, Gonzalez-Comadran M, Lopez GS, Brassesco R, Carreras R. Coenzyme Q10 and male infertility: A meta-analysis. Journal of Assisted Reproduction and Genetics. 2013;**30**:1147-1156

[145] Roveri A, Casasco A, Maiorino M, Dalan P, Calligaro A, Ursini F. Phospholipid hydroperoxide glutathione peroxidase of rat testis. Gonadotropin dependence and immunocytochemical identification. The Journal of Biological Chemistry. 1992;**267**:6142-6146

[146] Alvarez JG, Storey BT. Lipid peroxidation and the reactions of superoxide and hydrogen peroxide in mouse spermatozoa. Biology of Reproduction. 1984;**30**:833-841

[147] Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J. Dual function of the selenoprotein PHGPx during sperm maturation. Science. 1999;**285**:1393-1396

[148] Noack-Fuller G, Beer CD, Seibert H. Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. Andrologia. 1993;**25**:7-12

[149] Toman R, Hluchy S, Cabaj M, Massanyi P, Roychoudhury S, Tunegova M. Effect of separate and combined exposure of selenium and diazinon on rat sperm motility by computer assisted semen analysis. Journal of Trace Elements in Medicine and Biology. 2016;**38**:144-149

[150] Vezina D, Mauffette F, Roberts KD, Bleau G. Selenium-vitamin E supplementation in infertile men. Effects on semen parameters and micronutrient levels and distribution. Biological Trace Element Research. 1996;**53**:65-83

[151] Powell SR. The antioxidant properties of zinc. The Journal of Nutrition. 2000;**130**:S1447-S1454

[152] Chia SE, Ong CN, Chua LH, Ho LM, Tay SK. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. Journal of Andrology. 2000;**21**:53-57

[153] Harchegani AB, Dahan H, Tahmasbpour E, kaboutarakic HB, Shahriary A. Effects of zinc deficiency on impaired spermatogenesis and male infertility: The role of oxidative stress, inflammation and apoptosis. Human Fertility. 2018:1-12

[154] Hadwa MH, Almashhedy LA, Alsalman AR. Oral zinc supplementation restores superoxide radical scavengers to normal levels in spermatozoa of Iraqi asthenospermic patients. International Journal for Vitamin and Nutrition Research. 2015;**85**:165-173

[155] Kelkel M, Schumacher M, Dicato M, Diederich M. Antioxidant and anti-proliferative properties of lycopene. Free Radical Research. 2011;**45**:925-940

[156] Agarwal A, Sekhon LH. Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: Is it justified? Indian Journal of Urology. 2011;**27**:74-85