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Spinal Cord Injury, Oxidative Stress, and Antioxidants: Impact on Male Fertility

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Abstract

Objectives: Spinal cord injury (SCI) often occurs in young adult men. Factors such as erectile and ejaculation dysfunction and abnormal anomalies may contribute to the incidence of infertility. In healthy men, there is a balance between reactive oxygen species (ROS) and antioxidant scavenging systems and antioxidants have a positive impact on male fertility. Therefore, the present study examined the findings of different review studies on the role of oxidative stress (OS) and antioxidants in reducing the sperm function and treating the sperm components, respectively.

Materials and Methods: The relevant keywords were used to search for related articles in different databases such as Medline, PubMed, ISI, Scopus, and Google Scholar, which resulted in retrieving 28 articles about OS, antioxidants, DNA damage, apoptosis, and sperm parameters.

Results: Based on the findings of previous studies, antioxidant supplementation improved the number, motility, morphology, and the DNA integrity of the sperm. In addition, the combination of several antioxidants was found to perform better compared to using only one antioxidant.

Conclusions: In general, the heightened levels of uncontrolled OS is modified through the antioxidant supplements or their combination. Therefore, antioxidant supplementation can be considered in the treatment of infertile men.

Keywords: Spinal cord injury, Oxidative stress, Antioxidants, Men fertility

Introduction

Spinal cord injury (SCI) occurs in 20-50 people per year and is followed by clinical, social, and economic problems. About half of these cases occur in 16-30-year-old individuals of whom 80% are males (1).

SCI with neurological symptoms must be promptly treated to prevent secondary injuries from inflammation, edema, or hemorrhage (2). Reactive oxygen species (ROS) is one of the posttraumatic physical injuries in SCI and oxidative injuries from free radicals are considered as oxygen releasers. Most men with SCI are infertile because of a combination of factors including erectile dysfunction, muscle dysfunction, and poor semen quality (3).

Poor semen parameters and remarkable sperm DNA damages are reported in both men with SCI and animal species. ROS, as a posttraumatic physical injury in SCI, is neutrailized and oxygen species is decreased by antioxidants (4). Thus, the balance between ROS and antioxidants is essential for maintaining physiological oxidative mechanisms with the least cell damage. Antioxidants reduce ROS and act as a barrier against oxidative stress (OS). Oxidation stress reduces sperm function, indicating the toxic effect of hydrogen peroxide (H_2O_2) on sperm metabolism and motility (5). Although numerous clinical studies confirm the positive effects of antioxidants on male infertility, several others failed to support these findings (6). Accordingly, this review study discussed the effect of SCI on the male reproductive system and fertility. It further examined the role of antioxidants in reducing ROS and oxygen species alone or in combination with each other.

Search Strategy

Databases including PubMed, ISI, Scopus, and Google Scholar were searched using the related keywords. The selection of the articles was limited to those published during 2004-2018. In this review, keywords included 'spinal cord injury', 'oxidative stress', 'antioxidants', 'DNA damage', and 'sperm parameters'. Finally, 28 related articles were retrieved and used for data analysis.

The inclusion criteria for the articles were:

- Studies on SCI and various effects of SCI on male fertility;
- Studies related to antioxidants, different types of antioxidants, and their therapeutic mechanism.

Data Extraction

Five main categories were organized by the authors

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after reviewing the articles, including SCI, the causes of infertility after SCI, ROS, and DNA damage and apoptosis, neuroprotective effects of antioxidants on SCI injury, and antioxidants and the effect of antioxidants on sperm parameters.

Results

Spinal Cord Injury

SCI changes the life of an affected individual and is accompanied by reduced sperm motility and sexual dysfunctions (7). Because of losing motor and sensory function, SCI may cause chronic complications such as coronary artery diseases, urinary tract infections (UTIs), bladder dysfunction, and infertility as well (1).

In addition, SCI can lead to long-term impairments in many organ systems and with a permanent change in functions, can increase mortalities associated with a poor quality of life. Further, it has become a relatively large social epidemic in the public health system and, as a devastating disease, has an annual incidence rate of 12.1-57.8 cases per million people. There is little information about the epidemiology of SCI in 100 developing countries in which 80% of the world population live (8). Forty cases of SCI per million people occur annually in the United States except for those in the armed forces and those who die in accidents. Furthermore, there are about 12 500 new cases of SCI each year, with about 240 000-337 000 people with SCI who currently live in the United States (9). In developing countries, the prevalence of SCI was reported about 25.5 million per year. In a systematic review study of 64 articles from 28 developing countries from 1989 to 2012, Iran ranked the second country. The majority of patients with SCI were single men (10) with a mean age of 32.4 years. Depending on the severity and level of injury, life expectancy varies from 12.4% to 34.2% for the injured men at the age of 40 and they survive at least one year post-injury (9). SCI is divided into primary and secondary types. Primary SCI is caused by physical injury and is often the most important factor in determining the severity of the injury. Moreover, this type of disease leads to increased damage to neurological tissues and worsens the nerve condition. Secondary SCI follows worsening damaged tissue and precedes primary SCI. Throughout this continued injury, inflammatory cells such as macrophages, microglia, T cell, and neutrophils accumulate in the injured area. Additionally, these cells release inflammatory cytokines such as tumor necrosis factor, as well as interleukin (IL) -1α, IL-1β, and IL-6, which reach their peak within 6 to 12 hours after the injury and remain elevated for the following four days. In addition, the loss of homeostasis after SCI leads to intracellular hypercalcemia, which activates the calcium-dependent protein, disrupts mitochondrial function, and causes cell death or apoptosis (11). Oxidation stress is a secondary complication of SCI (12).

Causes of Infertility After SCI

The SCI can cause fertility disorder due to neurological damages and is characterized by erectile dysfunction and ejaculation with testicular alterations indicating spermatogenesis deficiency (12). Sperm motility, concentration, and morphology disorders develop because of OS and excessive production of ROS which is the most important predictor of an individual's ability to produce live sperms. Moreover, recurrent UTIs are considered as other important complications. Autonomic changes lead to the loss of homeostasis in the central nervous system. Further, cortical stimulus transmission to the spinal cord is prevented by the SCI after the complete modular lesion. Furthermore, the presence of a persistent inflammatory process generates a cytotoxic agent in the semen such as ROS, indicating high leukospermia and DNA fragmentation of the sperm (13).

According to a prospective study, men without SCI with high levels of ROS in semen were seven times less fertile and were unable to carry spontaneous pregnancy without therapeutic intervention compared to men with lower levels of ROS. However, those men suffering from SCI and having higher levels of ROS in their semen had less sperm motility. The SCI asthenospermia patients had degenerative changes and significant axonemal deficiencies in their sperms. Necrospermia and DNA fragmentation were significantly higher in the semen of patients with SCI compared to those without SCI (14, 15), the details of which are represented in Figure 1.

Hearn et al. showed that UTIs after SCI were the source of physical, emotional, and social disorders. UTIs were found to have a broader effect than merely physical changes (16). Basu et al in their study reported a large number of granulocytes and lymphocytes in men with SCI compared to the control group. Their results further revealed that the highest levels of leukocytes were lymphocytes and more T cells while no significant number of B cell was identified (17). Furthermore, Da Silva et al found that gene coding for PSA (KLK3) in seminal plasma at high concentrations in physiological conditions (0.5 to 0.3 mg/mL) reduced in the semen plasma of patients with SCI (18). Various studies demonstrated that ROS production is high in men with SCI. The normal functioning of sperm and acrosome function influence the physiological level of ROS, but high ROS in semen causes OS which has a negative effect on spermatozoa (15). Moreover, semen ROS affects the functioning of essential elements including protein, lipid, carbohydrates, and nucleic acids. It may also reduce the movement, count, and motility of sperm with DNA damage. Lipid peroxidation (LP) of the sperm plasma membrane by ROS reduces the fluidity of cell membrane (19).

Free radicals caused by OS significantly contribute to the production and increase of abnormal sperm while they reduce the number of sperms, as well as the transformation and fragmentation of sperm DNA. These

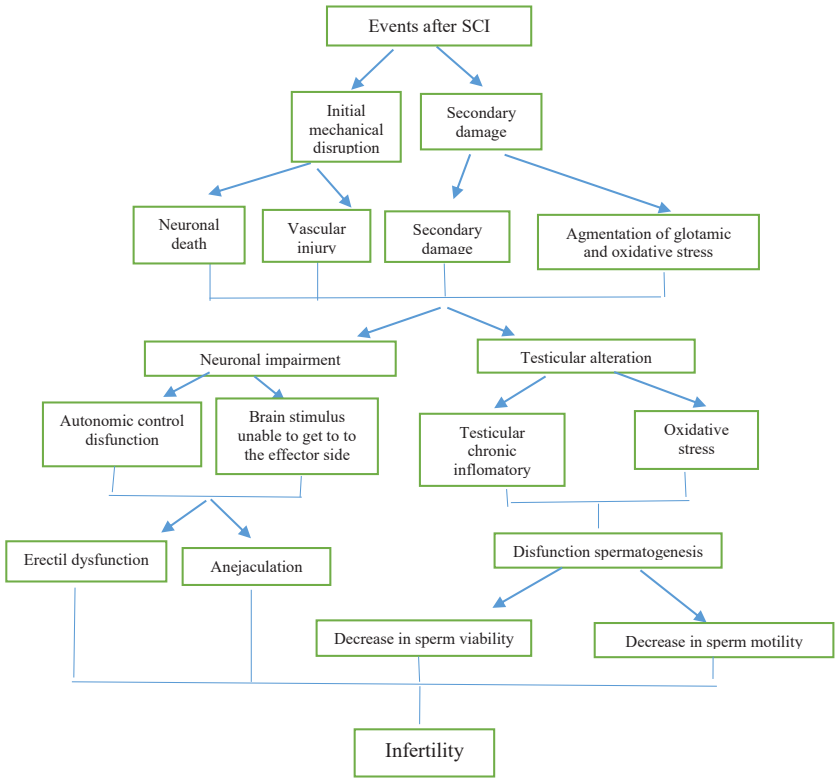

Figure 1. Events After Spinal Cord Injury Leading to Infertility. Reprinted with permission from Falavigna et al (15).

changes in sperm DNA can lead to infertility. However, increasing the production of hydrogen peroxide by spermatozoa under high oxygen pressure can reduce sperm motility (20). The high rate of cytotoxic ROS leads to sperm stability and survival (1).

What are Radicals and ROS?

The production of ROS and oxidative damage caused by oxygen free radicals are the main mechanisms of secondary injury in SCI. Radicals are chemical agents with one or more unpaired electrons. This chemical condition causes an unstable electron state and thus an extreme reaction of relevant molecules. Additionally, free radical derivatives of oxygen and nitrogen are biologically important. After acute SCI, neurons damaged by primary damage are degenerated and continue to die through a cascade of secondary damage (21). Reactive species (RS) are ROS, superoxide anion (O_2–), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH), and reactive nitrogen species such as nitric oxide (NO) and peroxynitrite (ONOO–). The secondary central nervous system injury is believed to damage the oxidation of the original parts of the cell and increase the production of free radicals and oxidants, as well as anti-oxidant systems as OS. In addition, the sperm function of infertile men is damaged by ROS produced by leukocytes or spermatozoa (22).

Infection and inflammation activate main ROS sources through different intracellular or extracellular stimuli, which are increased up to 100 times than the normal amount (23). Heightened proinflammatory cytokines such as IL-8 and lowered antioxidant superoxide dismutase can cause respiratory explosions, high levels of ROS, and ultimately, OS, which in turn, leads to sperm damage. Proinflammatory cytokines is created when there is an abnormal concentration of leukocytes for leukocytospermia, defined as the presence of more than one million peroxidase-positive cells per milliliter of semen by the World Health Organization. Leukocytes or granulocytes damage the human sperm causing the loss of sperm motility and morphology (23). It is shown that infertile men have higher NO concentrations compared to fertile men, resulting in the prevention of capacitation and sperm-oocyte binding. Active leukocytes are the main producer of ROS in the semen following inflammation and infection. Therefore, the stability of the sperm plasma membrane and its function are highly dependent on excessive ROS production 777. Premature spermatozoids with cytoplasm and abnormal head morphology are other important sources of the ROS pathophysiological outcome of OS due to the interaction of species with lipids, proteins, DNA, and other vital macromolecules (24). Krämer-Albers in a study reported that inflammation and ROS production after damage to the central nervous system increase tissue damage and inhibit neuronal regeneration. Evidence suggests that the delivery of NADPH2 oxidase from macrophages to extracellular

vesicles damages neurons and improves ROS signaling and axon recovery (25). Neutrophils are very potent inflammatory factors. Elastic cytokines, inflammatory proteins, or myelo prokidases are some factors which influence the inflammation of the tissue. The study further concluded that mitochondria are responsible for producing accelerators for significant types of reactions. Electron-damaged transport chain causes a disturbance of motion that not only stops the ion-specific channels but also prevents the specialized components of electron transport, which leads to the production of highly reactive radicals (26).

ROS can be induced internally or externally. Internal factors can be varicocele (high ROS production), cryptorchidism, and old age. In addition, external factors include smoking (27), alcohol use, exposure to radiation, and other risk factors coupled with a heightened biological or toxic level of ROS. Common sources of ROS in semen and their adverse effects are illustrated in Figure 2 (28, 29).

DNA Damage

Several studies compared semen quality in SCI patients and healthy people in terms of normal sperm count, lower sperm motility, low sperm life, sperm morphology change, and plasma components (14). The DNA of the sperm gives half of the genomic material to the offspring. Therefore, the natural genetic material of the sperm is essential for fertilization, the development of the fetus and embryo, and child health after birth, each of which can be disrupted under the influence of abnormal DNA. Anomalies and the lack of genetic materials act as an obstacle to prevent nucleus decrease, DNA break, or the arrangement of aneuploidy DNA of the sperm chromosome (30). The etiological factors related to the increased human sperm damage are summarized in Figure 3.

Samplaski et al in a study entitled "the relationship between sperm viability and DNA fragmentation rates" observed a strong reverse relationship between the survival rate of sperm and the rate of DNA degradation. Further, the survival rate of sperm was highly correlated with the rate of DNA fragmentation (31).

Similarly, Talebi et al, using cytochemical tests, examined the DNA integrity of ejaculated sperms and concluded that intra-testicular and post-testicular factors, as well as external factors increase sperm DNA damage that may affect male fertility. Furthermore, sperm fertility and DNA integrity may be damaged by medical disorders such as diabetes and varicocele that have a high proportion of sperm with abnormal DNA and immature chromatin

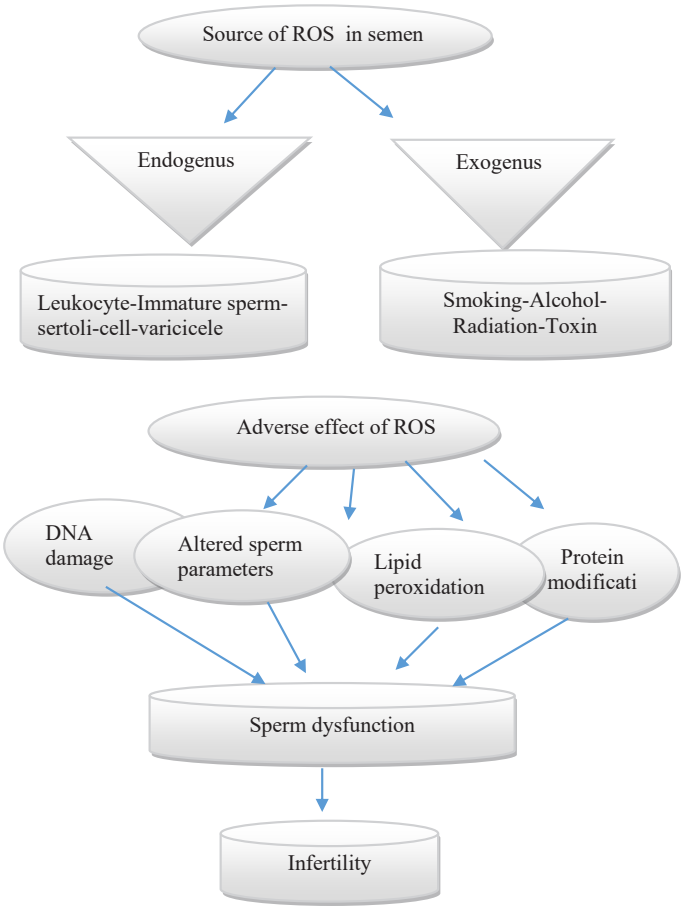

Figure 3. Common Sources Of Excessive Reactive Oxygen Species in Semen and Their Deleterious Effects.

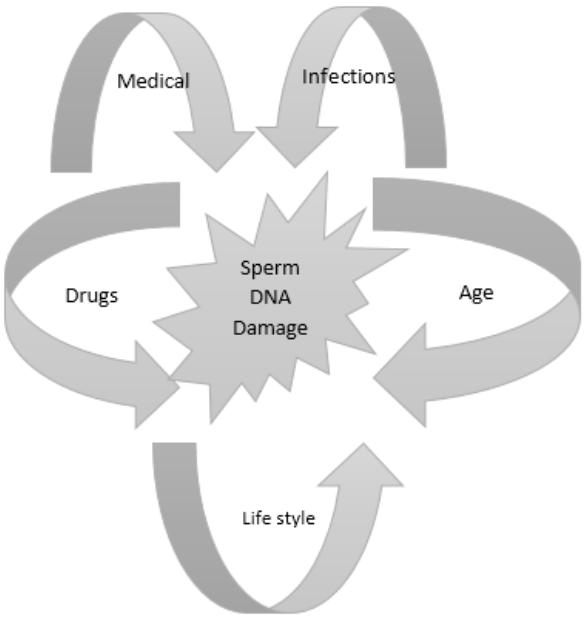

Figure 3. Etiological Factors Associated With the Increased Human Sperm Damage.

in men with medical disorders compared to fertile men. Therefore, the production of spermatozoa which is caused by varicocele with low-compacted chromatin leads to infertility (32).

In addition, ROS may have negative effects on fertility through modulating cell proliferation, differentiation, and function. Accordingly, these reactions damage any cell structure including the DNA molecule. Further, the pathogenic effects of ROS occur as a result of lower antioxidant potential, the male reproductive system, or seminal plasma (33).

In the study on transiently broken DNA strands during spermatogenesis of human and mouse, Marcon et al developed a new theory of chromatin remodeling and showed that chromatin remodeling in mammals is complicated and transient.

Although the DNA strand is broken, further research is needed to better understand the molecular mechanism. It is obvious that this could be an important step in controlling the genetic integrity of male gametes that might provide essential clues to the cause of sperm ejaculation in infertile men. In addition, the results can give us deeper insights into the cause of ROS in human (34).

Apoptosis

Unsuccessful apoptosis is another theory of DNA damage of sperm and fertility dysfunction, which is known as programmed and physiological cell death in a controlled manner (35). The ROS pathological levels are associated with spermatozoa apoptosis, which causes cell death and reduced sperm count. Previous research indicated a higher percentage of apoptotic spermatozoa in oligoasthenozoospermic men compared to normozoospermic men (36). Likewise, Restelli et al reported increased sperm apoptosis in SCI patients. There is some information regarding the effect of SCI on chromatin condensation of human sperm and apoptosis (37). "Abortive apoptosis" mechanism occurs in the sperm of infertile men thus the normal clearance of apoptotic marked spermatozoa fails to occur properly. Further, sperm DNA damage and apoptosis are useful indicators of male factor fertility and have significant relationships with the infertility of men (38). Furthermore, sperm DNA injury and apoptosis are beneficial indices of fertility and have a meaningful relationship with male infertility. Moreover, ROS is important in the metabolism of apoptosis by inducing cytochrome C and Caspase 9 and 3, which, in turn, raises single and double strand breaks of DNA (39). Therefore, seminal OS, sperm DNA damage, and apoptosis create a unified pathogenic molecular mechanism for male infertility. Apoptosis is a process of programmed cell death, which plays a fundamental regulatory function in the control of the overall size of cell populations. Additionally, apoptosis develops in male reproductive organ similar to other organs and occurs with ejaculation. The signs of apoptosis include caspase activation, the externalization of phosphatidylserine, changes in mitochondrial membrane potential, and DNA dispersion. DNA fragmentation, namely, caspase activation, the externalization of phosphatidylserine, and the alteration of mitochondrial membrane potential, represents apoptosis in the human sperm. These markers are more common in infertile men and are accompanied by an undesirable sperm function. Several indications of apoptotic pathway show its significance in spermatogenesis and sperm maturation in the testes and epididymis, respectively. In addition, apoptosis or planned cell death is the main cause of DNA damage of sperm before and after spermatogenesis. Unnecessary or damaged cells are naturally destroyed by apoptosis maintaining homeostasis in the tissues. In fact, abnormal sperm growth may be caused by abnormal apoptosis processes. Further, apoptosis involves the stages of induction, execution, and degeneration, as well as the signaling pathways of intrinsic and extrinsic sources (40).

Several genes and molecules play a major role in the onset and regulation of apoptosis. For example, BAX, BAK, PUMA, p53, c-Myc, and Bcl-2 family members that consist of pro- and anti-apoptotic factors also release other caspases. Tumor necrosis factor receptors activate extrinsic pathway signals while stress oxidation and nuclear or mitochondrial DNA damage lead to signals for the intrinsic pathway. The activation of BAX/BAK1 proteins releases cytochrome c and other factors of apoptosis from mitochondria, which develops apoptosomes and activates caspase-9, along with caspase-3 and 7 (41). Phosphatidylserine dislocation from apoptotic cell membrane occurs during apoptosis. Sperm DNA damage from apoptosis is illustrated in Figure 4 (42,43).

Moskovtsev et al compared DNA damage in ejaculated and testicular spermatozoa in patients with previously unsuccessful oral antioxidant treatment and concluded that the reconstituted sperm had the highest degree of DNA damage compared to ejaculated sperm of the same day (44). Furthermore, Muratori et al argued that the major path is to split the sperm DNA as the mechanism of apoptosis due to testicular conditions and OS during the transition in the male genital tract. The DNA-broken mechanisms may help conduct further studies concerning the role of medications in treating infertility in men. The effect of antioxidants should be evaluated in alive sperms as well (45).

Neuroprotective Effects of Antioxidants in SCI

After SCI, the inflammatory response occurs through cell activation in order to repair damaged tissues, which increases the volume and intensity of the lesions. This intracerebral dissonance activates apoptosis, neurological damage, the loss of motor function, sensitivity, autoimmune functions, or even death (46). Antioxidant therapy impedes this cascade through scavenging free radicals and inhibiting various enzymes such as superoxide dismutase, glutathione peroxidase, and catalase (47).

The antioxidant mechanistic approach involves oxidative damage caused by radical oxygen, and especially LP (48). Secondary hypoxia of SCI leads to the formation of free radicals and LP, resulting in converting lipids from plasma and intracellular membranes by reactive species into malondialdehyde, which leads to the destruction of the membrane structure (49). Some probable procedures for its containment are clear and put into four general categories: (i) Compositions preventing the start of LP and other sorts of oxidative damage through inhibiting the formation of ROS or reactive nitrogen species (50); (ii) LP-chemically inhibitor removes the beginning of radical species to steal electrons from unsaturated fatty acids and initiate LP (51); (iii) The release of LP chain reaction is stopped after its initiation; (iv) Alkoxyl ($LO^{\bullet}$) or peroxyl ($LOO^{\bullet}$) radicals are eliminated directly. The prototype is alpha-tocopherol (vitamin E), which inhibits LP through donating an electron from its phenolic hydroxyl (OH) moiety to quench a $LOO^{\bullet}$ radical. Moreover, this trilateral $LOO^{\bullet}$ antioxidant defense system functions effectively when it is exposed to post-traumatic OS. Previous research demonstrated that each of these antioxidants was quickly consumed in several minutes and hours after SCI (48).

A systematic review and meta-analysis by Bjelakovic et al revealed that prolonged treatment by beta-carotene and vitamins A and E increases the rate of mortality. However, it is not clear whether the increased mortality rate is associated with the introduced iron, the prevention of response to stress, the immune system, or any other mechanism. The findings are indirect evidence to the hypothesis that "antioxidation stress" affects the production of ROS and shows an inappropriate induction

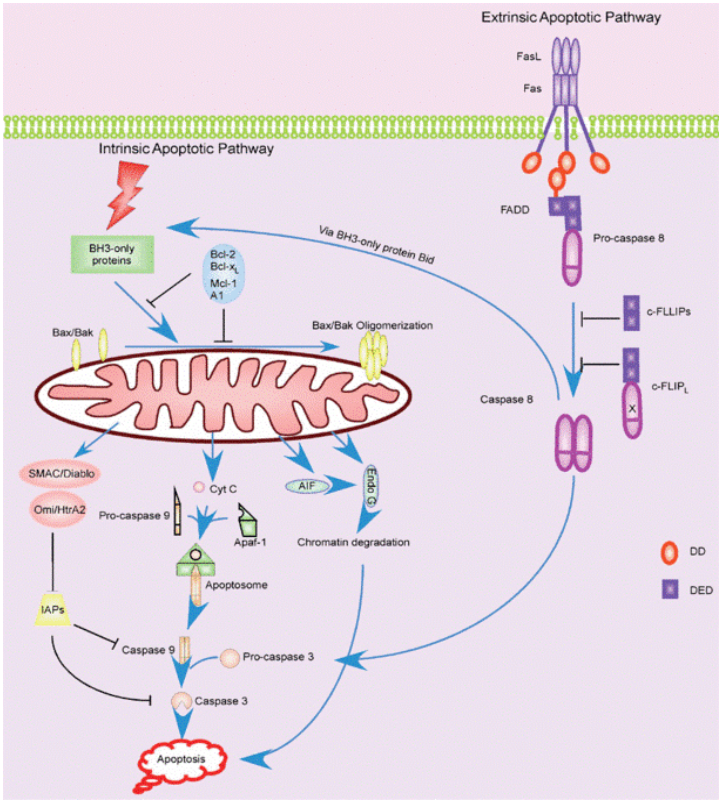

Figure 4. Apoptotic Pathways. Two Major Pathways Lead to Apoptosis: The Intrinsic Cell Death Pathway Controlled by Bcl-2 Family Members and the Extrinsic Cell Death Pathway Controlled by Death Receptor Signaling. Reprinted with permission from Zhang et al (43).

of ROS defense (52).

Antioxidants

Long-term effects of OS are due to the low antioxidant status and high free radical levels. No specific clinical symptoms or signs with OS are related to early imbalances. As a result, OS is not identified unless there is inevitable damage and its consequences continue for decades. The ROS defense mechanisms are suppressed by long-term actions or increased free radical contributing to disease development and aging (4). The oxidative damage of our cells is increased by aging and thus the consumption of exogenous antioxidants from fruits and vegetables help the endogenous antioxidant defense. The human body can absorb antioxidants while preventing or eliminating the formation of free radicals or carbonate chelate redox metals at the physiological levels. This should positively act in water and/or membrane areas and gene (53). Additionally, human antioxidant protection can minimize the ROS levels, but permit the useful roles of ROS in cellular signaling and redox regulations. ROS production and antioxidant defense activity are more or less balanced in the in vivo environment (54). On the other hand, the ROS balance is always beneficial because ROS is permanently produced in the body as low oxidative damage which requires the second type of endogenous antioxidant defense system. This system destroys ROS before the damaged bio-molecules are gathered, which causes stem cell changes and eternal damages. Furthermore, a large number of supplements with an antioxidant potential are consumed by many consumers, leading to prooxidine or antioxidative stress (4). It is essential to estimate the level of the individual OS before taking supplemental therapies. However, there is no reference value for a typical individual OS state thus its measuring is difficult (55). Antioxidant compounds in foods play an important role in protecting the human body. They are also widely used as additives in fats and oils, as well as food processing to prevent or delay the spoilage of foods. Spices and some herbs are considered as the sources of many effective antioxidants (56).

Antioxidants are divided into preventive and chain categories based on their functioning. Preventive antioxidants encompass superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, DNA repair enzymes, as well as several metal ions like albumin. In addition, the obviation of antioxidants or chain antioxidants includes ascorbic acid (vitamin C), carotenoids (e.g., retinol and vitamin A), uric acid, α-tocopherol (vitamin E), glutathione, and polyphenols (flavonoids).

Vitamins C and E, carotenoids, and polyphenols (e.g., flavonoids) are currently recognized as the main exogenous antioxidants. The dose of synthetic antioxidants shows that the amount of the recommended daily allowance levels of vitamin C and E is low for preventing OS (57). High body OS can be modified through antioxidant supplements which are uncontrolled by endogenous antioxidants. Further, antioxidants are very important to food scientists since they delay or inhibit food oxidation. Clinical studies demonstrated that eating a diet rich in fruits, vegetables, whole grains, legumes, and omega-3 fatty acids can improve the disease condition. Antioxidants are naturally present in low levels in foods (58) and can also protect food through disabling metallic ions and oxygen singlet. On the other hand, OS is due to the relative increase in free radicals and a reduction in the physiological activity of antioxidant defense against these radicals and thus can even lead to cell death. The inhibitory function of ROS production and the ROS drainage activity can control the amount of OS produced by the cell. In physiological conditions, the balance between pro-oxidant and antioxidants is low in favor of pro-oxidant products. Antioxidants and OS that cause antioxidant imbalance can be harmful to the body, and therefore, cause cancer and accelerate the aging process. It is worth mentioning that the balance between ROS and antioxidants exists in normal physiological conditions and OS develops only when the antioxidant system cannot neutralize high ROS production (59). Natural antioxidants are usually extracted from plant sources, and their effects are enhanced through plant species, diversity, extraction and/or processing methods, and the environment. The mode of action for such materials varies depending on the source material, the presence of synergists and antagonists, and the feeding of the matrix to which it is applied (58). Thus, it is important to diagnose oxidative imbalance at an early stage in the long term for preventing oxidative and antioxidant activities (Figure 1). These points need to determine the individual oxidation status before starting or ending the treatment with antioxidants. This may prevent or reverse the development of diseases which arise because of chronic oxidative imbalance (4).

The Effect of Antioxidants on Sperm Parameters

Selenium

Selenium is an essential element for many physiological processes, especially for the functions of immunity and reproduction, along with the metabolism of thyroid hormones and antioxidant defense (60). Humans and animals keep at least 25 selenoproteins. It helps maintain the normal sperm structure and integrity. Selenium is a component of deionidase (D) enzymes (61) that are divided into three types with different tissue distributions as well as gene expression regulation and function. D1 is primarily expressed in the liver, kidney, and thyroid and can reduce thyroxin (62). Furthermore, D2 can be found in a variety of tissues including skeletal muscle, bone, pituitary, retina, cochlea, central nervous system, thyroid, and brown adipose tissue, which is involved in the conversion of Thyroxine (T4) to the more active Triiodothyronine (T3) by 5′-deiodination. On the contrary, D3 deactivates

T3 through reducing T4 activation. Moreover, selenium is important for the regulation of immune functions such that selenium reduction and the selenoid biosynthesis of proteins are impaired in inflammatory diseases (63). Additionally, selenium has a major role in the growth and development of the processes involved in regulating production-related processes and reproduction abilities of the animals. Further, this element defends the antioxidant present in the organism and substantially modulates male ejaculation quality. Several selenoproteins such as synthetic selenophosphate and selenoprotein mitochondrial capsule were localized in the testes. In low amounts, such selenoproteins are essential for fertilization, acrosome reaction, overactive, movement, and capacity (6). Safarinejad et al examined the impact of selenium and N-acetyl cysteine with a 30-week treatment period on 468 infertile men with idiopathic oligo-asthenoteratospermia. In response, the serum follicle stimulating hormone decreased, but serum testosterone and inhibin B increased.

In addition, all semen parameters were improved with the treatment of selenium and N-acetyl-cysteine. In other words, selenium injection with N-acetylcysteine resulted in more beneficial effects on sperm parameters (64). Hosnedlova et al, in a study on the biological effects of selenium in selected animal species reported that selenium was involved in the antioxidant defense of the body and significantly modulated the quality of human ejaculation. Several selenoproteins such as selenophosphate synthase and mitochondrial capsule selenoprotein were localized in the testes. As previously mentioned, OS is an important factor that influences the fertilization of sperm through LP (60). Selenium deficiency is often observed as a deficiency of other nutrients with antioxidant activities such as vitamin E. Accordingly, a variety of antioxidant defense systems can benefit from various types of antioxidants. Lack of these 2 nutrients, namely selenophosphate synthase and mitochondrial capsule selenoprotein, are the cause of certain diseases, and the exclusion of each one is asymptomatic, but the shortage of both leads to the disease (65). Mohammadi et al conducted a study on the "Up-regulation of CatSper genes family by selenium" and found a high level of CatSper gene in the experimental group compared to the control group. Furthermore, the sperm analysis showed that sperm parameters improved after the treatment in aging and male adults. Moreover, selenium treatment in older people can regulate the expression of CatSper genes and increase sperm motility. In addition, it can improve sperm parameters, especially morphology and survival rates (65).

Keskes-Ammar et al, evaluating sperm OS and the effect of oral vitamin E and selenium in infertile men, confirmed the effects of vitamin E and selenium on skin quality and LP. The antioxidant protein is based on the fact that the metabolism of vitamin E is close to that of the selenium and this diluted element is an antiperspirant. In this study, sperm motility improved significantly after treatment using vitamin E-Se. However, no effect was observed respecting viability or morphology and sperm concentration. The beneficial effects of treatment by vitamin E cannot be explained through seasonal variations since a significant improvement in mobility is obtained during the time period when the least amount is observed theoretically (66).

Vitamins C and E

The semen plays a role in protecting the sperms against antioxidants such as glutamine peroxidase, superoxide dismutase, vitamins E and C, selenium, and carnitine (67). In their study regarding the effect of cyclodextrins, cholesterol, and vitamin E and their complexation on cryopreserved epididymal ram semen, Benhenia et al (68) introduced a new method for improving post-cooling sperm quality using vitamin E and cholesterol loaded-cyclodextrins in semen extender.

Cyclodextrins of vitamin E increase the sperm motility while decreasing the peroxidation of membrane lipid. Additionally, cholesterol-loaded cyclodextrin leads to membrane integrity (with the hypo-osmotic swelling test or HOST) and sperm motility. Both vitamin E and cholesterol have cyclodextrins and substantive effects on motility, protein, and lipid (68).

Amini et al investigated the effects of vitamins C and E on the rate of sperm motility, lifespan, and malondialdehyde (MDA) level. Semen samples from 10 sexually-mature Ross were divided into nine equal parts and diluted using 'Beltsville addition' containing no antioxidants. In addition, the parts encompassed 100 (C100), 200 (C200), 400 (C400), and 800 (C800) μg/mL vitamin C, as well as 2 (E2), 5 (E5), 10 (E10), and 15 (E15) μg/mL vitamin E. The sperm motility, sperm development, and growth, sperm viability and semen MDA sperm levels were evaluated after thawing. The results showed that the C200 and E5 extenders further increased compared to other extenders except for the E10 developer (P<0.05). Further, the advanced motility in the E5 extender (P<0.05) was higher than that of the other extenders except for the C200 and E10. Furthermore, the addition of C200 and E5 increased sperm viability post-thawed spermatozoa (P<0.05) compared to other extenders. Finally, the results revealed that MDA levels were lower in other products of C100 and C200 extenders than those of other extenders (P<0.05) except for E5. The results of this study indicated that C200 and E5 could improve the function of post-thawed rooster spermatozoa (69).

Ascorbic acid, which is called vitamin C, is a water-soluble antioxidant and acts as a key factor in various hydroxylation and amidation processes. It is applied in the synthesis of collagen, proteoglycan, and the ingredients of the intercellular matrix, along with vitamin E. Vitamin C can be found in high concentrations in semen and its intake increases its concentration in seminal plasma and prohibits DNA damage (70). In the study on semen

samples of normozoospermic men, Mangoli et al evaluated the effects of vitamin C on sperm parameters, sperm chromatin, and apoptosis. To this end, they divided 40 semen samples into five groups; group 1: control, group 2: in which semen was prepared by means of swim-up method and vitrified later, group 3: the neat semen was vitrified, group 4: vitamin C (600 mM) was added to the prepared spermatozoa and then vitrified, and group 5: vitamin C (600 mM) was added to the neat semen. Their results suggested that all the sperm parameters (i.e., count, motility, morphology, and viability) were present in all groups, especially group 4. Accordingly, sperm chromatin damage and acrosome reaction dysfunction were high in vitamin C-added groups, particularly in group 4. Therefore, vitamin C can mitigate the adverse effects of the vitrification of sperm parameters, chromatin quality, and apoptosis in both the spermatozoa and the sperm which were produced from normozoospermic samples (71).

Similarly, Sonmez et al examined the quality of sperm, LP, and plasma testosterone of the male rats by adding ascorbic acid to drinking water. They categorized 24 Wistar rats to three groups and, for 8 weeks, added 500 and 250 mg/kg/d ascorbic acid to the drinking water of the first and second group, respectively, while the third group was only given drinking water. Based on their results, sperm motility was not significantly different in the three groups whereas sperm volume in the epididymis and plasma testosterone significantly increased in rats that received ascorbic acid (P<0.05). Accordingly, the findings showed that ascorbic acid was correlated with high fertility in rats (72).

Greco et al studied the reduced incidence of sperm DNA fragmentation through oral antioxidant treatment and observed a significantly reduced incidence of DNA fragmentation in ejaculated spermatozoa following two months of oral antioxidant therapy. Unlike the effects on sperm DNA integrity, no significant improvement was found in sperm count, movement, and morphology after in vivo antioxidant treatment. The knowledge of the effects of antioxidants on sperm, movement, and morphology is different. Such a difference between the studies is likely associated with the type and amount of antioxidants, the characteristics of the patient group, and the duration of the treatment. The findings of this study demonstrated that, in infertile non-smoker men with unexplained high levels of DNA fragmentation, the percentage of DNA-fragmented spermatozoa in ejaculation can be significantly reduced through relatively short oral treatment using the combination of vitamins C and E (73).

Carnitine

L-carnitine (LC) or 3-aminobutyric acid is a natural compound, as well as a semi-essential vitamin-like substance that is necessary for metabolism. LC participation in intermediate metabolism is essential for biological processes, which plays an important role in the formation of sterile acylcarnitine esters from chain fatty acids. The highest concentration of LC in the epididymis is 2000 times more than the total blood concentration. Moreover, the high level of LC in the epididymis results from an active secretory process. A positive relationship exists between the initial sperm motility and increased LC in epididymis and L-acetyl in sperm (74). Garolla et al examined the role of carnitine supplements in idiopathic asthenospermia and the reason for their use for osteoporosis patients. In this study, 30 osteoporosis patients were assigned to two groups based on the level of glutathione peroxidase fluoride (PHGPx) and were given a placebo for the first three months. They were then given oral carnitine L (2 g/d) for another three months, followed by receiving no medication for three months. Based on the results, the sperm motility improved only in patients with normal levels of PHGPx. Additionally, the results showed that phospholipid-hydroperoxide glutathione peroxidase has an important role in male infertility and that carnitine therapy increases sperm motility in the presence of normal functioning of mitochondria (75). Wu et al evaluated the pregnancy promoting effect of LC combined with intracytoplasmic sperm injection (ICSI) on treating infertility in males with oligoasthenozoospermia. A total of 129 patients with oligoasthenozoospermia received oral LC for 2 weeks, along with ICSI (drug group, n=42) and only ICSI (control group, n=87). Then, the amount of sperm concentration and motility, sperm deformity, as well as the rate of fertilization, cleavage, available embryo, and clinical pregnancy was measured in the groups before and after LC. Based on the reports, short-acting drugs containing LC improved sperm quality and increased ICSI success rates (76).

Zinc

Zinc (Zn) is considered as the second most abundant metal in the body after iron. Although red meat, fish, and milk are rich in Zn, the World Health Organization suggested that Zn deficiency influences about one-third of the world population. It is indicated that Zn supplementation typically protects against spermatozoa and bacteria and prevents damage to chromosomes. In addition, it is essential for the development of testes and mature sperms. The male hypogonadism and incomplete development of sexual characteristics in humans can be positively associated with Zn deficiency. It is further found that low levels of Zn in semen can reduce sperm fertilization capacity (77). Ebisch et al reported that sperm concentration increased in patients who received folic acid 5 mg and zinc 66 mg for 26 weeks. However, there was no improvement in other semen parameters. Finally, a positive correlation was found between Zn serum and sperm concentration, motility, and inhibin B (78). Furthermore, Giacone et al investigated the effects of Zn, D-aspartic acid, and coenzyme-Q10 on

the sperm function. A number of 24 males (5.5 ± 3.32 years) including 12 men with normozoospermie and 12 men with asthenozoospermia participated in this study. Spermatozoa from each sample were divided into 2 control (A) and a Zn, D-aspartic acid (B) groups and coenzyme Q10 group (C). Progressive mobility, the number of spermatogenesis with progressive motion after the swim, LP, and DNA fragmentation were evaluated after three hours of incubation. This led to a significant increase (P<0.01) in the number of sperms in post-swelling among both normozoospermic and asthenozoospermic patients. Moreover, a statistically significant difference was reported regarding D-aspartic acid and coenzyme Q10 in both normozoospermic and asthenozoospermic patients after incubation with Zn (P<0.05). However, there was no statistically significant effect on sperm DNA parameters (79).

Colagar et al assessed the relationship between Zn levels in seminal plasma and sperm quality in fertile and infertile men. Sperm samples were collected from fertile and infertile men. After sperm analysis, the concentration of Zn, Mg, Ca, Na, and K in the toxic plasma of all groups was determined using the atomic absorption spectrometry. The concentration of the elements in the seminal plasma of all groups was Na> K> Ca> Zn> Mg, respectively. In fertile men, they were significantly higher than those of mint nitrogen (P<0.001). Fertile subjects demonstrated significantly higher seminal Zn levels than those of the infertile group (P<0.001). Additionally, Zn seminal in fertile and infertile men was significantly associated with sperm count (P<0.01) and natural morphology of the sperm (P<0.001). In addition, there was a significantly positive correlation between seminal Zn and Ca (P<0.01) and K (P<0.01) levels in all specimens. Based on the results, feeding Zn was a major risk factor for sperm quality and male infertility (80).

Astaxanthin

As a carotenoid xanthophyll, astaxanthin (AX) is found in different microorganisms and marine animals. Further, it is a red fat-soluble pigment without pro-vitamin A activity that has a stronger biological activity than other carotenoids. Salmon, trout, and shrimp use this carotenoid as a pigment in their feed. Furthermore, AX is derived from seafood or extracted from *Haematococcus pluvialis*

for food supplements in humans and animals. Its natural sources include algae, yeast, salmon, trout, krill, shrimp, and crayfish. *H. pluvialis* is one of the most important sources of natural AX. Moreover, AX is a lipophilic compound which is dissolved in solvents and oils. Solvents, acids, edible oils, as well as microwave assisted and enzymatic methods are employed for its extraction. Additionally, this carotenoid is accumulated in *Haematococcus* encysted cells and contains bilateral bonds, along with hydroxyl and keto with lipophilic and hydrophilic characteristics. In addition, its red color is constructed by the conjugated double bonds at the center of the composite (81). An antioxidant is a molecule that inhibits oxidation.

Basioura et al investigated the effect of AX antioxidant on the boar semen. A total of 20 ejaculations from 10 boars were divided into three groups: control (SC), solvent control (semen with dimethyl sulfoxide, the thinner of astaxanthin), and sperm with AX in the concentration of 0.5 µmol/L. Then, sperm parameters were examined 0, 24, and 48 hours after storage at 17°C in three experiments encompassing before (0 hour) and after (1 hour) sperm resistance tests at 37°C (experiments I and II, respectively) and before (0 hour) and after (1 hour) sperm in vitro incubation spectrometer (experiment III). Experiment I was generally better than group SC and viability was higher in group C. Further, fast sperm motility was higher in group A while it decreased in other groups. In experiment II, group A showed better long-term results regarding motility and viability compared to the other two groups. In experiment III, the rates of viability and motility decreased in groups SC and C whereas these parameters failed to differ in group A in terms of the experiment times. Therefore, the results of this study confirmed that AX had a favorable and protective impact on the quality of boar semen at the time of the study (82).

Vahidinia et al assessed the effect of calorie-restricted and antioxidant supplemented diet separately and in combination, on the quality and quantity of Wistar rat sperm. A total of 40 Wistar rats were randomly divided into four groups of 10. Group 1 had a routine diet for at least 86 days while group 2 had a restricted diet. Furthermore, group 3 received routine diet plus AX, along with vitamins E and C supplementation) and group 4 had restricted diet plus AX, as well as vitamins E and C supplementation. After 86 days, sperm count in group 4 was significantly higher than that of the other groups. However, the percentages of sperm motility significantly reduced in groups 2, 3, and 4 compared to group 1. As regards the antioxidant capacity, the results demonstrated a significant increase in groups 3 and 4 (P=0.20 and P=0.0, respectively) compared to groups 1 and 2. Therefore, an antioxidant supplement with or without caloric restriction has no significant effect on serum isoprostates in each group. However, AX, together with vitamins E and C and caloric restriction could improve infertility in rats in some cases (83). Similarly, Simson et al evaluated the effect of AX on sperm quality of Karan Fries bulls during storage at 5°C. AX was found to help sperm maintain or preserve sperm from damage when stored at 5°C. Moreover, the percentage of live sperm at 0, 24, 48, and 72 hours was higher in the AX group compared to the control group. Conversely, the concentration of superoxide dismutase decreased (P<0.05) only after 24 hours of preservation in AX samples compared with the control ones. Based on the findings of this study, the supplementation of AX to the semen extender helped to preserve or protect

spermatozoa from damage when they were stored at 5°C oxidative molecules prevented by endogenous and exogenous antioxidants such as carotenoids with its esters, which had 80% anti-lipid peroxidation activity and prevents lipid peroxidation in biological samples working as an anti-inflammatory agent (84). Algal cell extracts of *Haematococcus* and *Chlorococcum* were found to significantly reduce the bacterial load and gastric inflammation in *H. pylori*-infected mice (85). Cancer, bleeding, diabetes, heart disease, liver, neurodegenerative, and skin diseases can be enormously affected by AX giving numerous neuroprotective effects of protection in various experimental models of neurological diseases including both acute injury and chronic nerve failure. It has anti-oxidative, anti-inflammatory, and anti-apoptotic effects as well. When consumed with food, AX is a confidential nutrient with no toxic effects. Moreover, as a lipid-soluble compound, it acts effectively via the blood-brain barrier, therefore, it is of great help for the treatment of neurological diseases. Further evaluation of protective characteristics and underlying mechanisms of AX is necessary thus it should be highlighted as a novel neuroprotective element. Several studies examined the neuroprotective effects of AX on different models of neurological disorders. However, it is still subject to further research. However, the effect of AX esters on the treatment of neurological complications is under doubt, especially due to the fact that Ax diesters can be easily absorbed into metabolism with more effective biological activity compared to its free form (86). Furthermore, it is prominent to consider that current information on the protection of the AX-mediated neuroprotection mainly comes from ischemic stroke, subarachnoid hemorrhage, Alzheimer disease, and Parkinson disease for other neurological diseases such as traumatic brain damage, intracerebral hemorrhage, and hemodialysis. Therapeutic time windows, the reliability of drug injection routes, and AX optimal doses need further analyses. The development of clinical trials for assessing AX as a therapy for neurological diseases is essential considering that there are various enlightening general safety results, neurological experimental model studies, and clinical trials regarding other diseases (87). Furthermore, the meta-analysis of the increased mortality rate from the additional iron load, response to stress, immune system suppression, or any other mechanism is unknown. Thus, the findings provide indirect evidence for a hypothesis that "anti-oxidative stress" influences ROS production and inappropriate ROS induction. On the other hand, the excess of OS for a long time increases the oxidation damage to the negative effects of health and longevity (52).

Conclusions

The adverse effects of SCI in young adult men include physical, mental, and social problems.

The post-traumatic production of ROS and the resulting oxygen free radical-induced oxidative damages are the secondary injury mechanism in SCI. In addition, OS is considered a major contributory factor to male infertility. Normal sperm physiological processes depend on the low and controlled concentrations of ROS. However, sperm function can be significantly impaired by an increase in OS. Excessive ROS is the results of damaged, deficient, or abnormal spermatozoa. These defects cause male infertility through peroxidation damage to the sperm plasma membrane, DNA damage, and apoptosis. Moreover, the apoptosis of spermatozoa can lead to cell death and reduced sperm count. As a matter of fact, the elevated level of ROS is known to induce apoptosis and is tracked in mature spermatozoa of infertile men. The heightened levels of the uncontrolled OS by the endogenous antioxidants are corrected by antioxidant supplements. The current level of safety and quality of supplements with inappropriate antioxidant properties is scrimpy. Although advancements are observed in bioengineering mechanisms, bioconversion, and the action of these supplements, there are still many gaps in our knowledge. Further, the current knowledge of the safety and quality of supplements with antioxidant effects is scarce. Ideal antioxidants should be easily absorbed by the body and prevent or resolve free radicals or chelate redox metals. Based on the findings of various studies, the excessive use of antioxidants raises mortality as well. The amount of exogenous antioxidant cells may reduce production or the uptake of endogenous antioxidants such that the overall antioxidant potential remains intact.

Another issue is the use of a single or combination of antioxidants. Previous research shows that the combination of several antioxidants acts better than just one antioxidant and such combination can improve the parameters of the sperm. However, due to the controversial results, further studies are needed concerning using a single or a combination of antioxidants, as well as their doses.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

Not applicable.

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